# Behavior, Ecology, and Evolution of the Cicada-Hunting Parasitoid Flies (Diptera: Sarcophagidae: Emblemasomatini)

by

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Behavior, Ecology, and Evolution of the Cicada-Hunting Parasitoid Flies

(Diptera: Sarcophagidae: Emblemasomatini)

Dissertation directed by Professor Robert P. Guralnick and Professor M. Deane Bowers

## Abstract

Many species of parasitoid insects find their hosts by "eavesdropping" on the communication signals of other insects. Eavesdropping parasitoids are abundant and taxonomically diverse, but relatively little is known about the ecology and evolution of eavesdropping as a host-finding strategy. Furthermore, most such research has focused on a very small number of comparatively well-studied species. For my dissertation research, I developed a new eavesdropping parasitoid study system, and I used this system to investigate several key questions about the ecology and evolution of eavesdropping parasitoids in general. Specifically, I studied the parasitoid fly *Emblemasoma erro* (Diptera: Sarcophagidae), as well as other species of *Emblemasoma*, all of which eavesdrop on the acoustic mating calls of their cicada hosts (Hemiptera: Cicadidae). First, I demonstrated that *E. erro* is, in fact, an eavesdropping parasitoid, and I provided the first detailed descriptions of the life history, infection behavior, and host parasitism rates of this species. Next, I investigated the role of male hearing in *E. erro*. I found that both male and female *E. erro* use the calls of their hosts to locate mates, which is the first known example of intraspecific acoustic eavesdropping for the purpose of mate finding. With a third set of experiments, I investigated the signal preferences and host range of *E. erro*. I discovered that, unlike some other acoustic parasitoids, *E. erro* can exploit a large number of host species by listening for acoustic signal characteristics that are not species-specific. Fourth,

through a variety of laboratory and field experiments, I demonstrated that larval *E. erro* avoid intraspecific competition by altering the mating calls of their host cicadas. These call changes make parasitized hosts much less likely to be discovered, and superparasitized, by foraging female *E. erro*. Finally, I used multi-locus DNA sequence data from many sarcophagid species, including multiple species of *Emblemasoma*, to reconstruct the phylogeny of Sarcophagidae. I then inferred the evolution of feeding behaviors in this ecologically diverse family of flies and confirmed that hearing, which is only found in *Emblemasoma*, most likely evolved in response to the selective pressure of parasitoid host finding.

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# CHAPTER 1

## EAVESDROPPING PARASITOIDS: AN OVERVIEW

### 1.1 Introduction

Anyone who has heard the mid-day choruses of cicadas or watched the twilight displays of fireflies knows that insects are accomplished communicators, but these attention-grabbing acoustic and visual performances reveal only a fraction of insect communication. If we could perceive the substrate vibratory signals or pheromones deployed by countless other insect species (Greenfield 2002), the natural world would surely be abuzz with an almost constant din of insects talking to each another. Much long-distance insect communication, whether by sound, sight, or smell, is directed toward finding mates, but insects use intraspecifc communication for a host of other reasons, too, such as responding to threats, exploiting food resources, facilitating migration, or avoiding competition (Lewis 1984, Nufio and Papaj 2001, Greenfield 2002). In these roles, signaling is vital. But communication can be hazardous, too. Any time an insect chooses to communicate, it risks informing enemies that might also be listening (Haynes and Yeargan 1999).

Indeed, insects known as *eavesdropping parasitoids* make their living by listening in on the communications of other insects. *Parasitoids* are insects whose offspring feed parasitically on other animals (usually other insects) but eventually kill their hosts, thus occupying an ecological middle ground between true parasites and true predators (Godfray 1994). This remarkably successful strategy is found in at least six insect orders, has evolved independently more than 100 times, and accounts for up to 25 percent of all insect species (Eggleton and Belshaw 1992, Godfray 1994). Parasitoids use a wide variety of search techniques to locate their host animals, but many parasitoids rely on indirect methods, such as locating their hosts' food

plants or preferred habitats (Godfray 1994). Such strategies are inherently unreliable, because even if the correct habitat can be located, there is no guarantee that the host will actually be there. Eavesdropping parasitoids have a completely different approach: By intercepting their hosts' communication signals, they simply let their hosts *tell* them where to look (Zuk and Kolluru 1998, Haynes and Yeargan 1999).

This dissertation explores the ecology and evolution of eavesdropping parasitoids. In the remaining chapters, I first elucidate the behavior and natural history of a particular eavesdropping parasitoid, *Emblemasoma erro* Aldrich (Diptera: Sarcophagidae). Then, I use *E. erro* and its cicada hosts (Hemiptera: Cicadidae), along with other species of *Emblemasoma*, to address several key questions about the ecology and evolution of eavesdropping parasitoids. This work combines descriptive life history and behavior studies, field behavior experiments, molecular systematics and phylogenetic inference, and model-based evolutionary analyses, among other techniques.

With this introductory chapter, I establish a basic context for the chapters that follow by providing a brief, yet comprehensive, overview of eavesdropping and eavesdropping parasitoids. I first discuss parasitoid host finding and the advantages of eavesdropping, and I then survey the extensive literature concerning eavesdropping parasitoids and summarize our current knowledge of these insects. As part of this review, I also introduce the main questions that motivate the remaining chapters.

### 1.2 The problem of host finding and the benefits of eavesdropping

At some point in their life cycles, all parasitoid insects eventually face the same, fundamental challenge: They must find suitable host animals to ensure the survival of the next generation. This task, which usually is the responsibility of adult female parasitoids (Eggleton

and Belshaw 1992, Godfray 1994), is truly a case of trying to find the proverbial needle in a haystack. Parasitoids live in complex environments in which they must find host animals that are often small, scattered, and cryptic.

Parasitoids cope with this challenge by exploiting many different sources of information to guide them to their hosts. Godfray (1994) recognized three broad categories of information that parasitoids can use for host finding: 1) cues derived from the host's microhabitat, such as detecting a host's potential food source; 2) indirect cues derived from the host's activities, such as detecting frass or chemicals released by feeding-damaged plants; and 3) cues derived directly from the host itself, such as detecting a potential host's movement or smell.

Ideally, parasitoids should focus on cues that come directly from their hosts, because, unlike the other two categories of information, such cues are almost certain indicators of a potential host's presence. In reality, this is often extremely difficult because potential hosts usually do their best to avoid giving away any information that could lead to their detection. After all, for host animals, the consequences of discovery by a searching parasitoid are usually disastrous: parasitization is almost always fatal and results in either complete or partial loss of reproductive fitness. With such extreme consequences, host animals are strongly selected to minimize their chances of being found (Vet et al. 1991, Vet and Dicke 1992, Godfray 1994, Vinson 1998). At the same time, the stakes for parasitoids are nearly as high as for their hosts. Failure to find suitable hosts means that a female parasitoid's offspring might not survive, so parasitoids experience intense natural selection for efficient, accurate host finding.

This evolutionary cat and mouse game has created a predicament for host-foraging parasitoids that has been referred to as the “reliability-detectability problem” (Vet et al. 1991). As selection pressure from host-seeking parasitoids causes host animals to minimize their

detectability, cues coming directly from the host animals become less and less useful for long-range host location. The end result is that the sources of information that most reliably reveal a potential host's presence – that is, the cues emanating directly from the host animals themselves – also tend to be the sources of information that are the most difficult to detect. (Note that, in this discussion, "difficult to detect" does not refer to the physiological problem of detecting a particular cue. Rather, it simply means that low amplitude cues are harder to detect than high amplitude cues.) Consequently, parasitoids are often forced to rely on cues that are detectable from a long distance but are also much less reliable indicators of host presence. For example, many parasitoid species are known to orient to volatiles emitted by their hosts' food plants (Vinson 1976, Godfray 1994). These volatiles might be suitable for long-range detection, but they provide no guarantee that the host animal will actually be present when the parasitoid arrives.

Some parasitoids have discovered, however, that intraspecific insect communication can provide a convenient solution to the reliability-detectability problem. Many insects that are otherwise cryptic and inconspicuous produce communication signals that are both highly detectable and provide reliable information about the signaler's location, which makes them ideal cues for host-searching parasitoids. Sexual communication signals, especially those produced by males, might be particularly useful in this regard because they are often selected for high amplitude and long-range detectability (Burk 1988, Greenfield 2002). Furthermore, even though eavesdropping obviously makes communication costly for a parasitoid's host animals, choosing not to communicate might also result in severe fitness consequences (loss of mating opportunities, for example), and this could help ensure the persistence of the host-finding cue even under the pressure of parasitoid exploitation (Vinson 1998). Eavesdropping, then, provides

a way for parasitoids to neatly sidestep the trade-offs implied by the reliability-detectability problem. This is undoubtedly the chief advantage of eavesdropping, and it is now clear that a considerable number of parasitoids use this strategy to locate their hosts (see the next section).

### 1.3 A literature survey of eavesdropping parasitoids

The idea that long-range communication signals can expose an insect to its natural enemies is certainly not a new one, having been discussed by naturalists at various times in the 1800s and even earlier (e.g., Lord 1866, Swinton 1880, Distant 1897a, 1897b, references in Lloyd 1973). Nevertheless, parasitoids that eavesdrop on their hosts are a relatively recent discovery.[1] In 1964, Arthur et al. discussed circumstantial evidence that the parasitoid wasp *Temelucha interruptor* (Gravenhorst) located its hosts by eavesdropping on the host-marking pheromones of another parasitoid wasp species, but it was not entirely clear whether or not true eavesdropping was involved (evidently, this has still not been determined). Soon after, Bedard (1965) and Rice (1968, 1969) reported a series of experiments that demonstrated the pteromalid wasp *Tomicobia tibialis* Ashmead eavesdrops on the sex/aggregation pheromones of its bark beetle hosts (*Ips* spp.). Their results provided the first strong evidence that parasitoids could use the pheromone signals of other insects as cues for long-range host location. For about a decade, eavesdropping parasitoids were only known to exploit pheromone communications; the first reports of parasitoids using acoustic sexual signals for host location came in the mid 1970s (Cade 1975, Soper et al. 1976).

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1 Decades before the first eavesdropping parasitoids were discovered, Donisthorpe (1927) reported that the phorid parasitoid fly *Pseudacteon formicarum* Verrall was attracted by the formic acid secretions of its *Lasius* spp. ant hosts, an observation that was only recently experimentally confirmed (Maschwitz et al. 2008). Although formic acid acts as both venom and alarm pheromone for many species of formicine ants (Schmidt 1986), it does not seem to serve a communicative function in the genus *Lasius* (Regnier and Wilson 1969, Schmidt 1986), so this is probably not a case of true eavesdropping.

Since the earliest demonstrations of eavesdropping some 40 years ago, the number of parasitoid species that are known to eavesdrop on their hosts has increased dramatically, but, to my knowledge, the widely scattered literature reports of eavesdropping behavior by parasitoids have never been comprehensively reviewed. Without such an overview, little can be said about the current state of knowledge concerning these insects or their taxonomic and ecological diversity. To address this, I completed an extensive literature survey in which I attempted to gather all known examples of parasitoids that definitely, or probably, eavesdrop in some way on their hosts' communication signals. I also included species that I report as eavesdroppers for the first time in this dissertation. (Note that subsets of the species treated here have also been included in earlier reviews that relate in some way to eavesdropping. Haynes and Yeargan (1999) and Zuk and Kulluru (1998) are especially relevant, but also see Conti and Colazza (2012), Fatouros et al. (2008), Mizutani (2006), and Vinson (1984). Godfray (1994) includes a brief discussion of host signal interception by parasitoids. Also, at least a few examples of eavesdropping have been included in most reviews of parasitoid host-finding behavior.)

A complete summary of the results of this literature survey, with essential life history details for all species of eavesdropping parasitoids, is provided in Table 1.1. In total, I estimated that we currently know of about 100 species of likely eavesdropping parasitoids. The number of known eavesdropping parasitoid species has grown steadily and approximately linearly since the first examples were discovered in the 1960s and 1970s (Figure 1.1). Together, these accumulated data give at least a rough idea of the probable taxonomic and ecological diversity of these insects. I next discuss five general observations about eavesdropping parasitoids that can be inferred from this survey.

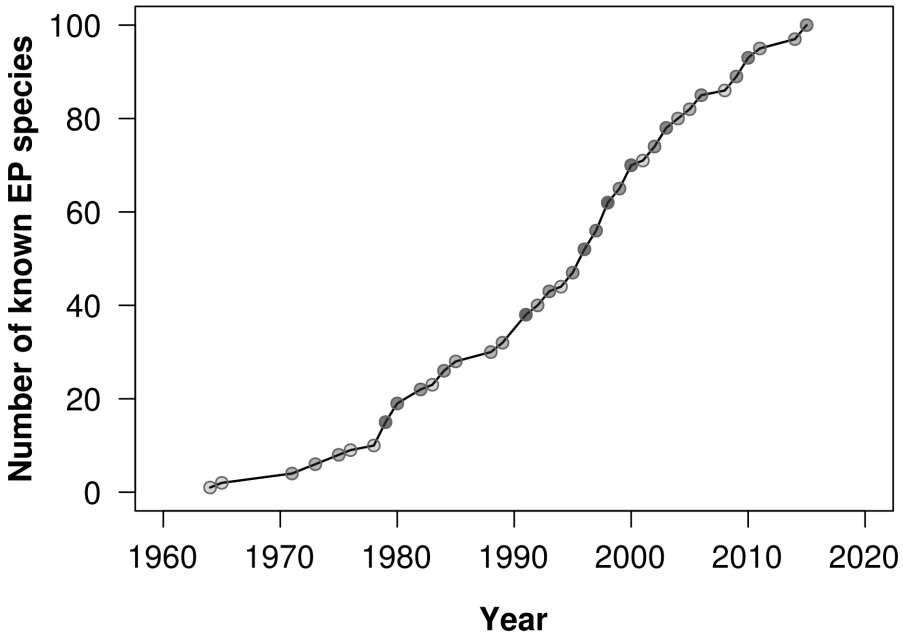


**Figure 1.1** Growth of the total number of known (or probable) eavesdropping parasitoid (EP) species since their initial discovery in the 1960s and 1970s. The shading of each point corresponds with the total number of parasitoid species that were discovered to eavesdrop in a given year; darker colors indicate more species.

***1. Eavesdropping is both relatively common and phylogenetically diverse.***

As far as is currently known, eavesdropping parasitoids are found in at least 13 families in two orders of insects (Table 1.1). Approximately 71% of known species belong to 10 families of the order Hymenoptera, while the remaining 29% belong to three families of Diptera. Considering that Hymenoptera and Diptera are estimated to account for about 74% and 22% of all parasitoid species, respectively (Godfray 1994), the order-level taxonomic diversity of eavesdropping parasitoids appears to roughly mirror that of parasitoids in general. Host use by eavesdropping parasitoids is also diverse; these parasitoids are known to use host species from at least six insect orders (Table 1.1).

Given this taxonomic diversity, there can be no doubt that eavesdropping, just like the parasitoid lifestyle itself (Eggleton and Belshaw 1992), has independently evolved multiple

(probably many) times among the insects. Consider, for example, eavesdropping dipterans. Even though the dipteran families Sarcophagidae and Tachinidae are closely related, eavesdropping undoubtedly evolved independently in each family (Lakes-Harlan et al. 1999), and the occurrence of eavesdropping parasitoids in a third, much more distantly related family, Phoridae, means that eavesdropping has evolved at least three times among the flies alone.

Regarding species-level diversity, it is probably impossible, for now, to estimate with any accuracy how widespread eavesdropping might be among all parasitoid species. Several studies have clearly shown that some parasitoids do *not* eavesdrop (e.g., Krupke and Brunner 2003, Bayoumy et al. 2011). Still, considering the higher-level taxonomic diversity of eavesdropping parasitoids, the steady growth in the number of species known to eavesdrop (Figure 1.1), and the fact that host-finding mechanisms are unknown for the vast majority of parasitoid species, it seems reasonable to conclude that eavesdropping is not an uncommon host-finding strategy. The extreme bias in our current knowledge of eavesdropping parasitoid diversity in favor of species that host on economically important insects (see below) also suggests that there must be many more eavesdropping parasitoids with economically unimportant hosts waiting to be discovered.

***2. There are important biases in our knowledge of eavesdropping parasitoids.***

Of the eavesdropping parasitoids that have so far been discovered, approximately 83% attack economically important host insects, the vast majority of which are agricultural or other pests. Indeed, the eavesdropping habits of several parasitoid species were first discovered when they were captured in traps baited with synthetic sex pheromones of their hosts during studies of agricultural pests (e.g., Rice and Jones 1982, Samways 1988, Hardie et al. 1991). Considering that fewer than 1 percent of all insect species could be considered pests of any kind (Pedigo and Rice 2006), this represents an obvious and severe bias in our current knowledge of

eavesdropping parasitoids. It is impossible to know exactly how this sampling bias effects the conclusions drawn from this literature survey, but at the very least, it is clear that much more, basic investigative work is needed to better understand the diversity and life histories of eavesdropping parasitoids that attack non-economically important hosts.

Apart from this overall emphasis on species relevant to applied entomology, our knowledge of the behavior, ecology, and evolution of eavesdropping parasitoids that exploit acoustic communication is affected by a different kind of bias. Because of the relative ease of working with acoustic communications in an experimental setting, parasitoids that eavesdrop on acoustic signals have proven to be especially valuable for investigating basic questions about parasitoid behavior, host behavior, and sexual signal exploitation and its consequences (e.g., Cade 1984, Aldrich et al. 1989, S.A. Adamo et al. 1995, Wagner 1996, Allen 1998, Gray and Cade 1999, Lakes-Harlan et al. 2000, Köhler and Lakes-Harlan 2001, Müller and Robert 2002, Hedrick and Kortet 2006, Lehmann and Lehmann 2006, Zuk et al. 2006, Beckers and Wagner 2011). However, the vast majority of this research effort has focused on a single species of parasitoid, *Ormia ochracea* (Bigot), and, to a lesser extent, a handful of its close relatives, all of which are parasitoids of crickets and katydids (Orthoptera: Ensifera) (reviewed in Lehmann 2003; see many of the preceeding references as well). Thus, much of what we know about acoustic eavesdropping parasitoids comes from studies of a single lineage of parasitoids, and in many cases, a single species.

A major objective of my dissertation research was to take a small step toward remedying these biases by elucidating the natural history of a different species of acoustic eavesdropping parasitoid, the sarcophagid fly *Emblemasoma erro* Aldrich, as well as some of its congeners, and then use these parasitoids as study organisms to investigate broader questions about the ecology

and evolution of acoustic parasitoids and eavesdropping parasitoids in general. It was already known that another species of *Emblemasoma*, *E. auditrix* (Shewell), is a highly specialized eavesdropping parasitoid of cicadas (Soper et al. 1976, Lakes-Harlan et al. 2000), and *E. auditrix* has been the subject of several illuminating behavioral and physiological studies (e.g., Lakes-Harlan et al. 1999, 2014, Köhler and Lakes-Harlan 2001, Lakes-Harlan and Kohler 2003, Schniederkötter and Lakes-Harlan 2004, de Vries and Lakes-Harlan 2005). However, virtually nothing was known about the natural history of any of the other 15 species of this genus. I address this primarily in Chapters 2 and 3. In Chapter 2, I present the life history, infection behaviors, and host parasitism prevalence of *E. erro* in the central Great Plains of North America. In Chapter 3, I analyze the function of hearing in male *E. erro*, and I present the first evidence that an acoustic parasitoid can exploit the calls of its hosts as a means for finding mates.

***3. Eavesdropping parasitoids usually exploit either acoustic or olfactory communication, and they mostly exploit long-range sexual or sexual/aggregation signals.***

All known eavesdropping parasitoids intercept either olfactory or acoustic communication signals (rarely both) (Table 1.1). Exploitation of olfactory communication is evidently most common, accounting for about 90% of known species. This could be because pheromones are behaviorally or physiologically easier to exploit than other signaling modalities for some reason (e.g., because of parasitoid preadaptations; see observation 5 below), but it probably also reflects the ubiquity of olfaction in insect communication. Olfactory signals are easily the most common form of insect communication (Greenfield 2002), so it is not surprising that they are also the signals most commonly targeted by eavesdropping parasitoids.

It is, however, somewhat surprising that only a single species of parasitoid is known to exploit substrate vibratory acoustic signals for host finding. This parasitoid, the platygastrid

wasp *Telenomus podisi* (Ashmead), intercepts the vibratory signals of female stinkbugs (Laumann et al. 2007) but also eavesdrops on the sex pheromones of male stinkbugs (Borges et al. 1998, Silva et al. 2006). All other known acoustic eavesdroppers listen for airborne sounds (e.g., Cade 1975, Soper et al. 1976). Substrate vibratory communication is quite common among insects (Greenfield 2002, Virant-Doberlet and Čokl 2004, Cocroft and Rodríguez 2005), so why it should be so infrequently exploited by parasitoids is puzzling. Substrate vibrations are usually constrained to be short-range communication signals (Cocroft and Rodríguez 2005), which might limit their usefulness to host-searching parasitoids and explain why vibratory eavesdropping is relatively uncommon. On the other hand, it could be that eavesdropping on vibratory communication is not rare at all, and we simply need to spend more time looking for it. The relative difficulty of studying substrate vibratory signals in comparison to other modes of communication lends some credence to this explanation.

Whether they prefer olfactory or acoustic cues, eavesdropping parasitoids are able to take advantage of an impressive semantic range of insect communications, including aggregation, alarm, anti-aphrodisiac, density-regulating, host-marking, sexual, and trail-marking signals (Table 1.1 and references therein). However, the vast majority of eavesdropping parasitoids – about 83% of the species in this survey – intercept long-range sexual advertisement signals or long-range signals that serve both sexual and aggregation functions. As discussed earlier, sexual advertisement signals should often be selected for maximum amplitude and long-distance detectability, so this emphasis on sexual communication is exactly what might be expected.

Long-range sexual signals are usually species-specific (Alexander 1967, West-Eberhard 1984), which raises the question of how exploitation of species-specific communication signals might constrain a parasitoid's host range. The mechanisms a parasitoid uses to find its host can

have a strong influence on its ultimate host range (Salt 1935, 1938, Shaw 1994, Stireman and Singer 2003, Stireman et al. 2006), so one possibility is that parasitoids that eavesdrop on species-specific sexual signals tend to be extreme specialists with very narrow host ranges. On the other hand, eavesdropping parasitoid signal recognition is subject to different selective pressures than host signal recognition, so parasitoids might be able to ignore species-specific signal traits and instead focus on signal characteristics that are shared among a group of potential host species. This topic is investigated in detail in Chapter 4.

***4. Eavesdropping parasitoids use both "direct" and "indirect" eavesdropping strategies.***

Just under half of eavesdropping parasitoids (about 45% of the analyzed species) use what could be called a direct eavesdropping host search strategy, in that these parasitoids eavesdrop on communication signals from the same host life stages that they parasitize. To give but a few examples, the tachinid fly *Ormia ochracea* parasitizes adult crickets and eavesdrops on the acoustic calls of adult males (Cade 1975), the pteromalid wasp *Tomicobia tibialis* Ashmead parasitizes adult *Ips confusus* (LeConte) bark beetles and eavesdrops on the sex/aggregation pheromones produced by adult males (Bedard 1965, Rice 1968, 1969), and the pteromalid wasp *Mastrus ridens* (Horstmann) parasitizes late instar larvae and prepupae of the moth *Cydia pomonella* (Linnaeus), which it finds by eavesdropping on the aggregation pheromone produced by cocoon-spinning larvae (Jumean et al. 2005).

A slightly larger proportion of eavesdropping parasitoid species (about 55%) use an indirect[2] eavesdropping strategy in which they intercept communication signals from one life stage of their host (usually the adult) but actually parasitize a different life stage. All known

2 Many examples of what I refer to as "indirect eavesdropping" could also be described by the "infochemical detour" concept discussed by Vet et al. (1991) and Vet and Dicke (1992). However, the term seems inadequate to describe the behaviors of eavesdropping parasitoids because it explicitly applies only to olfactory cues, so I use "indirect eavesdropping" instead.

indirect eavesdroppers are hymenopterans, many of which are egg parasitoids that eavesdrop on the sexual signals of the adults of their host insects (e.g., Kennedy 1979, Lewis et al. 1982, Nordlund et al. 1983, Aldrich et al. 1984, Zaki 1985, Battisti 1989, Frenoy et al. 1992, Leal et al. 1995, Yasuda and Tsurumachi 1995, Borges et al. 1998). Rarely, indirect eavesdropping can involve a parasitoid exploiting communication signals from one, non-host species as a means of locating a second, host species. For example, the pteromalid wasp *Cerocephala eccoptogastri* Masi is a hyperparasitoid of several species of braconid wasps that parasitize larvae of the bark beetle *Scolytus multistriatus* (Marsham) (Kennedy 1979, Grissell 1981). *C. eccoptogastri* finds parasitized beetle larvae, and its wasp hosts, by eavesdropping on the sex/aggregation pheromones of adult *S. multistriatus* (Kennedy 1979, 1984).

In some cases, indirect eavesdropping has led to fantastically complex (and convoluted) search behaviors. A good example is provided by the platygastrid wasp *Telenomus calvus* Johnson, which parasitizes the eggs of the predacious stink bug, *Podisus maculiventris* (Say). To find its hosts' eggs, *T. calvus* first eavesdrops on the sex/aggregation pheromones of adult male stink bugs and follows these signals to their source. Once the calling male bugs are located, *T. calvus* waits for a female stink bug, and upon encountering a female, climbs onto the female bug's body. The parasitoid then becomes phoretic, riding on the female stink bug until the she oviposits, at which point the parasitoid dismounts and quickly oviposits into the bug's eggs (Buschman and Whitcomb 1980, Aldrich 1985, Orr et al. 1986).

Often, though, indirect eavesdropping can lead parasitoids more or less directly to their hosts without the need for such complex secondary search behaviors. This is especially so for parasitoids whose host insects are typically found in dense aggregations that include multiple life stages. Examples are the multiple species of parasitoid wasps that attack eggs or larvae of the

bark beetle *Scolytus multistriatus* (Marsham) but eavesdrop on adult sex/aggregation pheromones (Kennedy 1979, 1984), or the figitid parasitoid wasps of the genus *Leptopilina* that parasitize larval drosophilid flies but eavesdrop on the aggregation pheromones of adult males (Wiskerke et al. 1993, Hedlund et al. 1996). In all of these cases, adult host insects are often closely associated with immature stages at the time the adults produce the relevant communication signals.

For this reason, it is important not to overstate the differences between direct and indirect eavesdropping, at least as far as host finding is concerned. In both strategies, eavesdropping serves the same basic purpose of allowing the parasitoid to efficiently move to a location where it is highly likely to encounter a potential host (or, perhaps, find a free ride to a potential host). The only consistent difference is that indirect eavesdropping necessarily requires that the initial, long-range search based on a host species' communication signals is followed by a secondary, short-range search using alternative sources of information. Even this difference might not be as great as it initially seems, however, because many parasitoids that directly eavesdrop on their hosts also require additional sources of information for short-range host finding and orientation (see, e.g., Chapter 2). In general, then, while indirect eavesdropping often does require more complex host searching behaviors than direct eavesdropping, the differences can also be relatively minor.

There is another, more subtle reason that the indirect/direct dichotomy might be of significance to parasitoid ecology: The difference between these two eavesdropping strategies could be of great importance in avoiding intraspecific competition. In general, once parasitoid larvae occupy a host animal, it is usually in their best interest to keep their host to themselves. If another female parasitoid discovers the host and *superparasitizes* it with her own eggs or larvae,

then there might no longer be enough food inside the host to go around, leading to reduced fitness or even death for the larval parasitoids due to competition. This concern applies to both direct and indirect eavesdropping parasitoids, of course, but the important difference is that the larvae of direct eavesdroppers might be able to take action to avoid superparasitism. If the larvae of direct eavesdroppers could alter the signals of their hosts to "hide" them from other female parasitoids by making the hosts less conspicuous or attractive, they could reduce their risk of superparasitism. There is ample evidence that larval parasitoids can manipulate their hosts in a variety of other contexts (Vinson and Iwantsch 1980a, Godfray 1994, Brodeur and Boivin 2004), so it seems reasonable to hypothesize that eavesdropping parasitoids could manipulate their hosts' communication signals, too. This hypothesis is the subject of Chapter 5.

***5. Eavesdropping parasitoids usually intercept communication signals that are highly divergent from their own, intraspecific communication signals and systems.***

For a parasitoid to eavesdrop on the communication signals of another species of insect, an obvious requirement is that the parasitoid must possess sensory organs that are approximately convergent in function with those of its host. How do parasitoids acquire the sensory structures needed to detect the often species-specific signals of their hosts? One possibility is that nascent eavesdropping parasitoids already possess intraspecific communication systems that are similar in some essential way (or in multiple ways) to those of their hosts. Then, with only relatively minor modification, the parasitoids' communication systems can be repurposed for eavesdropping. The evidence, however, suggests that this has not been the usual evolutionary path to eavesdropping. Instead, it seems that the host signal reception and taxis behaviors of most eavesdropping parasitoids have little in common with the parasitoids' own intraspecific communication systems.

For example, the females of nearly all of the eavesdropping parasitoid wasps included in this survey exploit airborne pheromones from their hosts for long-distance host location (Table 1.1). If these wasps' intraspecific communication systems provided the foundations for eavesdropping, then we might expect most of these species to have females that use male-produced pheromones for long-range mate finding. To the contrary, although many parasitoid wasps do use pheromones for intraspecific communication, the most common pattern is that female wasps produce pheromones which males use as mate-locating signals (Godfray and Cook 1997, Ruther 2013). Mate locating mechanisms are not known for all of the parasitoid wasp species in Table 1.1, but the generalization that males search for pheromone-producing females does seem to apply in many cases. For instance, females wasps of at least seven species of the genera *Aphidius*, *Diaeretiella*, and *Praon* (all of the family Braconidae) eavesdrop on airborne aphid sex pheromones (Table 1.1), yet all of these wasps probably have intraspecific communication systems in which males, not females, search for mates via chemoreception (Askari and Alishah 1979, Decker et al. 1993, Nazzi et al. 1996, McClure et al. 2007). It does not, therefore, seem very likely that the intraspecific communication systems of these parasitoid wasps originally provided the sensory and behavioral preadaptations for female eavesdropping and host location.

Instead, a more plausible explanation is that eavesdropping in parasitoid wasps arose from evolutionary co-option of ancestral, olfactory host-searching mechanisms. Parasitoid wasps in general are known to rely on chemoreception for host finding, and they are able to orient to an extraordinary variety of olfactory cues originating from their hosts' food plants and microhabitats (Godfray 1994). If the ancestors of eavesdropping wasp lineages had similar capabilities, then these host-finding mechanisms could have been elaborated by natural selection

to eventually permit females to eavesdrop on their hosts. (Note that in this brief treatment I am only considering eavesdropping as an innate response, although there is evidence that eavesdropping by some parasitoid wasps can also be a learned behavior (Kaiser et al. 1989, Hedlund et al. 1996, Schöller and Prozell 2002, Huigens et al. 2009).)

Far more puzzling are cases in which eavesdropping is associated with completely novel sensory structures, and in which the sensory modalities exploited for eavesdropping might not be used for any other purpose by either the eavesdropping parasitoids or their closest relatives. This seems to be the situation for at least two groups of eavesdropping parasitoid flies. Both the ormiine tachinid flies (e.g., *Ormia ochracea* and its relatives) and some species of the sarcophagid fly genus *Emblemasoma* use long-range, airborne acoustic signals to find their hosts (Soper et al. 1976, Lehmann 2003, Farris et al. 2008, Stucky 2015, Chapters 2, 6, and Appendix C of this dissertation). In both of these parasitoid lineages, the "ears" that are used for eavesdropping are not found anywhere else in either of their respective families (Lakes-Harlan et al. 1999). And, in at least some species of Ormiini and *Emblemasoma*, hearing does not appear to be associated in any way with mating or intraspecific communication (R. C. Lederhouse et al. 1976, Burk 1982b, Lakes-Harlan et al. 2014). These observations all seem to suggest that the "ears" of these flies were evolutionary innovations that arose purely for the purpose of finding hosts. However, this is not the only possible explanation. In Chapter 6, I investigate the question of how eavesdropping, and the ears of *Emblemasoma*, might have evolved in the fly family Sarcophagidae. Specifically, I test the hypothesis that host finding provided the selective pressure for the development of complex tympanal hearing in the ancestors of modern *Emblemasoma*.

## 1.4 Conclusions

For the first few decades after their discovery, one might have concluded that eavesdropping parasitoids presented interesting, but unusual, exceptions to the typical strategies that parasitoids use to find their hosts. Today, it is clear that eavesdropping parasitoids are not just biological curiosities. They are abundant and diverse, and eavesdropping truly *is* one of the typical strategies that parasitoids use to find their hosts. As I hope this review has shown, despite great variety in the particular details of their life histories, it is still possible to make some generalizations about eavesdropping parasitoids: They usually intercept acoustic or olfactory signals, they mostly exploit sexual advertisement or sex/aggregation communication signals, direct and indirect eavesdropping strategies are about equally common, and the interception of host communication signals is usually not related to the parasitoids' own intraspecific communication systems. I also hope to have shown that these basic, summative observations about eavesdropping parasitoids lead to interesting questions about the ecology and evolution of these insects, several of which I investigate in the following chapters.

**Table 1.1** Summary of all eavesdropping parasitoids currently known from the literature. Note that this table only includes the earliest reference or references indicating that a parasitoid eavesdrops on its host or that it exploits a particular type of host signal. Thus, the list of hosts for each parasitoid species does not necessarily include all known hosts for that species. Also, to make this compendium as broadly useful as possible, the criteria for inclusion were liberal in the sense that for some included species, the evidence for eavesdropping is mostly circumstantial.

| Parasitoid taxonomy | | | | Host taxonomy | | |
|---|---|---|---|---|---|---|
| **Order** | **Family** | **Species** | **Host signal** | **Order** | **Species** | **Reference(s)** |
| Diptera | Phoridae | *Apocephalus paraponerae* | alarm pheromone | Hymenoptera | *Paraponera clavata* | Brown and Feener 1991, Feener et al. 1996 |
| | | *Neodohrniophora elongata* | trail pheromone | Hymenoptera | *Atta sexdens rubropilosa* | Gazal et al. 2009 |
| | | *Pseudacteon brevicauda* | alarm pheromone | Hymenoptera | *Myrmica rubra* | Witte et al. 2010 |
| | | *Pseudacteon tricuspis* | alarm pheromone | Hymenoptera | *Solenopsis invicta* | Vander Meer and Porter 2002 |
| | | *Pseudacteon* sp. | alarm pheromone | Hymenoptera | *Azteca instabilis* | Mathis et al. 2011 |
| | Sarcophagidae | *Emblemasoma auditrix* | acoustic mating call | Hemiptera | *Okanagana rimosa* | Soper et al. 1976 |
| | | *Emblemasoma emblemasoma* | acoustic mating call | Hemiptera | various cicada species | Chapter 6 and Appendix C of this dissertation |
| | | *Emblemasoma erro* | acoustic mating call | Hemiptera | various cicada species | Stucky 2015, Chapters 2 and 4 of this dissertation |
| | | *Emblemasoma faciale* | acoustic mating call | Hemiptera | *Neotibicen* spp. | Chapter 6 and Appendix C of this dissertation |
| | Tachinidae | *Cylindromyia fumipennis* | sex/aggregation pheromone | Hemiptera | *Podisus maculiventris* | Aldrich et al. 2006 |
| | | *Cylindromyia* sp. | sex/aggregation pheromone | Hemiptera | *Euschistus servus* | Tillman et al. 2010 |
| | | *Euclytia flava* | sex/aggregation pheromone | Hemiptera | *Podisus maculiventris* | Aldrich et al. 1984 |
| | | *Euthera tentatrix* | sex/aggregation pheromone | Hemiptera | *Thyanta custator accerra*, *Euschistus* spp. | Aldrich et al. 2006 |
| | | *Euthera* sp. | sex/aggregation pheromone | Hemiptera | *Euschistus* sp. | Aldrich et al. 1991 |
| | | *Gymnosoma occidentalis* | sex/aggregation pheromone | Hemiptera | *Euchistus conspersus* | Krupke and Brunner 2003 |

| Parasitoid taxonomy | | | | Host taxonomy | | |
|---|---|---|---|---|---|---|
| **Order** | **Family** | **Species** | **Host signal** | **Order** | **Species** | **Reference(s)** |
| Diptera (continued) | Tachinidae (continued) | *Gymnosoma occidua* | sex/aggregation pheromone | Hemiptera | *Euschistus* sp. | Aldrich et al. 1991 |
| | | *Gymnosoma par* | sex/aggregation pheromone | Hemiptera | *Thyanta custator accerra*, *Euschistus* spp., *Plautia stali* | Aldrich et al. 2006 |
| | | *Gymnosoma rotundatum* | sex/aggregation pheromone | Hemiptera | *Plautia stali* | Moriya and Shiga 1984, Jang and Park 2010 |
| | | *Gymnosoma* sp. | sex/aggregation pheromone | Hemiptera | *Euschistus* sp. | Aldrich et al. 1991 |
| | | *Hemyda aurata* | sex/aggregation pheromone | Hemiptera | *Podisus maculiventris* | Aldrich et al. 1984 |
| | | *Homotrixa alleni* | acoustic mating call | Orthoptera | *Sciarasaga quadrata* | Allen 1998 |
| | | *Leucostoma gravipes* | sex/aggregation pheromone | Hemiptera | *Neacoryphus bicrucis*, *Lygaeus kalmii*, *Oncopeltus fasciatus* | Aldrich et al. 1999 |
| | | *Ormia depleta* | acoustic mating call | Orthoptera | *Neoscapteriscus borellii* | Fowler and Kochalka 1985 |
| | | *Ormia dominicana* | acoustic mating call | Orthoptera | *Orocharis luteolira* | Walker 1993 |
| | | *Ormia lineifrons* | acoustic mating call | Orthoptera | *Neoconocephalus triops* | Burk 1982 |
| | | *Ormia ochracea* | acoustic mating call | Orthoptera | *Gryllus texensis* | Cade 1975 |
| | | *Therobia leonidei* | acoustic mating call | Orthoptera | *Poecilimon veluchianus* | Lakes-Harlan and Heller 1992 |
| | | *Trichopoda pennipes* | sex/aggregation pheromone | Hemiptera | *Nezara viridula* | Mitchell and Mau 1971, Harris and Todd 1980, Aldrich et al. 1987 |
| | | *Trichopoda pilipes* | sex/aggregation pheromone | Hemiptera | *Coleotichus blackburniae* | Johnson et al. 2005 |
| Hymenoptera | Aphelinidae | *Aphytis coheni* | sex pheromone | Hemiptera | *Aonidiella aurantii* | Sternlicht 1973 |
| | | *Aphytis melinus* | sex pheromone | Hemiptera | *Aonidiella aurantii* | Sternlicht 1973, but see Morgan and Hare 1998 |
| | | *Aphytis mytilaspidis* | sex pheromone | Hemiptera | *Epidiaspis leperii* | Abdel-Kareim and Kozar 1988 |

| Parasitoid taxonomy | | | | Host taxonomy | | |
|---|---|---|---|---|---|---|
| **Order** | **Family** | **Species** | **Host signal** | **Order** | **Species** | **Reference(s)** |
| Hymenoptera (continued) | Aphelinidae (continued) | *Aphytis* spp. | sex pheromone | Hemiptera | *Aonidiella aurantii* | Samways 1988 |
| | | *Encarsia perniciosi* | sex pheromone | Hemiptera | *Quadraspidiotus perniciosus* | Rice and Jones 1982 |
| | Braconidae | *Aphidius eadyi* | sex pheromone | Hemiptera | *Acyrthosiphon pisum* | Poppy et al. 1997, Glinwood et al. 1999 |
| | | *Aphidius ervi* | sex pheromone | Hemiptera | *various aphid species* | Poppy et al. 1997 |
| | | *Aphidius rhopalosiphi* | sex pheromone | Hemiptera | *Sitobion avenae* | Glinwood et al. 1998 |
| | | *Aphidius uzbekistanicus* | alarm pheromone | Hemiptera | *Sitobion avenae*, *other aphid species* | Micha and Wyss 1996 |
| | | *Asobara tabida* | aggregation pheromone | Diptera | 7 drosophilid species | Hedlund et al. 1996 |
| | | *Bracon hebetor* | sex pheromone | Lepidoptera | *Galleria mellonella* | Dweck et al. 2010 |
| | | *Coeloides pissodis* | sex/aggregation pheromone | Coleoptera | *Dendroctonus frontalis* | Dixon and Payne 1980 |
| | | *Cotesia vestalis* | sex pheromone | Lepidoptera | *Plutella xylostella* | Reddy et al. 2002 |
| | | *Dendrosoter protuberans* | sex/aggregation pheromone | Coleoptera | *Scolytus multistriatus* | Kennedy 1979 |
| | | *Diaeretiella rapae* | sex, alarm pheromones | Hemiptera | *Brevicoryne brassicae*, *other aphid species* | Gabryś et al. 1997, Foster et al. 2005 |
| | | *Opius lectus* | host marking pheromone | Diptera | *Rhagoletis pomonella* | Prokopy and Webster 1978 |
| | | *Praon abjectum* | sex pheromone | Hemiptera | various aphid species | Hardie et al. 1991 |
| | | *Praon dorsale* | sex pheromone | Hemiptera | various aphid species | Hardie et al. 1991 |
| | | *Praon volucre* | sex pheromone | Hemiptera | various aphid species | Powell et al. 1993 |
| | | *Psyttalia concolor* | sex pheromone | Diptera | *Bactrocera oleae* | Benelli et al. 2014 |
| | | *Spathius benefactor* | sex/aggregation pheromone | Coleoptera | *Scolytus multistriatus* | Kennedy 1979 |
| | | *Spathius pallidus* | sex/aggregation pheromone | Coleoptera | *Dendroctonus frontalis* and/or *D. brevicomis* | Dixon and Payne 1980 |

| Parasitoid taxonomy | | | | Host taxonomy | | |
|---|---|---|---|---|---|---|
| **Order** | **Family** | **Species** | **Host signal** | **Order** | **Species** | **Reference(s)** |
| Hymenoptera (continued) | Diapriidae | *Psilus* sp. | sex/aggregation pheromone | Coleoptera | *Dendroctonus frontalis*, also *D. brevicomis*? | Dixon and Payne 1980 |
| | Encyrtidae | *Anagyrus fujikona* | sex pheromone | Hemiptera | *Planococcus kraunhiae* | Tsueda 2014 |
| | | *Anagyrus* sp. near *pseudococci* | sex pheromone | Hemiptera | *Planococcus ficus* | Franco et al. 2008 |
| | | *Ooencyrtus nezarae* | sex/aggregation pheromone | Hemiptera | *Riptortus clavatus* | Leal et al. 1995 |
| | | *Ooencyrtus pityocampae* | sex pheromone | Lepidoptera | *Thaumetopoea pityocampa* | Battisti 1989 |
| | | *Ooencyrtus telenomicida* | sex/aggregation pheromone | Hemiptera | *Nezara viridula* | Peri et al. 2011 |
| | | *Tetracnemoidea peregrina* | sex pheromone | Hemiptera | *Pseudococcus calceolariae*, *Planococcus citri* | Rotundo and Tremblay 1975 |
| | | *Thomsonisca amathus* | sex pheromone | Hemiptera | *Pseudaulacaspis pentagona* | Matuhira and Kouzaki 2001, Bayoumy et al 2011 |
| | Eulophidae | *Closterocerus ruforum* | sex pheromone | Hymenoptera | *Diprion pini*, *Neodiprion sertifer* | Hilker et al. 2000 |
| | | *Entedon leucogramma* | sex/aggregation pheromone | Coleoptera | *Scolytus multistriatus* | Kennedy 1979 |
| | Figitidae | *Leptopilina boulardi* | aggregation pheromone | Diptera | *7 drosophilid species* | Hedlund et al. 1996 |
| | | *Leptopilina heterotoma* | aggregation pheromone | Diptera | *Drosophila simulans* | Wiskerke et al. 1993 |
| | Ichneumonidae | *Mastrus ridens* | aggregation pheromone | Lepidoptera | *Cydia pomonella* | Jumean et al. 2005 |
| | | *Temelucha interruptor* | host marking pheromone | Lepidoptera | *Rhyacionia buoliana* | Arthur et al. 1964, Schröder 1974 |
| | | *Venturia canescens* | density-regulating pheromone | Lepidoptera | *Ephestia kuehniella* | Corbet 1971 |
| | Platygastridae | *Gryon japonicum* | sex/aggregation pheromone | Hemiptera | *Riptortus clavatus* | Paik et al. 2009 |
| | | *Gryon pennsylvanicum* | sex/aggregation pheromone | Hemiptera | *Leptoglossus australis* | Yasuda and Tsurumachi 1995 |

| Parasitoid taxonomy | | | Host signal | Host taxonomy | | Reference(s) |
|---|---|---|---|---|---|---|
| **Order** | **Family** | **Species** | | **Order** | **Species** | |
| Hymenoptera (continued) | Platygastridae (continued) | *Telenomus busseolae* | sex pheromone | Lepidoptera | *Sesamia nonagrioides* | Colazza et al. 1997 |
| | | *Telenomus calvus* | sex/aggregation pheromone | Hemiptera | *Podisus maculiventris* | Aldrich et al. 1984 |
| | | *Telenomus euproctidis* | sex pheromone | Lepidoptera | *Euproctis taiwana* | Arakaki et al. 1996 |
| | | *Telenomus isis* | sex pheromone | Lepidoptera | *Sesamia calamistis* | Fiaboe et al. 2003 |
| | | *Telenomus podisi* | sex/aggregation pheromone, vibratory signals | Hemiptera | *Euchistus heros* | Borges et al. 1998, Laumann et al. 2007 |
| | | *Telenomus remus* | sex pheromone | Lepidoptera | *Spodoptera frugiperda* | Nordlund et al. 1983 |
| | | *Telenomus triptus* | sex/aggregation pheromone | Hemiptera | *Piezodorus hybneri* | Leal et al. 1995 |
| | | *Trissolcus basalis* | sex/aggregation pheromone | Hemiptera | *Nezara viridula* | Colazza et al. 1999 |
| | | *Trissolcus teretis* | sex/aggregation pheromone | Hemiptera | *Euchistus heros* | Borges et al. 1998 |
| | | *Trissolcus urichi* | sex/aggregation pheromone | Hemiptera | *Euchistus heros* | Borges et al. 1998 |
| | Pteromalidae | *Anisopteromalus calandrae* | host marking pheromone | Coleoptera | *Callosobruchus chinensis* | Onodera et al. 2002 |
| | | *Cerocephala eccoptogastri* | sex/aggregation pheromone | Coleoptera | *Scolytus multistriatus* | Kennedy 1979 |
| | | *Cheiropachus quadrum* | sex/aggregation pheromone | Coleoptera | *Phloeotribus scarabaeoides* | Lozano et al. 2000 |
| | | *Dinarmus basalis* | host marking pheromone | Coleoptera | *Callosobruchus chinensis* | Kumazaki et al. 2000 |
| | | *Dinotiscus colon* | sex/aggregation pheromone | Coleoptera | *Scolytus multistriatus* | Kennedy 1979 |
| | | *Halticoptera laevigata* | host marking pheromone | Diptera | *Myoleja lucida* | Hoffmeister and Gienapp 1999 |
| | | *Halticoptera rosae* | host marking pheromone | Diptera | *Rhagoletis basiola* | Roitberg and Lalonde 1991 |

| Parasitoid taxonomy | | | | Host taxonomy | | |
|---|---|---|---|---|---|---|
| **Order** | **Family** | **Species** | **Host signal** | **Order** | **Species** | **Reference(s)** |
| Hymenoptera (continued) | Pteromalidae (continued) | *Heydenia unica* | sex/aggregation pheromone | Coleoptera | *Dendroctonus frontalis* | Dixon and Payne 1980 |
| | | *Lariophagus distinguendus* | sex/aggregation pheromone | Coleoptera | *Rhyzopertha dominica* | Steidle et al. 2003 |
| | | *Pteromalus cerealellae* | sex, marking pheromones | Coleoptera | *Callosobruchus maculatus* | Mbata et al. 2004 |
| | | *Tomicobia tibialis* | sex/aggregation pheromone | Coleoptera | *Ips confusus* | Bedard 1965, Rice 1968 |
| | Tetracampidae | *Dipriocampe diprioni* | sex pheromone | Hymenoptera | *Diprion pini* | Hilker et al. 2000 |
| | Trichogramma-tidae | *Lathromeris ovicida* | sex pheromone | Lepidoptera | *Sesamia calamistis* | Fiaboe et al. 2003 |
| | | *Trichogramma brassicae* | sex, antiaphrodisiac pheromones | Lepidoptera | *Ostrinia nubilalis, Pieris brassicae* | Frenoy et al. 1992, Fatouros et al. 2005 |
| | | *Trichogramma chilonis* | sex pheromone | Lepidoptera | *Helicoverpa assulta*, *Ostrinia furnacalis* | Boo and Yang 2000 |
| | | *Trichogramma evanescens* | sex pheromone | Lepidoptera | *Pieris brassicae*, *Mamestra brassicae*, *Pectinophora gossypiella*, *Spodoptera littoralis*, *Earias insulana* | Noldus and van Lenteren 1985, Zaki 1985 |
| | | *Trichogramma maidis* | sex pheromone | Lepidoptera | *Ostrinia nubilalis* | Kaiser et al. 1989 |
| | | *Trichogramma oleae* | sex pheromone | Lepidoptera | *Prays oleae*, *Palpita unionalis* | Milonas et al. 2009 |
| | | *Trichogramma ostriniae* | sex pheromone | Lepidoptera | *Ostrinia furnacalis* | Bai et al. 2004 |
| | | *Trichogramma pretiosum* | sex pheromone | Lepidoptera | *Helicoverpa zea* | Lewis et al. 1982 |
| | | *Trichogramma sibericum* | sex pheromone | Lepidoptera | *Rhopobota naevana* | McGregor and Henderson 1998 |
| | | *Uscana lariophaga* | sex pheromone | Coleoptera | *Callosobruchus maculatus* | van Huis et al. 1994 |

# CHAPTER 2

## INFECTION BEHAVIOR, LIFE HISTORY, AND HOST PARASITISM RATES OF *EMBLEMASOMA ERRO*[3]

### 2.1 Introduction

For female parasitoids, successful reproduction usually requires finding suitable hosts for their offspring. The problem, of course, is that potential hosts generally do their best *not* to be found. Sometimes, however, even well-hidden host insects must produce intraspecific communication signals, and these communication signals can be exploited by specialist parasitoids for use in efficient, long-range host location (Godfray 1994, Zuk and Kolluru 1998, Haynes and Yeargan 1999). Most often, such "eavesdropping" parasitoids intercept chemical communications, but several species of flies (Diptera) from two families, Sarcophagidae and Tachinidae, use acoustic signals to find their hosts (Cade 1975, Soper et al. 1976, Lakes-Harlan and Lehmann 2015). Acoustically orienting tachinid parasitoids (tribe Ormiini) parasitize crickets and katydids (Orthoptera) (Lehmann 2003), while sarcophagid acoustic parasitoids, which are currently placed in the genus *Emblemasoma* (*sensu* Pape (1990)), parasitize cicadas (Hemiptera: Cicadidae) (Soper et al. 1976, Schniederkötter and Lakes-Harlan 2004). Because acoustic signals are often more amenable to experimental manipulation than pheromones, acoustic parasitoids have become valuable model organisms for investigating sexual signal exploitation and its consequences (e.g., S.A. Adamo et al. 1995, Allen 1998, Gray and Cade 1999, Müller and Robert 2002, Lehmann and Lehmann 2006, Beckers and Wagner 2011).

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However, current knowledge of acoustic parasitoids is heavily biased toward the ormiine tachinids, which have received the majority of research (reviewed in Lehmann 2003). In comparison, our understanding of sarcophagid acoustic parasitoids is far more limited. *Emblemasoma* includes 16 described species (Pape 1996), but nearly everything known about the basic biology, behaviors, and ecology of these flies comes from study of a single species, *E. auditrix* (Shewell), which is a specialist parasitoid of the cicada *Okanagana rimosa* (Say) (e.g., Lakes-Harlan et al. 2000, Köhler and Lakes-Harlan 2001, Schniederkötter and Lakes-Harlan 2004). No detailed information is available about the infection behaviors or life histories of any other *Emblemasoma*, and the only other record of phonotactic behavior comes from a study in which the species of *Emblemasoma* was not determined (Farris et al. 2008). Furthermore, no information about host parasitism rates or parasitoid loads is available for any species besides *E. auditrix* and its host *O. rimosa*.

Considering that *Emblemasoma* are frequently referenced in discussions of insect hearing and parasitoid biology (e.g., Godfray 1994, Feener and Brown 1997, Yager 1999, Yack 2004, Robert 2005, Hedwig and Robert 2014, Strauß and Lakes-Harlan 2014) and that their "ears" have been the focus of multiple physiological investigations (Lakes-Harlan et al. 1999, Robert et al. 1999, Farris et al. 2008), it is perhaps surprising how little we actually know about the basic biology and ecology of any of these flies besides *E. auditrix*. As a consequence, it is nearly impossible to make meaningful generalizations about sarcophagid acoustic parasitoids, and drawing broader conclusions about acoustically orienting parasitoids in general is similarly difficult.

**Figure 2.1** Male *Neotibicen dorsatus*, Harvey Co., KS.

**Figure 2.2** Female *Emblemasoma erro*, Ellsworth Co., KS.

In 2008, I discovered that adults of the cicada *Neotibicen dorsatus* (Say) (Figure 2.1), a large cicada that is common in the grasslands of central North America (Cole 2008), were sometimes infected with the larvae of a sarcophagid parasitoid. After a preliminary investigation revealed that this parasitoid was *Emblemasoma erro* Aldrich (Figure 2.2) and that these flies were most likely locating their hosts acoustically, I began a comprehensive study of the basic biology of this fly. The only information previously available about the biology of *E. erro* was a record of a single female fly that had been reared from a specimen of the cicada *Quesada gigas* (Olivier) in Brazil (Lopes 1981) and a report of rearings from *Neotibicen* sp. in Texas, USA (Lakes-Harlan 2009).

In this paper, I 1) describe the host locating and larviposition behaviors of *E. erro*; 2) describe this parasitoid's life history; and 3) report the results of an investigation of parasitism rates and parasitoid loads in natural populations of the host cicada, *N. dorsatus*. I then discuss: the infection behaviors of *E. erro* in comparison with other acoustic parasitoids and other sarcophagid parasitoids, potential host defenses, and the causes of variation in host population parasitism rates and parasitoid loads, including empirical evidence that superparasitism might contribute to high parasitoid loads in some host populations. The results show not only that the behaviors and life histories of sarcophagid acoustic parasitoids are more diverse than previously recognized, but also that the infection behaviors of *E. erro* are unlike those known for any other acoustic parasitoid.

## 2.2 Methods

### *Study sites*

Surveys of host populations, collections of adult hosts and parasitoids, and field behavioral observations were conducted at six primary study sites located in Ellsworth, Harvey,

McPherson, and Reno counties in central Kansas, Hamilton County in western Kansas, and Prowers County in eastern Colorado (Figure 2.3). The central Kansas sites are located within the Central Great Plains level III ecoregion, while the western Kansas and eastern Colorado sites are located near the boundary between the High Plains and Southwestern Tablelands ecoregions (US Environmental Protection Agency 2013). All sites consisted of native midgrass or shortgrass prairie vegetation intermixed with riparian, woody vegetation or planted trees. Supplemental collections of host cicadas and adult flies for life history and behavioral study were made at five additional locations in Kansas with habitat that was similar to that at the primary study sites (Figure 2.3.)

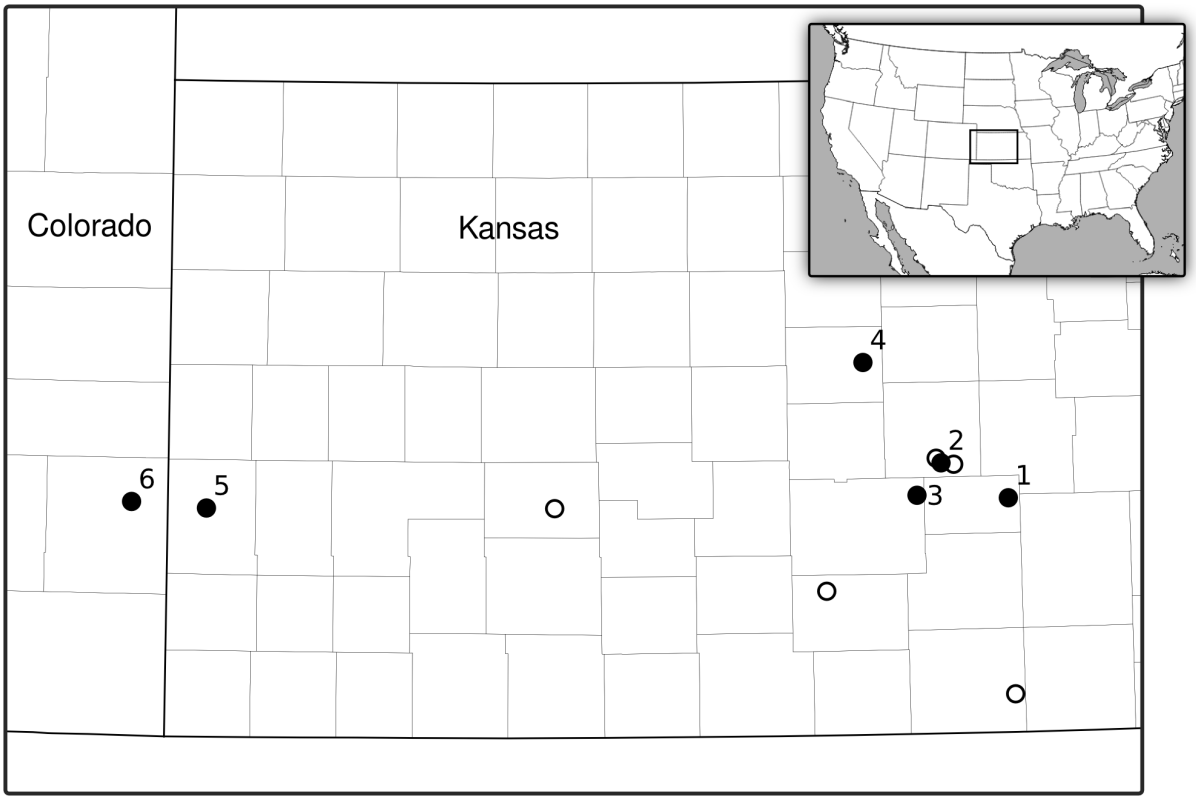


**Figure 2.3** Locations of study sites. Filled circles indicate the primary sites used for estimating host parasitism rates and open circles indicate secondary sites used for additional collections of cicadas and flies. Primary sites are referenced in the text by the counties in which they were located: 1) Harvey Co., 2) McPherson Co., 3) Reno Co., 4) Ellsworth Co., 5) Hamilton Co., and 6) Prowers Co. Both *N. dorsatus* and *E. erro* were found at all 11 sites. The inset map indicates the location of the main map in the United States.

### *Host locating and larviposition behaviors of* **E. erro**

The host locating and larviposition behaviors of *E. erro* were studied in three ways. First, natural cicada/fly interactions were observed directly in the field whenever possible during the summers of 2010-2014. Second, artificial broadcasts of acoustic stimuli were used to test for phonotactic behavior by female flies in the field; and third, cicada/fly interactions were observed in a controlled, outdoor laboratory environment. The methods for these latter two approaches are next described in more detail.

#### *Field broadcasts of acoustic stimuli*

Preliminary observations suggested that male cicadas' acoustic signals played a role in host location by *E. erro*, but such observations cannot assess whether acoustic cues by themselves are sufficient to attract female parasitoids. To separate acoustic stimuli from other possible sources of information about the location of potential hosts (e.g., visual or olfactory), a loudspeaker was used in the field to broadcast audio that mimicked the calling song of a typical male *N. dorsatus*.

Acoustic signals for attracting *E. erro* were generated by gathering audio recordings of calling male *N. dorsatus*, analyzing these recordings to estimate the mean values of several acoustic parameters, then constructing model acoustic signals that matched, as closely as possible, the mean calling song of the species. To ensure that the model acoustic signals were broadly representative of *N. dorsatus* from the general study area, I obtained recordings of 20 different individuals of *N. dorsatus* from six field sites in Kansas. All recordings were made as uncompressed, 16-bit PCM audio at a sampling rate of 44.1 kHz using a highly directional shotgun microphone (Sennheiser ME66 or ME67 with a matched windscreen) and a digital audio recorder (Sony MZ-M200 or PCM-M10). To minimize background noise while also avoiding

near-field acoustic effects in the frequency range of the cicadas' calling songs (Michelsen and Nocke 1974, Peterson 1980), recordings were typically made with the microphone held at a distance of between 0.5 and 2 meters from the calling cicada. Most recordings were made directly in the field, but in some cases, cicadas were captured and placed in mesh cages, then recorded once they resumed normal acoustic activity.

Each recording was analyzed to determine the values of three acoustic variables: peak frequency, pulse group (PG) length, and PG rate. A "pulse group" is defined as a first-order assemblage of sound pulses (i.e., a train of sound pulses) that is separated by silence from the rest of the audio signal. It is the basic unit of temporal structure in the call of *N. dorsatus* (see Cole (2008) for a spectrogram and oscillogram of the *N. dorsatus* calling song; note that Cole refers to PGs as "syllables"). Peak frequency was estimated by identifying the highest peak in a power spectral density plot generated by Audacity® (Audacity Team 2014) using a 512-sample Fast Fourier Transform with the Hann window function. If there were two or more peak frequencies that differed by less than 0.5 dB, their average was taken as the overall peak frequency. PG length and rate were determined using custom-written software to analyze 10 seconds of audio from the middle of each calling song recording. Following these analyses, a single model acoustic signal was constructed using the *N. dorsatus* recording that was as close as possible to the mean calling song observed for the species (mean peak frequency=4308 Hz [$s$ = 444]; mean PG length=20.3 ms [$s$ = 1.45]; mean PG rate=37.04 PG/s [$s$ = 2.25]). I also generated synthetic acoustic signals constructed from amplitude-modulated sine waves that exactly matched the observed mean acoustic variable values.

Acoustic signals were broadcast in the field with a custom-built, portable broadcasting system consisting of a 12-volt audio amplifier and a high-output, horn-loaded tweeter speaker

(PylePro PH44) mounted in the top of a wooden box. Acoustic signals were fed to the amplifier from either a portable CD player or a flash memory-based digital audio player. Broadcasts in the field were conducted either in the late morning or afternoon when cicadas were naturally active, usually for a duration of 4 to 8 minutes at one time. Flies that were attracted to the broadcast speaker were captured by hand.

*Laboratory observations*

Although natural cicada/fly interactions were occasionally observed in the field, opportunities for such observation were unpredictable and infrequent. Furthermore, close-range observation was often impossible, and cicadas could rarely be captured after an encounter with a fly to determine whether larviposition occurred. Consequently, observation of cicada/fly interactions in a more controlled setting was also necessary.

Initial attempts to observe the infection behavior of *E. erro* in 2010 used restrained cicadas and audio broadcasts in an approach similar to that used by Schniederkötter and Lakes-Harlan (2004) for their study of *E. auditrix*. This technique was unsuccessful for *E. erro*, however, so further experiments with immobilized cicadas were abandoned.

Instead, unrestrained cicadas and flies were allowed to freely interact in outdoor cages during behavioral experiments in 2012 and 2013. For each infection behavior trial, one female fly was released into a mesh cage containing one or two uninfected male cicadas. Three types of cage were used: a cylindrical cloth mesh cage approximately 27 cm in diameter and 39 cm high; a larger cylindrical cloth mesh cage approximately 46 cm in diameter and 66 cm high; and a much larger, rectangular screen "flight cage" with a square base and walls approximately 1.8 m on each side and just over 2.1 m high at the center of the top. The behaviors of the fly and cicada(s) were then observed carefully throughout the duration of the trial. If the fly appeared to

directly contact the cicada with the tip of her abdomen or otherwise attack the cicada, the cicada was immediately removed and inspected for the presence of fly larvae. In most trials, the cicada and fly were not physically disturbed inside of the cage, but in some cases the cicada was induced to flight by the experimenter to observe the fly's response. Trials ended when a fly larviposited upon a cicada or the fly no longer showed interest in the cicada(s) in the cage. To avoid overly stressing the animals, trials were also usually terminated after a fly made several attempts to attack a cicada even if larviposition was not observed. If the infection status of a cicada could not be determined by visual inspection immediately after a trial, the cicada was not used in further trials for at least 48 hours in order to verify whether it had become infected. No single female fly was used to infect more than two cicadas. Whenever a fly larviposited on a cicada, I attempted to immediately count the number of larvae deposited. This was not always possible, though, and in these cases the total number of larvae was determined by rearing the parasitoids or dissecting the host.

All cicadas used for this part of the study were mature adult male *N. dorsatus* that were captured directly in the field. Captured cicadas were maintained outdoors in large cloth mesh cages placed over live branches of green ash (*Fraxinus pennsylvanica*), which provided the cicadas with a suitable food source. After capture, and before exposing them to parasitoids, all cicadas were closely monitored for up to 9 days to determine whether they had already been parasitized in the field by *E. erro*. Only unparasitized cicadas were used for studying cicada/fly interactions. Adult female *E. erro* were obtained by broadcasting the model call of *N. dorsatus* in the field, as described above, and collecting attracted female flies by hand. Flies were kept in small mesh cages in the laboratory and provided with sucrose and water *ad libitum*.

***Life history of* E. erro**

The timings of key life history events for *E. erro* were estimated by rearing parasitoids from hosts that were naturally infected in the field, infected during the behavior studies described above, or artificially infected in the lab. To artificially infect cicadas in the laboratory, a female *E. erro* was first anesthetized by chilling the insect at approximately 4º C for several minutes. The fly was then decapitated, placed on a piece of moistened filter paper on a watch glass, and live first-instar larvae were carefully dissected from the fly's abdomen. Individual larvae were transferred to uninfected adult *N. dorsatus* cicadas using the moistened tip of a fine artist's brush. Most larvae were placed on the intersegmental membrane at the base of the cicada's wings, but some were placed at the lateral junction of the metathorax and mesothorax or the junction of the metathorax and the 1st abdominal tergum. A fine insect pin was sometimes used to make a small puncture in the membrane at the wing base in order to facilitate the larvae's entry into the host's body. Larvae from a single female fly were never used to infect more than two cicadas.

All infected cicadas were kept in outdoor, mesh cages as described above. Cicadas were checked several times daily, and any individuals that died or appeared moribund were moved indoors into small plastic emergence containers to capture emerging fly larvae. Once a host was moved to a larval emergence container, video recording was used to capture the precise time and location of larval egress from the host. If no larvae were observed in an emergence container approximately 48 hours after host death, or if only undersized larvae emerged, the dead cicada was dissected to check for additional fly larvae.

Parasitoid larvae that emerged from their host were provided with fine sand in which to burrow and pupariate. Once pupariation was complete, bits of moist paper towels were placed in the emergence containers to help maintain suitable humidity, and the puparia were kept at room

temperature (generally 24-28º C) and exposed to the approximate natural daily photoperiod. The emergence containers were fitted with screen tops to capture any eclosing adult flies.

To better understand how biotic and abiotic factors influence larval development, I evaluated the effects of two key variables – effective clutch size (the number of larvae from a clutch that successfully develop inside a host) and the ambient temperature experienced by the host – on the total time larval parasitoids spent inside their host (the "larval residence time"). These variables were chosen because temperature affects the development and growth rates of insects in general (Harrison et al. 2012), and the number of larvae inside a host might influence how rapidly the host is consumed. For this analysis, larval residence time was calculated as the total elapsed time, in hours, from the moment a larva was deposited on a cicada until the larva emerged from its host. Effective clutch size was taken as the total number of larvae that emerged from a host (because the host cicadas for this analysis were infected in the behavior studies or in the lab, all larvae inside a host were known to be from the same clutch). Temperature was calculated as the mean ambient air temperature experienced by each host during the course of infection. Temperature data were taken from the Daymet 1-km daily surface weather dataset (Thornton et al. 1997, 2014). The overall mean ambient air temperature experienced by an infected cicada was estimated by averaging the daily minimum and maximum temperatures for each day that the cicada was infected up to the time of larval egress from the host. The relationship among these three variables was analyzed using multiple linear regression with effective clutch size and temperature as the explanatory variables. To avoid potential non-independence problems caused by related larvae sharing the same host cicada, the data were summarized at the level of the host cicada; that is, for each host with multiple parasitoid larvae, the mean residence time for all larvae from the host was used in the analysis instead of the

residence time for each parasitoid larva. Diagnostic plots of the standardized residuals were used to verify the fit of the regression model. This, and all other statistical analyses, were conducted in R version 3.1.1 (R Core Team 2015). Note that larvae from parasitized cicadas captured in the field could not be included in this analysis because it was not known when these larvae were deposited on their hosts.

***Host parasitism rates and parasitoid loads***

To obtain population samples for estimating host parasitism rates, adult *N. dorsatus* were surveyed by walking through the habitat at a study site and attempting to capture all *N. dorsatus* that were observed perched in the vegetation or disturbed into flight. Teneral or recently emerged cicadas were excluded because male cicadas do not develop full calling capabilities or begin sexual acoustic behaviors until several days after eclosion (Maier 1982; B. Stucky, unpublished data). Captured cicadas were maintained in captivity to rear the parasitoids from all infected cicadas, determine the total number of infected cicadas in each sample, and determine the parasitoid load of each host, following the methods described above.

Cicada population surveys were conducted at the six primary study sites in July, August, or early September of 2011-2014, although not all sites were sampled all three years (Table 2.1). The survey dates were limited by when adult *N. dorsatus* were actually present in the field, which varied from year to year. In 2012, for example, adult *N. dorsatus* were abundant in central KS by July 1, but they did not reach similar abundance in 2013 until the latter half of July.

By the time the first population samples were collected in 2011, I had established that the male cicada's calling song was a critical cue used by female parasitoids to locate their hosts. Consequently, surveys from 2011-2013 focused on male cicadas only (females do not produce sound) in order to use the limited space available for housing these large insects as efficiently as

possible. In 2014, both female and male cicadas were sampled at the field sites in Harvey, McPherson, and Reno counties in central Kansas.

Logistic regression (generalized linear models with binomial-distributed response and logit link function) was used to evaluate whether host parasitism rates varied among the field sites and whether sample year or sample date also influenced parasitism rates. The proportion of parasitized cicadas in population samples was modeled with field site, year, and ordinal sample date as possible predictor variables. Both field site and year were treated as categorical variables. To test the effects of individual predictors and decide which variables to retain in the model, nested models were compared using the difference of their deviance statistics (i.e., likelihood-ratio tests) (Dobson and Barnett 2008). Standardized residuals plots were examined to check for any problems with model specification. To further assess the final model fit, the likelihood-ratio (also known as McFadden) pseudo $R^2$ was calculated (McFadden 1974, Menard 2000).

Early in this study, it became clear that parasitoid loads varied among the population samples. One possible cause of such variation is superparasitism, which, for gregarious parasitoids such as *E. erro*, is expected to occur more often when unparasitized hosts are rare (Godfray 1994). Unparasitized hosts are rare when parasitism rates are high, so to test for this causal relationship, I used simple linear regression to evaluate whether high host parasitism rates corresponded with high parasitoid loads. For this analysis, each data point was the estimated mean host parasitoid load and parasitism rate for a single study site in a given year. Yearly population samples for which fewer than 3 parasitized cicadas were available to estimate the mean parasitoid load were excluded from the analysis. Parasitism rate was used as the explanatory (i.e., X-axis) variable, and because parasitism rates were estimated from population

samples, some of which were small, there was the possibility of substantial measurement error. Consequently, the regression analysis was likely to suffer from slope attenuation bias, in which the slope estimator is biased to be less than the true slope (Bulmer 1979, Smith 2009). To compensate for this, I used the sizes of each population sample to estimate the mean measurement error variance across all population samples. I then used this estimate of the error variance with the method of moments estimator (MME) of the bias correction factor (Carroll and Ruppert 1996, Smith 2009) to calculate an attenuation-corrected slope estimate. Diagnostic plots of the standardized residuals were used to verify the fit of the simple linear regression model.

***Additional statistical methods***

All confidence intervals (CIs) for population proportion estimates were calculated using the Wilson method (also known as the score confidence interval) because of its good performance across a broad range of sample sizes (Wilson 1927, Agresti and Coull 1998). CIs for the estimates of population means were constructed using the standard *t*-distribution method when possible (Whitlock and Schluter 2009), but in cases where the population distribution appeared to be non-normal (as determined by examining plots of sample distributions), CIs were calculated using the bootstrap-*t* resampling method with 1,000,000 replicates (Efron and Tibshirani 1993, Carpenter and Bithell 2000). Bootstrap-*t* resampling with 1,000,000 replicates was also used to compare the means of non-normally distributed populations. Throughout this paper, "*s*" is used to indicate the sample standard deviation.

## 2.3 Results

### *Host locating and larviposition behaviors of* E. erro

*Host locating behavior*

Field and laboratory observations of cicada/fly interactions and field broadcasts of the *N. dorsatus* call all confirmed that *Emblemasoma erro* uses the calling songs of male cicadas as the primary cue for locating potential hosts. In the field, I was able to observe, at relatively close range, the interactions between 14 *N. dorsatus* cicadas and *E. erro* flies. All of the cicadas involved in these interactions were males, 13 of which were acoustically active while I observed them. 7 individual flies were observed in the process of locating a perched cicada (either by flight or walking), and in every case, the perched cicada was calling while the fly was moving toward it. In the other observed cicada/fly interactions, the flies were already perched near the cicada when I first saw them and I did not observe how, or when, the flies actually arrived near these cicadas. I never saw flies near perched female cicadas, which cannot produce sound.

During the infection behavior trials in outdoor cages in 2012 and 2013, female flies in the experiment cages typically showed an immediate, strong phonotactic response to calling cicadas. When a cicada in the cage began to call, a fly would either walk towards the calling cicada, fly to within several centimeters of the cicada and then walk towards it, or, in some cases, fly directly to, and land on, the calling cicada. Thus, both field and laboratory observations of cicada/fly interactions provided strong circumstantial evidence that *E. erro* use the acoustic calls of cicadas to locate their hosts, but such observations cannot definitively rule out the possibility that some other source of information was actually being used, such as visual or olfactory cues.

Field broadcasts of the model *N. dorsatus* calling song furnished unambiguous evidence that acoustic cues, by themselves, are sufficient to attract female flies. The model *N. dorsatus*

calling song was broadcast at least once at all 6 primary field sites. *E. erro* were attracted to the broadcast speaker at every location. Flies often arrived within a few seconds of the start of a broadcast, and it was not uncommon to see multiple flies perched on the top of the speaker box at the same time. At least 2 or more flies were collected at each primary field site, and more than 60 *E. erro* in total were captured during this study. Often, many more flies arrived at the broadcast speaker than could be captured by hand. I did not attempt to quantify the number of flies that arrived and were not captured, however, because individual flies will sometimes arrive at and leave the speaker multiple times during a single broadcast (B. Stucky, pers. observation), making any such counts unreliable. The broadcast apparatus never attracted any flies when the loudspeaker was not operating.

*Larviposition behavior*

Acoustic cues and phonotaxis are clearly critical for *E. erro* to locate its hosts, but the calling song by itself never induced *E. erro* to larviposit in the absence of a host. Despite the large numbers of flies that were attracted to the calling song broadcasts, no fly larvae were ever found on the speaker or surface of the speaker box following a broadcast of the model *N. dorsatus* call.

Furthermore, laboratory observations of infection behaviors revealed that, even when a potential host was present, the cicada's calling song was still not the stimulus that ultimately triggered larviposition. Although cicadas often called during the infection behavior trials in 2012 and 2013, in no case did this directly result in larviposition by a female fly. Instead, once a fly had moved to within a few centimeters of a calling cicada by orienting to the cicada's calling song, it would typically remain more or less stationary next to the cicada with its head facing toward the cicada's body. At this point, repeated calls from the cicada usually resulted in

relatively little additional movement from the fly. However, if the cicada moved either by walking or flight, the fly usually attempted to maintain its proximity to the potential host. Thus, if the cicada began walking, the fly would typically follow it from a short distance (e.g., 2-3 cm away). If the cicada took flight, the fly almost always immediately took flight as well and attempted to follow the cicada in the air.

Sometimes, cicada locomotion resulted in a larviposition attack by the fly, and all evidence suggested that at least some movement by the cicada was essential for larviposition. 17 incidents of successful larviposition were obtained during the infection behavior trials in 2012 and 2013, and in every case, larviposition was only observed when cicadas were in motion. I was able to determine the moment of larviposition for 15 of these attacks. Of these, 5 (33.3%) occurred while the cicada was in flight; the remaining 10 attacks (66.7%) occurred while the cicada was either walking, flapping its wings, or both. In a few cases, the cicadas that were attacked never called at all during the time the fly was in the cage. These cicadas were "discovered" by the flies purely due to their physical movement in the cage, further indicating that movement by a potential host, not sound, provides the visual cues that ultimately trigger larviposition. As a further example, in 2012 I experimented with releasing a female *N. dorsatus* (which cannot produce sound at all) in the air in front of a female fly. The fly eventually followed the cicada and larviposited on it in flight.

Successful larviposition attempts resulted in the deposition of one or more tiny first-instar larvae directly upon the exterior of the cicada's body (Figure 2.4). A sticky secretion also usually accompanied the larvae, presumably to help them adhere to the host. Flies appeared to briefly contact the cicada with their abdomens during larviposition, but high-speed video recording would be needed to reveal the exact mechanics of this process. After larviposition, the larvae

**Figure 2.4** Larviposition by *E. erro*. A 1st-instar larva of *E. erro* on the right fore wing of a male *N. dorsatus* moments after larviposition (larva indicated by blue arrow). The cicada's head and foreleg are at top center.

immediately began searching for an area of intersegmental membrane through which to burrow and enter the host's body. The larvae typically entered the host quite rapidly, disappearing after anywhere from a matter of seconds to a few minutes.

Although the location of larvae deposition could not be determined in all cases because the larvae sometimes disappeared into the host's body before they could be observed, the evidence suggests that flies prefer to attack the base of a host's wings. Of 15 attacks for which the exact location of larviposition was determined (out of 17 total successful attacks), 1 (6.7%) was on the base of a fore leg, 2 (13.3%) were on the abdomen, and 12 (80%) were either directly on the wings (usually near the base) or on the pterothorax or first two abdominal segments next

to the base of the wings. Left/right orientation was recorded for 14 of the 15 attacks, and of these, 9 (64.3%) were on the left side of the cicada's body, 3 (21.4%) were on the right side, and 2 (14.3%) were approximately medial. In their studies of the infection behaviors of *E. auditrix*, Schniederkötter and Lakes-Harlan (2004) discovered that *E. auditrix* preferentially attacked the left side of potential hosts. Most laterally-oriented attacks by *E. erro* also occurred on the left side of the cicada's body (9 of 12, or 75%), but this asymmetry was not statistically significant for this sample size (exact binomial test, $p = 0.146$).

The number of larvae deposited by a single female fly on a host cicada during the infection behavior trials (i.e., the clutch size) varied from a minimum of 1 to a maximum of 6, but more than 80% of the time, flies (14 of 17) deposited 3 or fewer larvae. The mean clutch size was 2.53 (95% bootstrap-*t* CI: 1.85–3.45 larvae/host, $s = 1.50$, $n = 17$ hosts) and the median was 3.

Observations of fly behavior in the field appeared to corroborate the infection behaviors in the lab. I observed 8 male *N. dorsatus* that each produced one or more complete calling songs (as many as 5 in one case) while an *E. erro* was perched next to the cicada. In no case did the calling song appear to trigger an attempt at larviposition. Just as in the laboratory, flies often waited, nearly motionless, next to calling cicadas, and if a cicada in the field crawled up or down the vegetation it was perched on, the fly usually followed it. If the cicada took flight, the fly usually also took flight and followed the cicada in the air.

As in the cage infection behavior trials, flies in the field only seemed to attack a cicada if the cicada was in motion or had just moved, and most apparent attacks occurred when the cicada was in flight. On two such occasions, cicadas that were evidently struck in the air by *E. erro* had their flight disrupted to such an extent that the cicadas crashed to the ground. Unfortunately, I

could not determine with absolute certainty whether any of the flies I observed in the field actually larviposited on the attacked cicadas. I was able to capture 8 cicadas shortly after their interactions with *E. erro*, but I was unable to locate 1st-instar fly larvae on any of them. Considering how rapidly larvae can burrow into their host's body, it is likely that they had already disappeared from view by the time I was able to look for them. Parasitoids were reared from all 8 cicadas, though, so it is very likely that at least some became infected during the observed cicada/fly interactions.

### *Life history of* E. erro

From the moment of larviposition until they completely exited the host's body, *E. erro* larvae spent, on average, 88.0 hours residing inside their host (95% CI: 81.19– 94.76 hours, $s$ = 17.1, $n$ = 27 larvae from 13 host cicadas and 10 female flies, range = 61.3–116.0). Multiple regression analysis of these data revealed that both temperature and effective clutch size had significant effects on larval residence time. Together, these two variables explained more than 93% of the observed variation in residence times ($R^2$ = 0.934; $p$-values for the coefficients of both explanatory variables were < 0.002). Increases in either ambient temperature or the number of larvae in a host were associated with a decrease in residence time (the estimated relationship was $residence\_time = 211.2 - 4.14 \cdot temperature - 5.50 \cdot effective\_clutch\_size$) (Figure 2.5). By the time all larvae left an infected host, it was common to find all soft tissues inside the cicada's body entirely consumed so that nothing but the exoskeleton remained.

To exit their host, larvae used their oral hooks to burrow through intersegmental membrane, and they usually emerged by squeezing between one of the cicada's opercula and its abdomen (Figure 2.6). The exact location of egress was observed for 83 larvae from 28 *N. dorsatus* hosts, and of these, 64 (77.1%) exited from behind one of the cicada's opercula. Of the

remaining larvae, 16 (19.3%) exited next to the pygofer or terminal abdominal segments at the apex of the abdomen, and 3 (3.6%) burrowed through the membrane between the head and prothorax.

After leaving their host, larvae immediately burrowed into the soil (or sand, in the case of the emergence containers) to pupariate. Although more than 300 *E. erro* larvae were obtained from infected *N. dorsatus* specimens during the course of this study, relatively few of these were successfully reared to the adult stage. 51 flies survived to adulthood, and the times of both larval egress from the host and adult eclosion were obtained for 31 of these flies. Adult flies eclosed 18.4 days, on average, after leaving their host (95% CI: 18.02–18.69 days, $s = 0.91$, $n = 31$ flies

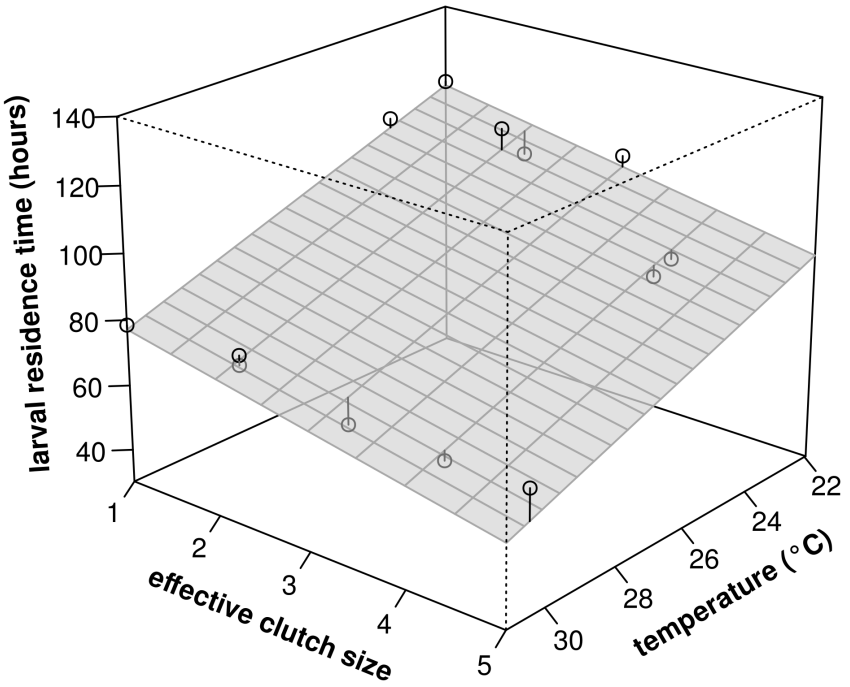


**Figure 2.5** Relationship of effective clutch size and temperature to larval residence time. Each data point represents the mean residence time of the parasitoid larvae inside a single host cicada along with the effective clutch size (number of larvae emerging from the host) and the mean air temperature experienced by the host during parasitoid development. The planar surface represents the multiple linear regression model of the effects of temperature and effective clutch size on larval residence time. Lines connected to the data points indicate the vertical distance of each data point from the regression surface (i.e., the residuals).

**Figure 2.6** Emergence of *E. erro* from its host. A mature larva of *E. erro* emerges from between the left operculum and the abdomen of a deceased male *N. dorsatus* from Prowers Co., CO.

from 15 host cicadas, range = 16–20 days). The lifespan of adult flies in the field is unknown. Adult flies maintained in the laboratory survived as long as 92 days.

The lifetime reproductive potential of female *E. erro* was not determined, but I did dissect 14 gravid female flies that were collected at audio broadcasts of the *N. dorsatus* calling song in 2013 and 2014 and counted all larvae contained within their abdomens. These flies carried as few as 3 and as many as 174 larvae in their incubatory pouches, with a mean of 60.7 larvae per fly ($s$ = 57.5). The observed distribution of larvae counts was strikingly bimodal: Three flies had more than 150 larvae, while all of the rest had fewer than 80. The larvae of the four flies with the largest larvae counts were noticeably smaller than those from the remaining flies and generally had less well-developed bristles. Remnants of eggshell were still visible in the incubatory pouches of three of these flies, suggesting that the larvae had recently hatched.

### *Host parasitism rates and parasitoid loads*

*Parasitism rates*

The results of the *N. dorsatus* population surveys for the prevalence of *E. erro* infection are presented in Table 2.1. Parasitized *Neotibicen dorsatus* were collected at all six of the primary study sites, although infected cicadas were not detected in all population surveys. All parasitoids that were reared to the adult stage were identified as *Emblemasoma erro*, and the morphologies of all other larvae and puparia that were obtained were also consistent with *E. erro*. No hyperparasitoids of *E. erro* were observed.

Across all four sampling years (2011-2014) and all six primary study sites, the overall observed parasitism rate for *N. dorsatus* males was 26.3% (95% CI: 21.4–31.9%, $n = 266$ cicadas). The surveys in 2014 also included a sample of 28 female *N. dorsatus* from the central KS field sites (in Harvey, McPherson, and Reno counties), and of these, 1 female cicada was infected with *E. erro* larvae (3.7%; 95% CI: 0.7%–18.3%).

There was substantial variation in observed parasitism rates among the population samples (summarized in Table 2.1). The results of the logistic regression analysis suggested that much of this variation was due to differences among field sites, with sampling year possibly also having a small effect (likelihood-ratio tests of *field site* and *year* as predictors: $p < 0.00001$ and $p = 0.0663$, respectively). The model including these two predictor variables seemed to explain the data reasonably well, with pseudo $R^2 = 0.549$. Nevertheless, this result must be interpreted with caution. The westernmost field sites often had the highest sample parasitism rates, but because of logistical constraints, these sites were always surveyed later in the summer than the other field sites (Table 2.1). Thus, the variable *field site* was at least partially collinear with

*sample date*. Consequently, the high parasitism rates observed at these sites could have been due, at least in part, to seasonal effects rather than inherent site differences.

**Table 2.1** Observed parasitism rates of male *N. dorsatus* in the field. Observed parasitism rates are given for each study site for all sample years combined, with the yearly observations for each site given below the site summary rows. The overall totals for all sites and years combined are given at the bottom of the table. "Infected" is the number of parasitized cicadas that were captured, "Uninfected" is the number of unparasitized cicadas, "Total" is the total number of cicadas captured, and "95% CI" is the Wilson 95% confidence interval for the population estimate of the percentage of infected male cicadas. Refer to Figure 2.3 for study site locations.

| Study site | Dates | Infected | Uninfected | Total | % infected | 95% CI |
|---|---|---|---|---|---|---|
| McPherson Co. | site summary (2012-14) | 3 | 45 | 48 | 6.3 | 2.1–16.8 |
| | *2012: July 2, 4* | *0* | *12* | *12* | *0.0* | |
| | *2013: Aug. 2, 20* | *0* | *10* | *10* | *0.0* | |
| | *2014: Aug. 3, 12* | *3* | *23* | *26* | *11.5* | |
| Prowers Co. | site summary (2013-14) | 27 | 9 | 36 | 75.0 | 58.9–86.2 |
| | *2013: Aug. 22, 28* | *11* | *4* | *15* | *73.3* | |
| | *2014: Aug. 21, Sept. 4* | *16* | *5* | *21* | *76.2* | |
| Hamilton Co. | site summary (2013-14) | 10 | 3 | 13 | 76.9 | 49.7–91.8 |
| | *2013: Aug. 22, 28* | *6* | *3* | *9* | *66.7* | |
| | *2014: Aug. 21* | *4* | *0* | *4* | *100.0* | |
| Harvey Co. | site summary (2011-14) | 11 | 51 | 62 | 17.7 | 10.2–29.0 |
| | *2011: Aug. 12* | *1* | *9* | *10* | *10.0* | |
| | *2012: July 7, 12* | *5* | *13* | *18* | *27.8* | |
| | *2013: Aug. 5, 12* | *3* | *14* | *17* | *17.6* | |
| | *2014: Aug. 9, 12* | *2* | *15* | *17* | *11.8* | |
| Reno Co. | site summary (2011-14) | 8 | 65 | 73 | 11.0 | 5.7–20.2 |
| | *2011: Aug. 14, 15* | *1* | *3* | *4* | *25.0* | |
| | *2012: July 16, Aug. 17* | *1* | *13* | *14* | *7.1* | |
| | *2013: July 23* | *0* | *15* | *15* | *0.0* | |
| | *2014: Aug. 4, 11* | *6* | *34* | *40* | *15.0* | |
| Ellsworth Co. | site summary (2011-13) | 11 | 23 | 34 | 32.4 | 19.1–49.2 |
| | *2011: Aug. 11* | *5* | *3* | *8* | *62.5* | |
| | *2012: Aug. 19* | *2* | *1* | *3* | *66.7* | |
| | *2013: Aug. 10, 17* | *4* | *19* | *23* | *17.4* | |
| **Total** | – | 70 | 196 | 266 | 26.3 | 21.4–31.9 |

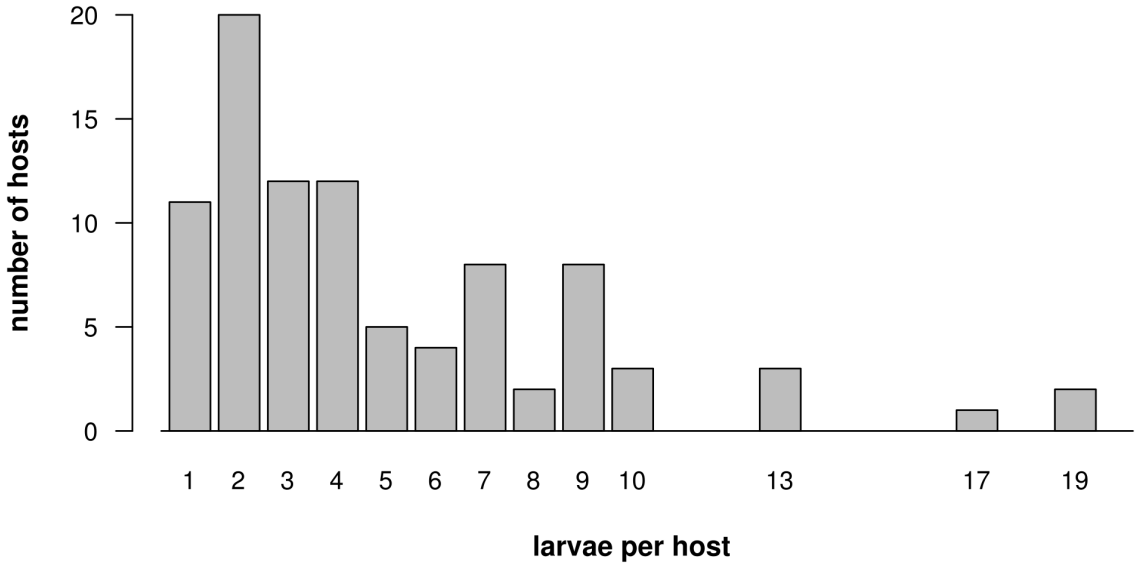


**Figure 2.7** The distribution of parasitoid loads (larvae per host) of infected cicadas in the field.

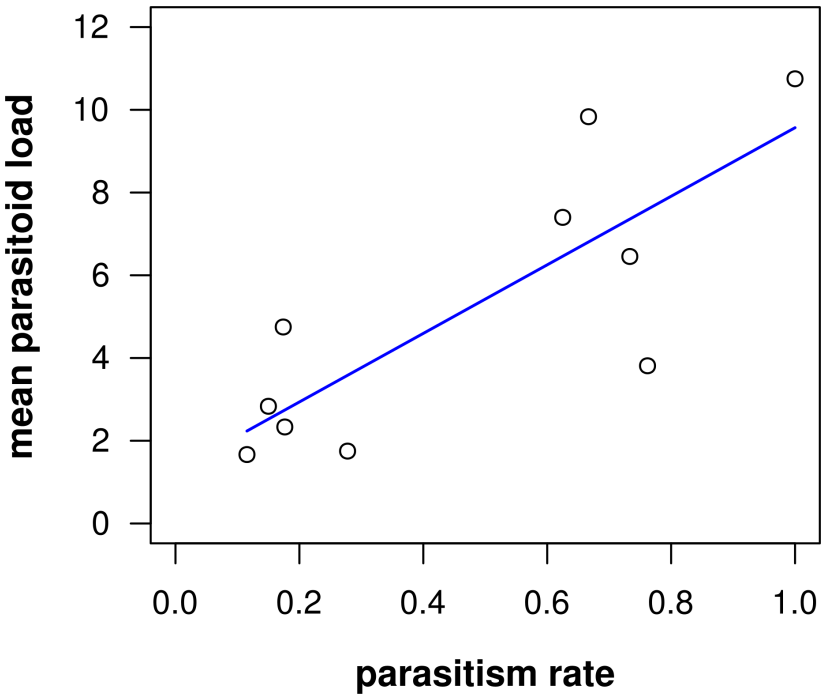


**Figure 2.8** Relationship between host parasitism rate and mean parasitoid load per host. Each data point represents one year of host population sampling data for a single study site. The solid blue line represents the linear regression model for the data.

*Parasitoid loads*

The mean parasitoid load of all field-collected infected cicadas was 4.97 larvae/host (95% bootstrap-*t* CI: 4.23–5.92 larvae/host, $s = 3.95$, $n = 91$ hosts, range = 1–19 larvae/host) and the median was 4, reflecting the strong right skew of the distribution (Figure 2.7).

The parasitoid loads of field-collected infected cicadas were often much higher than the clutch sizes of larvipositing females in the laboratory infection trials. A bootstrap-*t* comparison of means confirmed that the mean clutch size of female parasitoids (2.53 larvae/host) was significantly less than the mean parasitoid load of hosts in the field (4.97 larvae/host) (95% bootstrap-*t* CI: 1.32–3.52 fewer larvae/host, $p < 0.0001$).

Overall, there was a strong, positive relationship between host cicada parasitism rates and mean parasitoid loads per host (Figure 2.8), with parasitism rate explaining about 65% of the variation in mean parasitoid load (simple linear regression: $b = 8.29$, $R^2 = 0.650$, $p = 0.0048$). The estimated slope of the relationship was 8.29, but the imprecision of the parasitism rate estimates meant that this slope estimate likely suffered from attenuation bias. The estimated bias correction factor was approximately 1.117, giving an attenuation-corrected slope estimate of 9.25.

## 2.4 Discussion

The results of this study provide the first detailed information about the infection behaviors and life history of any species of *Emblemasoma* besides *E. auditrix*. Both laboratory and field observations reveal that *E. erro* find their hosts by eavesdropping on the sexual communication signals of male cicadas. When a female *E. erro* locates a calling cicada, she waits to attack until the host is in motion, and larviposition on flying cicadas is not uncommon. The results also show that male *N. dorsatus* are commonly parasitized by *E. erro* and that there

can be substantial variation in population parasitism rates and parasitoid loads. I next discuss the behavior and life history of *E. erro*, especially in comparison to other acoustic parasitoids and other sarcophagid parasitoids; assess possible host defenses; and discuss possible causes of variation in host parasitoid loads and parasitism rates.

### *Host locating and infection behaviors of* E. erro

*E. erro*'s use of phonotaxis to locate potential hosts is similar to that reported for other acoustically hunting parasitoids (Soper et al. 1976, Lehmann 2003, Lakes-Harlan and Lehmann 2015), but *E. erro*'s preference for attacking moving targets is apparently unique among known acoustic parasitoids. For example, *E. auditrix*, the only other *Emblemasoma* for which larviposition behaviors are known, will aggressively attack stationary or restrained cicadas. Upon finding a male cicada, female *E. auditrix* exhibit a stereotyped behavioral sequence in which the female fly immediately attempts to squeeze underneath the perched cicada's wings to gain access to the cicada's timbal region. She then uses specialized terminal abdominal sternites to cut through the cicada's timbal membrane and injects larvae directly into the host's body (Schniederkötter and Lakes-Harlan 2004). Female *E. erro* lack any comparable abdominal modifications, but larvipositing through the host's timbal would likely be impossible for *E. erro* anyway, because male *Neotibicen dorsatus* have timbals that are fully protected by well-developed timbal covers. In contrast, *E. auditrix*'s host cicada, *Okanagana rimosa*, lacks timbal covers entirely.

Tachinid acoustic parasitoids of the tribe Ormiini will also attack stationary hosts, and they will even larviposit without visual or tactile confirmation of a host's location. For example, *Homotrixa alleni* Barraclough, *Ormia depleta* (Wiedemann), and *O. ochracea* (Bigot) will all deposit larvae at a sound source regardless of whether or not a potential host insect is actually

present (Cade 1979, Fowler 1987, Allen et al. 1999). For *E. erro*, the host's calling song was never sufficient by itself to trigger larviposition, even when a potential host was present. In contrast to *E. erro*, ormiine tachinids are all nocturnal parasitoids of Orthoptera, and their willingness to larviposit in the absence of a host probably reflects an almost total reliance on acoustic cues at night. For acoustic parasitoids such as *E. erro* that are active during the day, requiring visual confirmation of a suitable host prior to larviposition allows for more precise placement of larvae and undoubtedly decreases the number of larvae that are wasted by the female fly.

In comparison to the larviposition behaviors of other acoustic parasitoids, *E. erro*'s tendency to attack flying cicadas is especially striking. One third of the successful attacks observed in the experiment cages took place while the cicada was in flight, but this is almost certainly an underestimate of the true frequency of flight-based attacks in nature. Due to the size of the cages used in the trials, most attempts by flies to follow cicadas in the air resulted in failure because the cicada crashed into a side of the cage before the fly could approach and orient itself to the flying cicada. It was hoped that the large "flight cage" would alleviate this problem, but even it appeared to be too small for most aerial attacks to succeed. Nevertheless, flies seemed much more reluctant to attack potential hosts that were not in flight.

This conclusion is further supported by observations in the field, where nearly all apparent larviposition attacks occurred while cicadas were in flight. Flies sometimes even followed a single cicada from perch to perch, waiting patiently next to the cicada each time it landed, but never attempting to attack while the cicada was not flying. As an example, in 2013 I observed a male *N. dorsatus* calling from a grass flowering culm with a female *E. erro* perched on the opposite side of the stalk near the cicada's abdomen. The fly was nearly motionless until

the cicada backed a short distance down the stalk, causing the fly to move with him nearly in unison, but the fly made no move to attack the cicada. When the cicada flew a short distance (approximately 1-2 meters) to a new perch, the fly closely followed him in the air, landed next to the cicada, and again remained nearly motionless while the cicada began calling. The cicada flew twice more, with the fly following both times, and after the final flight of at least 30 meters, I captured the cicada and later reared two *E. erro* larvae from it.

While *E. erro*'s behavior of larvipositing on hosts while they are in flight or otherwise in motion might be different from *E. auditrix* and tachinid acoustic parasitoids, it is remarkably similar to the larviposition behaviors reported for some sarcophagid parasitoids of the genus *Blaesoxipha* that parasitize acridid grasshoppers. *Blaesoxipha aculeata* (Aldrich), *B. caridei* (Brethes), *B. kellyi* (Aldrich), *B. redempta* (Pandellé), and *B. reversa* (Aldrich), among others, have all been reported to attack grasshoppers while in flight (Coquillett 1892, Kelly 1914, Aldrich 1916, Lloyd 1951, Rees 1973, Povolný and Verves 1997). Kelly (1914) provided a detailed description of the larviposition behaviors of *B. kellyi*, reporting that grasshoppers were attacked either on the wing or on the ground, and that grasshoppers were only attacked when they were in motion (but see Smith 1915). Furthermore, both *B. kellyi* and *B. reversa* typically place larvae near the base of a host's wings, much like *E. erro* (Kelly 1914, Rees 1973).

It is worth noting that early last century, Beamer (1928) and Kelly (1914), both working in Kansas, reported seeing cicadas pursued by flies while in flight. Beamer noted that "the flies follow but a few inches away, and sometimes seem almost to alight on the body of the cicada." Although their observations were largely adventitious and incidental, and neither author identified the flies involved, it seems plausible in retrospect that their papers might have been the first published records of *E. erro*'s host infection behavior.

### *Infection of female hosts*

Given *E. erro*'s primary host-finding mechanism, male cicadas are clearly the primary targets of infection by this parasitoid. However, the observation of a fly larvipositing on a female cicada in the laboratory, along with the 2014 survey of female *N. dorsatus* in the field, confirm that female cicadas are also sometimes attacked.

Since female cicadas are silent, how are they discovered by *E. erro* in the field? One possibility is that, simply by chance, they happen to fly within the visual range of a perched female *E. erro*. Perhaps more likely, though, female *N. dorsatus* and female *E. erro* might sometimes encounter one another because of a shared interest in male cicadas. Like *E. erro*, female cicadas perform phonotaxis in response to males' calls, so female cicadas could become parasitized if they were attracted to the same calling male as a female *E. erro*. In any case, despite many hours spent observing cicadas in the field, I never witnessed any interactions between female *E. erro* and female *N. dorsatus*, so such encounters must be rare in comparison to encounters between male cicadas and female *E. erro*. However, *E. erro*'s occasional use of female hosts is apparently not unusual. Several other species of acoustic parasitoids that primarily attack male hosts are also known to sometimes parasitize females (Soper et al. 1976, Lehmann 2003).

### *Phenology and fecundity of* **E. erro**

Little is known of the seasonal phenology of *E. erro*. In this study, adult flies were observed in the field as early as June 13 (in 2012) and as late as September 4 (in 2014), and these were also the earliest and latest dates that I attempted to find them. The rearing data strongly suggest that *E. erro* is multivoltine in the geographic area covered by this study. With a total development time from larviposition to adult eclosion of about 22 days, it seems possible that

there could be at least three generations per year. *E. auditrix*, in contrast, is apparently univoltine (Soper et al. 1976, de Vries and Lakes-Harlan 2005).

Female *E. erro* were observed with as many as 174 first-instar larvae, nearly 3.5 times the maximum of 50 observed for *E. auditrix* (de Vries and Lakes-Harlan 2005). The apparently large difference in fecundity between these two species might be at least partially explained by their larviposition behaviors and life histories. *E. auditrix* deposits larvae directly inside a host's body, one larva per host, and all available evidence suggests that *E. auditrix* is a solitary parasitoid (Soper et al. 1976). By injecting larvae into its hosts, *E. auditrix* likely "wastes" relatively few larvae during larviposition, and as a solitary parasitoid, it is plausible that multiple larvae inside a single host would physically attack one another (Godfray 1994). Under these conditions, females might benefit by producing fewer, larger larvae to increase their chances of survival. In contrast, because *E. erro* deposits its larvae on the exterior of a host, it is likely that some percentage of these larvae never manage to make it inside the host's body. Moreover, *E. erro* is a gregarious parasitoid, and as such, larvae probably face little direct physical aggression from conspecifics (Godfray 1994). For *E. erro*, then, investing fewer resources in more larvae might increase a female's lifetime reproductive success. Some tachinid acoustic parasitoid species, which deposit their larvae even more haphazardly, also have large larval complements (Wineriter and Walker 1990, Allen et al. 1999, Kolluru and Zuk 2001), and although behavioral data for other sarcophagid parasitoids is extremely limited, at least some parasitoid species in the genus *Blaesoxipha* also appear to follow this pattern (Middlekauff 1959).

***Host defenses and mortality***

Once discovered by a female *E. erro*, male *N. dorsatus* appeared to have relatively few viable options to defend themselves. When approached by a parasitoid fly, calling male *N.*

*dorsatus* cicadas responded either by flying, immediately terminating their call and remaining motionless on their perch (hereafter referred to as "hiding"), or simply continuing their calling behavior. The latter seemed to be the most common. Cicadas often called repeatedly and walked freely about the walls of the experiment cages despite being followed by a fly only a few centimeters away. Cicadas sometimes even called with a fly perched right on top of them. However, stationary cicadas that were directly contacted by a fly would often vigorously flick their wings to try to repel the parasitoid. Unfortunately, given the relatively small space inside the cages, evaluating the effectiveness of any of these behaviors was nearly impossible because a cicada could never truly escape from the fly.

Nevertheless, observations in the field suggested that both the flight and hiding strategies do sometimes work. In at least one case, a fly lost interest in a hiding cicada and left before the cicada resumed calling, and in another, a cicada that was contacted by an approaching fly managed to escape by flying away. Most of the time, though, flies simply waited until a hiding cicada became active again, and they usually had little difficulty in following a flying cicada from one perch to another. As a defensive strategy, flying seems especially risky given *E. erro*'s aptitude for aerial larviposition.

After being larviposited upon, cicadas had yet another option for defending themselves. I repeatedly observed cicadas perform "wing flipping" behavior immediately after being attacked, characterized by rapidly flapping their wings several times while perched. In this way, one cicada managed to completely dislodge the single larva that had been deposited on the cicada's right fore wing, thus avoiding infection completely. This was the only case for which I confirmed that a cicada was able to remove all larvae from its body, but it is possible that some larviposition events were not detected during the behavioral experiments. Wing flipping by *N.*

*dorsatus* appears to be functionally similar to the grooming behaviors used by the cricket *Gryllus texensis* Cade and Otte to prevent infection by the larvae of *Ormia ochracea* (Vincent and Bertram 2010).

Although the hosts of some other sarcophagid parasitoids have been reported to occasionally survive parasitism (Spencer and Buckell 1957, Danyk et al. 2000), infection by *E. erro* appears to be invariably fatal for *N. dorsatus*. In most cases, hosts died several hours before the parasitoid larvae emerged. Host death was usually preceded first by loss of wing function, then loss of leg function beginning with the hind legs and ending with the fore legs. Prior to death, a cicada's antennae were typically the last appendages to display a visible response to external touch. After a cicada died, small, rhythmic movements of the legs or head capsule were often visible as the parasitoid larvae used their oral hooks to scrape muscle and other soft tissue from the integument.

Sometimes, though, when a cicada was infected with only a single larva, the larva emerged before the cicada died, leaving the host in a severely weakened, moribund state. Cicadas in this condition usually succumbed after a few hours. In one exceptional case, a large male *N. dorsatus* from the Prowers Co., CO site that was infected with a single *E. erro* larva survived for more than 24 hours following parasitoid emergence. Although sluggish, it was still able to cling to and crawl on a perch, weakly flutter its wings (but not fly), and was even observed attempting to feed before its movements became uncoordinated and it, too, died. Overall, *E. erro* must be a major cause of mortality for adult male *N. dorsatus*, especially considering the very high parasitism rates observed in some cicada populations.

***Variation in host parasitism rates among study sites***

Host populations at the two westernmost field sites appeared to have consistently higher parasitism rates than sites further east (Table 2.1, Figure 2.3). The biogeography of potential host cicadas might offer one explanation for this pattern. The western sites were located on the semi-arid High Plains, where there are fewer species of large cicada present than on the more mesic midgrass prairies of the study sites further east. *E. erro* parasitizes other cicada species besides *N. dorsatus* (B. Stucky, in prep.), so higher parasitism rates of *N. dorsatus* on the High Plains could be a consequence of local differences in the communities of potential host species.

However, as noted in the Results, because these western sites were also sampled later in the season than the eastern sites, higher parasitism rates could have also been caused by seasonal effects rather than intrinsic differences among the sites. One might expect parasitism rates to increase throughout the season as *E. erro* populations reach their peak and host populations decline, as has been observed for several other species of dipteran parasitoids, including some acoustic parasitoids (e.g., Tamaki et al. 1983, Allen 1995, Lehmann 2008). It seems likely that this accounts for at least some of the among-site differences in parasitism rates found in this study. Furthermore, both host and parasitoid population sizes undoubtedly also play a role in determining parasitism rates. As evidenced by some of the small population sample sizes, host cicadas were uncommon and difficult to collect for some years at some field sites, which suggests that there was variation in host population sizes from year to year. Future studies that estimate host and parasitoid population sizes and sample both High Plains and central Plains sites multiple times throughout the season will be needed to fully disentangle the effects of these variables on host parasitism rates.

### *Superparasitism by* E. erro

The strong, positive relationship between parasitism rate and parasitoid load (Figure 2.8), as well as the significant difference between the mean parasitoid load of field-collected hosts and the mean clutch size of larvipositing females (4.97 and 2.53 larvae/host, respectively), can both be explained as a consequence of superparasitism in the field. If at least some host cicadas are superparasitized in the field, then we should expect the mean parasitoid load of host cicadas to be larger than the mean clutch size of individual female flies. Furthermore, for gregarious parasitoids such as *E. erro*, superparasitism is expected to be more common when unparasitized hosts are rare, simply because female parasitoids have a harder time finding hosts that have not already been infected (Godfray 1994). Unparasitized hosts are rare when parasitism rates are high, so higher parasitism rates should correspond with increasing rates of superparasitism. Increased superparasitism would, in turn, likely result in larger parasitoid loads per host, which means that higher population parasitism rates should correspond with higher parasitoid loads. This prediction matches the pattern of the data quite well (Figure 2.8).

Additional, anecdotal evidence of superparasitism was found in the relative sizes of larvae emerging from some of the most heavily parasitized hosts. In some cases, two distinct larval size classes were evident, presumably due to the smaller larvae having been deposited on the host later than the larger larvae. In other cases, though, all larvae emerging from heavily parasitized hosts were approximately the same size, suggesting that either a single female deposited all of the larvae at once, or more likely, that two (or more) female flies discovered an uninfected host at nearly the same time.

## 2.5 Conclusions

*Emblemasoma erro* is a widespread, common parasitoid of the cicada *Neotibicen dorsatus* on the grasslands of the Great Plains in the central United States. Female flies locate potential hosts by eavesdropping on the acoustic mating calls of male cicadas, then use visual cues to larviposit on the host while it is in motion. Larviposition often occurs while the cicada is in flight. Parasitization by *E. erro* is always fatal for *N. dorsatus*, which seems to have few consistently effective defenses against attack. Parasitism rates for male *N. dorsatus* can exceed 70% in some host populations. Parasitoid loads of infected cicadas average about 5 larvae per host but can be as high as 19 larvae per host. At least some variation in parasitoid loads is likely due to superparasitism in host populations with high parasitism rates.

Even though *E. erro* is, like *E. auditrix*, an acoustically orienting parasitoid of cicadas, the close-range infection behaviors of these two species are highly divergent, and the infection behaviors of both species are very different from tachinid acoustic parasitoids of the tribe Ormiini. Indeed, the infection behavior of *E. erro* is unlike that known for any other acoustic parasitoid. There are important life history differences between *E. erro* and *E. auditrix* as well: *E. auditrix* is apparently a solitary, univoltine parasitoid with relatively low larval production per female; *E. erro* is a gregarious, multivoltine parasitoid with high larval production per female. Given the marked differences between *E. erro* and *E. auditrix*, the results of this study suggest that more work is needed to characterize the diversity of *Emblemasoma* parasitoids. An improved understanding of sarcophagid acoustic parasitoids would make it possible to more meaningfully compare sarcophagid and tachinid acoustic parasitoid lineages, and it would also allow for more robust inferences about eavesdropping parasitoids in general.

# CHAPTER 3

## EAVESDROPPING TO FIND MATES: THE FUNCTION OF MALE HEARING IN *EMBLEMASOMA ERRO*

### 3.1 Introduction

At least six species of flies of the tachinid tribe Ormiini and two species of flies of the sarcophagid genus *Emblemasoma* (sensu Pape 1990) are parasitoids with a rather unusual method of finding their hosts: Female flies have highly sensitive "ears" that allow them to listen for, and home in on, the airborne acoustic signals of crickets, katydids, and cicadas (Cade 1975, Soper et al. 1976, Burk 1982a, Fowler and Kochalka 1985, Lakes-Harlan and Heller 1992, Walker 1993, Allen 1998, Stucky 2015). Females perform rapid, positive phonotaxis to the calling songs of their hosts, but all previous studies have found that males are not attracted by these sounds in the field (Fowler 1987, Walker 1993, Farris et al. 2008, Lakes-Harlan et al. 2014). Nevertheless, male flies also have ears. They possess the same tympanal structures as females, and the males' tympana are physiologically functional (Robert et al. 1992, Lakes-Harlan et al. 2014). So far, however, no definite adaptive function of male hearing has been demonstrated for any of these fly species.

This chapter presents the results of a behavioral investigation of the function of male hearing in the sarcophagid parasitoid *Emblemasoma erro* Aldrich. *Emblemasoma erro* is an acoustically orienting parasitoid of the cicada *Neotibicen dorsatus* (Say) in the central Great Plains region of North America, and as with other sarcophagid and tachinid acoustic parasitoids, females of *E. erro* orient to the calling songs of their hosts for larviposition (Stucky 2015). After informally observing that male *E. erro* were also attracted to the acoustic signals of cicadas, I hypothesized that both male and female *E. erro* might use the calling songs of their host cicadas as a means for locating potential mates. To test this hypothesis, I investigated the attractiveness

of host cicada calls to both male and female *E. erro*, and I tested whether non-gravid female *E. erro* also perform phonotaxis to cicada calls, as would be expected if females use cicada calls to find mates. I also observed the behaviors of male *E. erro* at a sound source and attempted to observe the mating behavior of *E. erro* in the field. All results suggested that cicada calls serve as mate-finding cues for this parasitoid. I discuss the mate locating behavior of *E. erro* in the context of the mating behaviors of other sarcophagid species, other acoustic parasitoids, and, more broadly, eavesdropping parasitoids in general.

### 3.2 Methods

Most of the field work for this study was conducted at three study sites located in Ellsworth, McPherson, and Reno counties in central Kansas. All acoustic trapping of *E. erro* (described below) was done at these three sites. Behavioral observations were also mostly from these three localities, with additional observations at some other nearby field sites. The habitat at all study sites was qualitatively similar and consisted of native midgrass prairie vegetation intermixed with both naturally occurring and planted trees. All research was conducted in late July, August, and early September of 2011-2013.

Phonotaxis by *E. erro* was studied in the field by broadcasting appropriate acoustic stimuli from a loudspeaker, which is a technique that has been widely used by researchers working on other species of acoustic parasitoids (e.g., Cade 1975, Soper et al. 1976, Lakes-Harlan et al. 2000, Lehmann 2003). The acoustic stimuli were designed to represent the calling song of the host cicada *Neotibicen dorsatus* from the study sites. Details of the methods used to construct these signals are provided in Stucky (2015). Briefly, the calling songs of 20 different male *N. dorsatus* were recorded, and the recordings were analyzed to determine the values of three acoustic parameters: peak frequency, pulse group (PG) length, and PG rate

("pulse group" is defined in Stucky (2015), and Cole (2008) includes a spectrogram and oscillograms of the *N. dorsatus* calling song). The mean values of these parameters were used to construct representative "model calls", either from the recordings themselves or by generating synthetic signals from amplitude-modulated sine waves, that represented the average mating call of male *N. dorsatus*. The synthetic signals used for this study had a peak frequency of 5.64 kHz, PG length of 20.3 ms, and a PG rate of 37 bursts/s. 5.64 kHz is higher than the observed mean peak frequency of *N. dorsatus* (4.31 kHz), but *E. erro* are much more responsive to signals of 5.64 kHz than 4.31 kHz (see Chapter 4), so the higher frequency was used. *N. dorsatus* has a broadband call with peak acoustic energy found from about 3 kHz to 7 kHz (Cole 2008), so the synthetic signals were still broadly representative of the natural calling song.

For direct behavioral observations, sounds were broadcast from a tweeter speaker (PylePro PH44) mounted in the top of a wooden box, and flies were observed as they arrived at the speaker and while they remained on or near the broadcast apparatus. I did not attempt to estimate the numbers of female and male flies that were attracted during these behavioral observations because some flies will depart and return to a sound source multiple times during a broadcast (B. Stucky, pers. observation), which makes manually counting flies as they arrive unreliable. Instead, acoustic live traps (Appendix A) were used to objectively quantify the comparative phonotactic responsiveness of male and female *E. erro*. For each trial, 3 traps were deployed at a field site. The synthetic *N. dorsatus* call was simultaneously broadcast from each trap for 30 minutes, after which all captured flies were counted and sexed. To provide additional information about *E. erro*'s phonotactic behavior, the relative signal amplitudes among the three traps were generally not the same and differed by as much as 18 dB between the loudest and quietest traps. Because of this, the signals were rotated among the traps every 10 minutes so that

each trap broadcast at each amplitude for the same amount of time. To avoid pseudoreplication, no captured flies were released until after trapping was completed at a location. Traps were only operated in the afternoons when the host cicadas were naturally active. Trapping in 2011 was part of a larger study of *E. erro*'s phonotactic behavior; the full results of that study are reported in Chapter 4.

To test whether non-gravid female flies (defined here as females with no live first-instar larvae in their incubatory pouches) perform positive phonotaxis to host calls, I dissected the abdomens of 20 female flies that were captured at broadcasts of the *N. dorsatus* calling song and noted the presence or absence of larvae. In addition, in 2012 I tested the phonotactic responses of two female flies that had been reared from infected *N. dorsatus* collected in the field. Neither fly had previously been exposed to either male flies or cicadas. Each fly was tested separately. For each test, the flies were released into a large, outdoor, rectangular screen cage (approximately 1.8 m wide on each side and 2.1 m high in the center) and given several minutes to acclimate to the enclosure. A portable digital audio player (Samsung Galaxy Player 4.0) was held on the side of the cage opposite to the side on which the fly was perched, and the model call of *N. dorsatus* was broadcast from the audio player's onboard speaker. Depending on the location of the fly, the distance between the fly and speaker at the start of the broadcast varied between approximately 1.8 m and 3.1 m. Playback was terminated once a fly performed complete phonotaxis to the speaker or after about 2 minutes if the fly did not travel to the sound source. The playback procedure was repeated up to four times for each fly.

The overall proportion of male flies captured by the acoustic traps across all years and study sites was analyzed using an exact binomial test. To further investigate the comparative phonotactic behaviors of female and male flies, the numbers of flies arriving at the call

broadcasts were analyzed using Poisson regression (i.e., generalized linear models with log link function and Poisson-distributed response) (Dobson and Barnett 2008). The predictor variable of primary interest was fly *sex*, but *field site*, *year*, and *amplitude* were also considered as predictors. To account for possible variations in male/female responses across the study conditions, the pairwise interactions of fly *sex* with each of *site*, *year*, and *amplitude* were also evaluated. The effect of each explanatory variable was tested by comparing a reduced model against the full model using the difference of their deviance statistics (i.e., likelihood ratio tests) (Dobson and Barnett 2008). Confidence intervals (CI) for the overall estimated proportions of males and non-gravid females arriving at call broadcasts were calculated using the Wilson method (also known as the score confidence interval) (Wilson 1927, Agresti and Coull 1998). All statistical analyses were done using R version 3.1.3 (R Core Team 2015).

## 3.3 Results

### *Phonotaxis by male flies*

The traps broadcasting the model *N. dorsatus* call were operated for a total of 24 trap-hours and captured a total of 110 *Emblemasoma erro* (Table 3.1). Of these, 76 (69.1%) were female and 34 (30.9%) were male. Considering all trapping data together, significantly more females than males were captured by the traps (exact binomial test: $p < 0.001$; 95% CI for the proportion of male flies: 0.230 – 0.401).

The results of the Poisson regression analysis of the numbers of flies captured in the acoustic traps is given in Table 3.2. As expected from the binomial test of the aggregated data, there were significant differences in the numbers of male and female flies that were captured ($p < 0.001$), but the proportions of males and females varied among the study years (interaction of fly *sex* and *year*: $p = 0.0177$). The proportion of male flies captured in 2013 was much higher than

in 2011 and 2012 (Table 3.1); the reason for this was not clear. There were no differences in the proportions of males and females trapped among the field sites (interaction of fly *sex* and *field site*: $p = 0.369$). Signal amplitude had a strong effect on the number of flies captured by a trap ($p < 0.001$), but amplitude had no effect on the proportions of male and female flies that were trapped (interaction of *amplitude* and fly *sex*: $p = 0.386$). Since broadcast amplitude had no effect on the comparative phonotactic responsiveness of male and female *E. erro*, the effect of amplitude on fly phonotaxis is not considered further in this chapter and is instead discussed in detail in Chapter 4.

On three occasions, an individual trap only caught male *E. erro*. No flies were ever captured by the traps when the loudspeakers were not broadcasting.

**Table 3.1** Total numbers of female and male *E. erro* captured in acoustic traps for each study year.

| Year | Female | Male | Total |
|---|---|---|---|
| 2011 | 22 | 9 | 31 |
| 2012 | 36 | 7 | 43 |
| 2013 | 18 | 18 | 36 |
| **Total** | 76 | 34 | 110 |

**Table 3.2** Analysis of deviance table for the Poisson regression model of the number of flies captured in the acoustic traps. Significance values for individual predictor variables were calculated using "type II" tests as implemented in the car package for R (Fox and Weisberg 2011).

| Variable | Deviance ($\chi^2$) | d.f. | *p* |
|---|---|---|---|
| *amplitude* | 124.325 | 1 | < 0.001 |
| *sex* | 16.451 | 1 | < 0.001 |
| *year* | 3.297 | 2 | 0.192 |
| *field site* | 0.215 | 2 | 0.898 |
| *sex*year* | 8.071 | 2 | 0.0177 |
| *sex*field site* | 1.993 | 2 | 0.369 |
| *sex*amplitude* | 0.751 | 1 | 0.386 |

Attraction of male flies was not just an artifact of the broadcast apparatus. Male flies were also occasionally captured in the field on the outside of mesh cages housing calling male *N. dorsatus* cicadas, which confirmed that they perform phonotaxis to the natural mating calls of male cicadas as well as to the loudspeakers.

***Phonotaxis by non-gravid female flies***

Twenty female flies were collected at model *N. dorsatus* call broadcasts and dissected to determine whether they were gravid (i.e., whether they contained first-instar larvae). Of these 20 flies, 6 (30%) had no larvae in their incubatory pouches and 14 (70%) contained first-instar larvae (95% CI for the proportion of non-gravid females: 0.145 – 0.519). The 6 non-gravid flies all contained eggs only. In some cases, the eggs were quite small and clearly at an early point in their development.

In the tests of the phonotactic responses of the two reared female *E. erro* that had not been exposed to either male flies or potential hosts, both flies performed complete flight phonotaxis to the broadcast speaker. The first fly was tested 3 times, and it traversed the width of the cage to arrive at the speaker in the first two tests. For the third test, the fly did not travel to the speaker, but the broadcast still appeared to trigger walking about the wall of the cage and several short flights. The second fly was tested 4 times, and performed complete flight phonotaxis to the speaker all 4 times. Neither fly showed any obvious response to the speaker when it was not broadcasting the model *N. dorsatus* call.

***Behavioral observations***

Male *E. erro* that were attracted by the broadcast loudspeaker typically either perched on the top of the loudspeaker box, apparently to await the arrival of other flies, or they immediately

pursued one or more flies that had previously arrived at the loudspeaker. When another fly was visually detected by a male, the male would often rapidly approach the second fly either on foot or in the air and make physical contact with it. Such contact generally appeared to be an attempt at copulation, because the pursuing male would grasp the second fly and mount it in the coupling position typical of many Diptera (McAlpine 1981). Male flies attempted to mount both males and females and seemed unable to discriminate between the sexes prior to making physical contact. When a male attempted to mount another fly, it usually resulted in both flies departing from the loudspeaker box, so it was rarely possible to determine whether attempted couplings were ultimately successful.

During these and other field experiments with *E. erro*, I observed three apparently successful matings between flies that had been attracted to model cicada call broadcasts. Two matings involved male and female files that were observed copulating inside one of the live traps after both had been captured. The durations of these matings were not precisely timed, but the second pair remained together for at least 25 minutes. In the third observation of mating in the field, two flies that had landed near the loudspeaker began copulating, then flew off joined together. Attempts were also made to observe mating behavior in captivity in the lab, but these efforts were mostly unsuccessful. However, putative male mating behavior in captivity was similar to that observed at the sound broadcasts in the field, with males pursuing potential mates either on foot or in the air. Only one successful mating was observed in captivity, between a pair of flies that had been reared from a parasitized *N. dorsatus* (Figure 3.1). These flies remained in copula for at least 90 minutes.

**Figure 3.1** Mating pair of *Emblemasoma erro*.

### 3.4 Discussion

All results of this study were consistent with the hypothesis that *Emblemasoma erro* uses the acoustic sexual signals of its host as a means for locating potential mates. In the field, both male flies and non-gravid female flies perform positive phonotaxis to acoustic stimuli mimicking the calls of their host cicadas. At least some unmated female flies with no previous exposure to males or host signals are also phonotactically responsive. Once male flies arrive at a sound source, they pursue and try to mate with other flies that are also attracted to the acoustic stimulus. Although it was not known with certainty whether the non-gravid female flies captured in the field were seeking mating opportunities, these flies were obviously incapable of infecting hosts, and the observation of three mating pairs in the field indicates that at least some receptive females perform phonotaxis to cicada calls. Traveling to calling cicadas must involve a

considerable expenditure of energy, and it is difficult to imagine any other compensatory benefit to either males or non-gravid females beyond finding mates.

While this study cannot rule out the possibility that female *E. erro* also play a role in attracting males for mating (with a pheromone signal, for example), it seems unlikely. Observations of male *E. erro* suggest that even at very close range, they cannot easily discriminate between receptive females and other females and males. Indeed, their approach to finding a suitable female appears to be mostly trial and error, as has been observed for some other sarcophagid species (Thomas 1950, Sharma 1975, Adham et al. 1980). In any case, the acoustic stimulus by itself is clearly sufficient to attract male flies. On three occasions, only male *E. erro* were captured by an acoustic trap, and at the beginning of a call broadcast, it was not uncommon for one or more male flies to arrive before any females.

There is remarkably little information available about the mate-finding behaviors of other sarcophagids. The rather limited literature suggests, though, that there are two main strategies used by these flies for locating mates. In the first strategy, male flies aggregate at visual markers that are unrelated to adult or larval food resources, such as the tops of hills, and females visit these locations to mate (Chapman 1954, Dodge and Seago 1954, Povolný and Verves 1997). This behavior, often referred to as "hilltopping", is common among many species of calyptrate flies (Chapman 1954, Dodge and Seago 1954, Alcock and Schaefer 1983). In the second strategy, both males and females travel to adult feeding sites or larviposition sites to seek mates. This has been observed, for example, in sarcophagid species that feed on dung and carrion (Thomas 1950, Martín-Vega and Baz 2013, Rivers and Dahlem 2013). Males of at least one species, *Sarcophaga bullata* Parker, also produce a pheromone that is attractive to females (Girard and Budris 1975, Girard et al. 1979).

The mate-finding behavior of *E. erro* appears to be a highly specialized version of the second sarcophagid mate finding strategy described above. Both males and females are attracted to larviposition sites (i.e., male cicadas) to find mates, and both sexes use cicadas' mating calls as the cue for locating these sites. The two mate-finding strategies used by sarcophagids are not mutually exclusive, of course, so it is entirely possible that *E. erro* males also aggregate at visual markers. The ability to facultatively switch strategies could be especially advantageous at times when hosts are not abundant, such as early in the season.

Once they arrive at a calling cicada, the close-range mating behaviors of male *E. erro* are similar to other members of their family. Although sarcophagid species vary in their strategies for initially bringing the sexes together, at close range, most sarcophagid mating systems seem to depend on males that actively search for females visually and, once a potential mate is spotted, attempt to intercept the female and mate with it (Thomas 1950, Moradeshaghi and Bohart 1968, Sharma 1975, Adham et al. 1980, Spofford and Kurczewski 1985, Alcock 2000, Gilbert and Kim 2007). Several authors have commented on the apparent inability of male sarcophagids to discriminate among receptive females, unreceptive females, other males, or even other species (Thomas 1950, Sharma 1975, Adham et al. 1980). Among the subfamily Sarcophaginae (which includes *Emblemasoma*), there is no evidence of elaborate courtship behaviors. Males rapidly grasp and attempt to mount and copulate with females. This is not the case for all sarcophagids, however, as at least one species of Miltogramminae, *Phrosinella aurifacies* Downes, engages in complex, ritualized courtship prior to mating (Spofford and Kurczewski 1985).

Only about 30% of the flies captured in the live traps were male, but this is probably an underestimate of the true proportion of males that were attracted to the sound broadcasts. During trap operation, males could often be seen perching on the outside of the trap, from where they

would watch for and attempt to intercept other flies that arrived. These males usually did not enter the trap and they sometimes persisted on the outside of the trap until the broadcast terminated. Carrion-feeding sarcophagid species in which both males and females are attracted to carcasses to seek mates can be caught with carrion-baited live traps, and, in at least one study, the percentage of males captured in this way was very similar to that observed here for *E. erro* (Martín-Vega and Baz 2013). As with *E. erro*, males station themselves near the food resource to intercept mates and do not always enter the trap (Martín-Vega and Baz 2013).

Among the species of acoustic parasitoids that have been studied so far, *E. erro* is the only one for which hearing seems to play a significant role in the lives of the adult males. Yet males of all species appear to have functional ears, so why have other acoustic parasitoids not evolved a similar strategy to locate mates? For tachinid acoustic parasitoids of the tribe Ormiini, the answer might lie with the biology of their hosts. As far as is known, these flies all parasitize crickets or katydids (Orthoptera: Ensifera), which produce their calling songs at night (Lehmann 2003). Thus, female ormiines must also search for their hosts nocturnally. However, like sarcophagids, males of many tachinid species also depend on visual cues for finding mates (Wood 1987), and this requirement might preclude them from using host calls as nocturnal mate finding cues. Indeed, there is evidence that some ormiines use hilltopping to locate mates (R.C. Lederhouse et al. 1976, Burk 1982a). As an aside, several authors have posited that the ormiine ear might also function to evade bat predation (Robert et al. 1992, 1996), and while there is experimental evidence supporting this hypothesis for females of one species, *Ormia ochracea* (Bigot) (Rosen et al. 2009), the behavioral response of males to bat ultrasound has not been reported.

There are no obvious explanations for the absence of acoustic mate finding in *Emblemasoma auditrix* (Shewell), the only other species of *Emblemasoma* for which any detailed life history information is available. As with *E. erro*, female *E. auditrix* hunt for host cicadas acoustically, and the host cicadas for both species are active during the day (Soper et al. 1976, Lakes-Harlan et al. 2000). Despite thorough investigation of multiple hypotheses, including the use of host signals to find mates, Lakes-Harlan et al. (2014) were unable to identify any adaptive function for hearing in male *E. auditrix*. The mating system of *E. auditrix* is not well understood, but it appears to be a variant of hilltopping behavior in which males aggregate in patches of vegetation and use visual cues to pursue potential mates (Lakes-Harlan et al. 2014).

Although they both host on cicadas, *E. auditrix* and *E. erro* are in several respects quite different from one another. *E. auditrix* is highly specialized and only attacks a single cicada species, *Okanagana rimosa* (Say) (Lakes-Harlan et al. 2000). *Emblemasoma erro*, on the other hand, has a broader host range, and its hosts usually have a considerably longer seasonal presence than *O. rimosa* (Chapter 4). *E. auditrix* is univoltine while *E. erro* in the central Great Plains is multivoltine (Stucky 2015). It seems plausible that these contrasting life history traits could favor one mate finding strategy over another, but the extent to which they, or other factors, might have influenced the evolution of these flies' mating systems is not known.

*E. erro*'s use of its hosts' communication signals as a mate finding cue appears to be unique among the few well-studied species of acoustic parasitoids, but there is convincing evidence that some species of non-acoustic eavesdropping parasitoids also use this strategy. Four species of tachinid flies (*Euclytia flava* (Townsend), *Gymnosoma rotundatum* (Linnaeus), *Hemyda aurata* Robineau-Desvoidy, and *Trichopoda pennipes* (Fabricius)) locate their hosts by eavesdropping on the olfactory sexual signals of adult male stinkbugs, and males of all four

species have been captured on host pheromone-baited traps or on cages housing live male host insects (Mitchell and Mau 1971, Harris and Todd 1980, Aldrich 1985, Jang and Park 2010). Males of these parasitoids have been observed stationing themselves near a pheromone source and exhibiting territorial behaviors or pursuing other flies that arrive, and *T. pennipes* is known to mate near potential hosts (Harris and Todd 1980, Aldrich 1985, Higaki and Adachi 2011). Another example is provided by phorid fly *Apocephalus paraponerae* Borgmeier which finds its hosts by eavesdropping on the alarm pheromone of injured bullet ants, *Paraponera clavata* (Fabricius) (Feener et al. 1996). It is not entirely clear whether this is truly a case of eavesdropping, though, because the chemicals involved might not play a communicatory role in *P. clavata* as they do in other ants (Hermann et al. 1984). In any case, male flies are apparently attracted by the same volatile cues that females use to find hosts, and mating has been observed on or near potential hosts (Feener et al. 1996). Some parasitoid wasps that eavesdrop on pheromones have also adopted this strategy. For instance, both males and females of the pteromalid *Tomicobia tibialis* Ashmead are attracted to the sex/aggregation pheromones of their *Ips* spp. bark beetle hosts, and mating occurs after the wasps arrive at a pheromone source (Bedard 1965, Rice 1969). A few other cases in which male eavesdropping parasitoids have been attracted to host pheromones have also been reported, but without direct observation of mating or sexual behaviors following chemotaxis (Kennedy 1979, 1984, Dixon and Payne 1980, Zaki 1985, Micha and Wyss 1996, Gabryś et al. 1997, Aldrich et al. 2006, Benelli et al. 2014). In some studies, male behavior is difficult to interpret because it is not always clear if the host signal by itself is attractive to males or if females that arrive at the signal source are subsequently responsible for attracting males.

Long-distance sexual advertising signals, such as the calls of male cicadas, are usually species-specific and provide a powerful premating reproductive isolating mechanism because females are only attracted to the calls of conspecific males (Alexander 1967). When parasitoids such as *E. erro* couple their mate seeking behavior to the species-specific calls of their hosts, then those calls could end up promoting reproductive isolation for the parasitoid, too. More generally, this phenomenon could arise whenever parasitoids link mate finding to any source of information that helps maintain reproductive isolation in their hosts. Long-range sexual signals are only one of many possibilities. For example, adults of the fruit flies (Tephritidae) *Rhagoletis mendax* and *R. pomonella* use olfactory cues from their preferred host fruits – blueberries and apples, respectively – to find oviposition sites and mating opportunities, and this appears to be an important premating isolating mechanism (Feder and Bush 1989). These flies are attacked by the specialist parasitoid wasp *Diachasma alloeum*, which is also attracted to, and mates near to, the preferred fruits of its host flies (Stelinski and Liburd 2005). Moreover, male and female *D. alloeum* that emerge from *R. mendax* or *R. pomonella* are much more strongly attracted by the preferred fruit (either blueberries or apples) of the host species from which they emerged, which suggests that there could be two distinct "host races" of *D. alloeum*, isolated in part by a dependence on olfactory cues that is convergent with their hosts (Stelinski and Liburd 2005). For *E. erro*, more thorough investigation of signal preferences and host ranges will be needed to evaluate how strongly mating behavior might be linked with host fidelity.

Every summer, the grasslands of the central Great Plains ring with the calls of male *Neotibicen dorsatus* trying to attract female cicadas. By producing their calls, these male cicadas unwittingly betray their locations to female *Emblemasoma erro* searching for hosts for their offspring. The results of this study strongly suggest that the acoustic signals of *N. dorsatus* serve

yet another purpose in *E. erro*'s life cycle. For these flies, the call of *N. dorsatus* is also a "love song" that both male and female *E. erro* exploit as a means for finding mating opportunities. *E. erro* and olfactory eavesdroppers with analogous mate-finding behaviors clearly demonstrate that for at least some eavesdropping parasitoids, insect communication signals do not merely provide an efficient way to find potential hosts – they provide an efficient way for these parasitoids to find each other, too.

# CHAPTER 4

## TO LISTEN FOR ONE HOST SPECIES OR MANY? SIGNAL PREFERENCES AND HOST RANGE OF *EMBLEMASOMA ERRO*

### 4.1 Introduction

The cues that a parasitoid uses to locate its hosts are likely to play an important role in determining the parasitoid's host range (Salt 1935, 1938, Shaw 1994, Stireman and Singer 2003, Stireman et al. 2006). Potential hosts that would otherwise be suitable for a parasitoid might be avoided entirely if they do not sufficiently match a parasitoid's host-finding behavior. After all, a parasitoid cannot exploit potential hosts that it cannot "see." Many other factors are also important in determining host range, of course. Phenology and biogeography determine which species are physically available as potential hosts for a parasitoid at a particular time and place. Physiological compatibility, host defenses, and body size determine whether these potential hosts are suitable for a parasitoid to complete its development (Vinson and Iwantsch 1980b, Godfray 1994). But host-searching behaviors ultimately determine which species in a given environment will be available to a parasitoid as potential hosts and which will be ignored.

Parasitoids therefore face a complex optimization problem. Ideally, they should be able to find and exploit as many suitable host species as possible, as quickly as possible, while minimizing time and energy spent in fruitless searching and in pursuit of unsuitable hosts. Different host finding strategies will often require a compromise between these two goals. For example, generalist, nonspecialized host searching behaviors might allow a parasitoid to exploit a wide variety of host animals, but could also result in wasted time and energy due to relatively inefficient searching, or even wasted eggs or larvae if unsuitable hosts are attacked. On the other hand, highly specialized host finding behaviors could allow for fast, efficient location of

high-quality hosts, but at the expense of a small host range and the inability to exploit other suitable hosts in the environment (see, e.g., Morehead and Feener 2000).

The trade-offs of a highly specialized host searching strategy could be especially significant for parasitoids that find their hosts by eavesdropping on other insects' sexual communication signals (see Chapter 1), because long-range sexual signals are usually species-specific and highly divergent among related species (Walker 1964a, Alexander 1967, West-Eberhard 1984). Thus, detecting and responding to these signals requires carefully tuned sensory receptors and signal recognition mechanisms that might be useful for finding only one or a small number of potential host species (Haynes and Yeargan 1999). This reasoning suggests that eavesdropping parasitoids that exploit species-specific communication signals will often have signal recognition mechanisms similar to those of their hosts and correspondingly narrow host ranges.

On the other hand, host signal recognition by eavesdropping parasitoids is obviously subjected to different selective pressures than intraspecific signal recognition by the hosts themselves. Whereas intraspecific sexual signaling and reception by hosts is expected to be strongly selected to avoid confusion with sympatric species (Alexander 1960, Alexander et al. 1997), signal recognition by eavesdropping parasitoids need not necessarily be so constrained. Instead, a parasitoid might be able to detect and exploit multiple host species with divergent communication signals by recognizing only the signal characteristics that those host species have in common.

These ideas have been most thoroughly investigated for parasitoids that eavesdrop on acoustic communications. Acoustic eavesdropping is an almost ideal system for testing hypotheses about signal preferences and host ranges, because species-specific recognition of

insect acoustic signals depends on a small suite of basic acoustic properties that are reasonably easy to measure: signal amplitude, carrier frequency, and temporal structure (which may involve either amplitude or frequency). Furthermore, these properties can be experimentally manipulated, and in comparison to pheromone-based communication, they make for a relatively well-defined signal space. For these reasons, I also focus on acoustic eavesdropping in this chapter.

Most evidence gathered so far suggests that acoustic eavesdropping parasitoids fit the prediction of highly specialized signal preferences and small host ranges, but detailed information about signal recognition is currently available for only two species of acoustic parasitoids. The first, *Emblemasoma auditrix* (Shewell) (Diptera: Sarcophagidae), parasitizes cicadas (Hemiptera: Cicadidae) (Soper et al. 1976), and the second, *Ormia ochracea* (Bigot) (Diptera: Tachinidae), parasitizes crickets (Orthoptera: Gryllidae) (Cade 1975). Both parasitoids have highly restricted host ranges: The cicada *Okanagana rimosa* (Say) is the only known host of *E. auditrix* (Lakes-Harlan et al. 2000, Köhler and Lakes-Harlan 2001), and even though *O. ochracea* parasitizes at least eight species of cricket (*Gryllus* spp. and *Teleogryllus oceanicus* (Le Guillou)) throughout its wide geographic range (Cade 1975, Walker and Wineriter 1991, Zuk et al. 1993, Wagner 1996, Hedrick and Kortet 2006, Gray et al. 2007), it appears to specialize on only one host species at any given locality (Walker 1986, 1993, Gray et al. 2007, Thomson et al. 2012). In accord with their narrow host ranges, the signal recognition mechanisms of both parasitoids are tuned primarily for detecting single host species. Both *E. auditrix* and *O. ochracea* use all three basic acoustic properties to identify their hosts (Walker 1993, Wagner 1996, Gray and Cade 1999, Lakes-Harlan et al. 2000, Köhler and Lakes-Harlan 2001, Wagner and Basolo 2007). In contrast, there is some evidence that a third species of acoustic parasitoid,

the tachinid *Therobia leonidei* (Mesnil), might have a relatively broad acoustic search template in comparison to *E. auditrix* and *O. ochracea*, but the signal preferences of *T. leonidei* are poorly understood (Stumpner and Lakes-Harlan 1996, Lehmann and Heller 1998).

Here, I investigate the relationship between signal preferences and host range for *Emblemasoma erro* Aldrich, which is, like *E. auditrix*, an acoustically orienting parasitoid of cicadas (Stucky 2015). In the central Great Plains of North America, the cicada *Neotibicen dorsatus* (Say) is a major host for *E. erro*, with infection rates among male cicadas exceeding 50% in some populations (Stucky 2015). However, during preliminary studies of the biology of this fly, I found that *E. erro* infects at least two other sympatric cicada species: *Neotibicen dealbatus* (Davis) and *N. pruinosus* (Say). These three species have calls that differ substantially in temporal structure but are at least somewhat similar in terms of frequency and amplitude, which led me to hypothesize that, unlike *E. auditrix*, *E. erro* might rely primarily on frequency and amplitude for host detection rather than a call's temporal structure, and could therefore have a much broader overall host range than *E. auditrix*. Alternatively, *E. erro* might depend on temporal elements of the call of *N. dorsatus* for long-range host detection. If this were correct, then cicada hosts besides *N. dorsatus*, such as *N. dealbatus* and *N. pruinosus*, would be infected only when they were opportunistically detected by visual cues at close range. Discriminating between these alternatives requires understanding which acoustic features are most important to *E. erro* when searching for hosts.

An additional goal of this study was to examine the signal preferences of both male and female *E. erro*. If, as previous work has suggested (see Chapter 3), male *E. erro* respond to the calling songs of hosts as a means for finding mates, then their signal preferences should be convergent with those of female flies. I report the results of experiments designed to test all of

these hypotheses by answering three main questions: First, which acoustic properties are most important in attracting *E. erro*, and what predictions about *E. erro*'s host range do they suggest? Second, are the signal preferences of female and male flies the same? Third, what is the actual host range of *E. erro* across a large geographic area, and does it match the predictions from the analysis of signal preferences? After answering these questions, I compare the signal preferences and host range of *E. erro* to other acoustic parasitoids and discuss what factors might account for different outcomes in the evolution of eavesdropping parasitoid host ranges.

## 4.2 Methods

### *Acoustic terminology*

In this chapter, I use the term "pulse" in the same sense as Broughton (1963) and Morris and Walker (1976). Application of the term is somewhat subjective, but usually poses little practical difficulty when analyzing cicada calling songs.

The term "chirp" is used to denote the basic unit of temporal structure in the calling songs of some cicadas, such as *N. dorsatus*. A single chirp in a calling song is defined as a first-order assemblage of pulses that is clearly separated by silence from the rest of the audio signal. Therefore, a calling song that is comprised of repeated chirps has regularly spaced silences that separate each chirp from the others. "Chirp", as used in this chapter, is equivalent to the term "pulse group" (PG) defined in Stucky (2015). An example is provided in Figure 4.1, which illustrates the chirp structure of the calling song of *N. dorsatus*.

In contrast, an "amplitude burst" (AB) is a single group of high-amplitude pulses in a continuous amplitude-modulated pulse train. ABs are not separated by silence from the rest of the signal. More detailed discussion, and illustrations of this term, are provided in Stucky

(2013). Both chirps and ABs can be described in terms of their individual lengths and rates of repetition in a calling song.

The term "syllable" has been used at various times to refer to the acoustic units of cicada calling songs for which the terms chirp and AB are used in this paper (e.g., Sanborn et al. 2002, 2011, Seabra et al. 2006, Cole 2008). In the context of insect bioacoustics, "syllable" has been given a precise definition based on the physiology of the sound-producing mechanism (Broughton 1963, Ragge and Reynolds 1998), but the application of "syllable" to the descriptions of cicada calls has often disregarded this definition because the required physiological information is rarely available for cicadas. Rather than risk the confusion of incorrect (or at least imprecise) usage, I prefer the somewhat more cumbersome terms chirp/PG and AB because their definitions are based purely on the acoustic signal itself and do not require detailed knowledge of how the components of the signal were produced.

### *Signal preferences of* E. erro

Three-way choice experiments of broadcast acoustic stimuli were used to evaluate the signal preferences of *E. erro* in the field. Test signals were broadcast from acoustic live traps designed to capture *E. erro* (Appendix A) and the numbers of flies captured in the traps were used to quantify the flies' preferences.

Because *Neotibicen dorsatus* was the only cicada that had been clearly documented as a major host for *E. erro* (Stucky 2015), this cicada's calling song was used as a model for constructing the test signals. As determined from recordings of 20 different individuals of *N. dorsatus* from six field sites in Kansas, the calling song of *N. dorsatus* is a sequence of chirps with an overall mean peak frequency of 4.31 kHz ($s$ = 0.444), mean chirp length of 20.3 ms

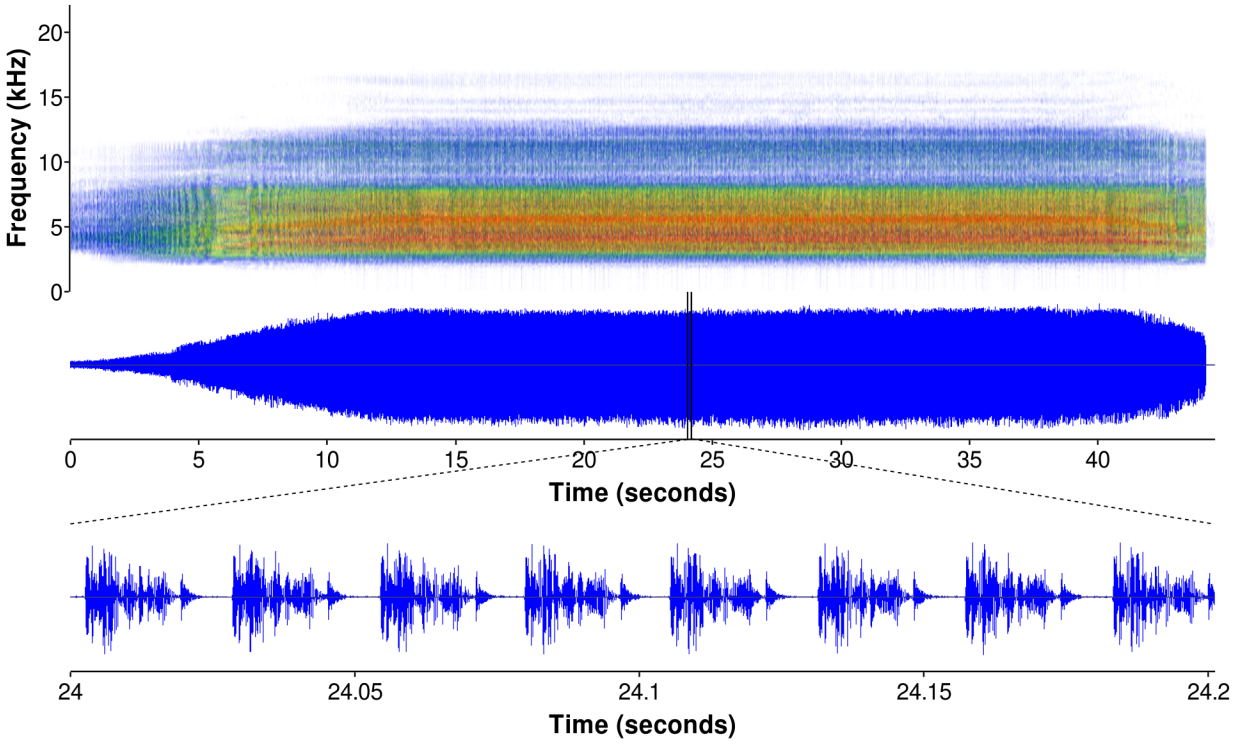


**Figure 4.1** Spectrogram and oscillograms of the calling song of *N. dorsatus*. The spectrogram and upper oscillogram illustrate the entire call. The bottom oscillogram illustrates 8 chirps (pulse groups) from the middle of the call.

($s$ = 1.45), and mean chirp rate of 37.04 chirp/s ($s$ = 2.25) (Stucky 2015). A spectrogram and oscillograms of the calling song of *N. dorsatus* are provided in Figure 4.1.

To represent the natural call of *N. dorsatus*, a synthetic signal that exactly matched the observed mean chirp length and chirp rate was generated from an amplitude-modulated sine wave that matched the observed mean peak frequency. The beginning and end of each synthetic chirp was smoothed to 0 amplitude using the Tukey window function with a 20% taper (Tukey 1967). This signal, hereafter referred to as the "average call", is illustrated in Figure 4.2. Sets of test signals were generated in the same way but varied in either frequency, chirp length, or chirp rate (Figure 4.2). For each set of test signals, one signal was 3 standard deviations above the observed mean for a single acoustic parameter, one was 3 standard deviations below, and the third was the average call in which all parameters matched their observed means. Preliminary

broadcast results revealed that the lowest frequency test signal failed to attract any flies, so a new set of frequency test signals was generated with the observed peak frequency as the lowest test value and the frequencies of the remaining two signals 3 and 6 standard deviations higher (5.64 and 6.97 kHz, respectively). In addition, the frequencies of the average call and all other test signals were also shifted to 5.64 kHz. *N. dorsatus* has a broad-spectrum call with overall peak acoustic power spanning a range from about 3 kHz to 7 kHz (Cole 2008, Figure 4.1), so this new "average" frequency was still a reasonable representation of the true call of *N. dorsatus*. The final test values used for each acoustic property were as follows: *peak frequency* – 4.31, 5.64, 6.97 kHz; *chirp length* – 15.9, 20.3, 24.6 ms; *chirp rate* – 30, 37, 44 chirp/s. A set of varying amplitude test signals was also generated in which the peak amplitudes of the three signals decreased sequentially by 9 dB. The amplitude test signals were otherwise identical and matched the average call in terms of frequency, chirp length, and chirp rate.

For each choice trial, three acoustic traps were placed in an approximate straight line at a field site with 10 meters between neighboring traps. One of the sets of test signals (amplitude, frequency, chirp rate, or chirp length) was randomly chosen, and the three signals of the test set were each randomly assigned to one of the acoustic traps. All three traps were then operated simultaneously for 10 minutes. Next, the three signals were rotated among the three traps and broadcast again for 10 minutes. This procedure was repeated a third time so that by the end of the broadcasting, each signal had been broadcast once for 10 minutes from each trap with 30 minutes of total broadcast time per test signal. All of the flies collected at each signal were counted, sexed, and then released. The broadcast amplitudes of all traps were adjusted to approximately 103 dB at 1 m using a sound pressure level (SPL) meter (RadioShack® model 33-2055 set to C weighting), with the average call serving as the calibration signal. This made

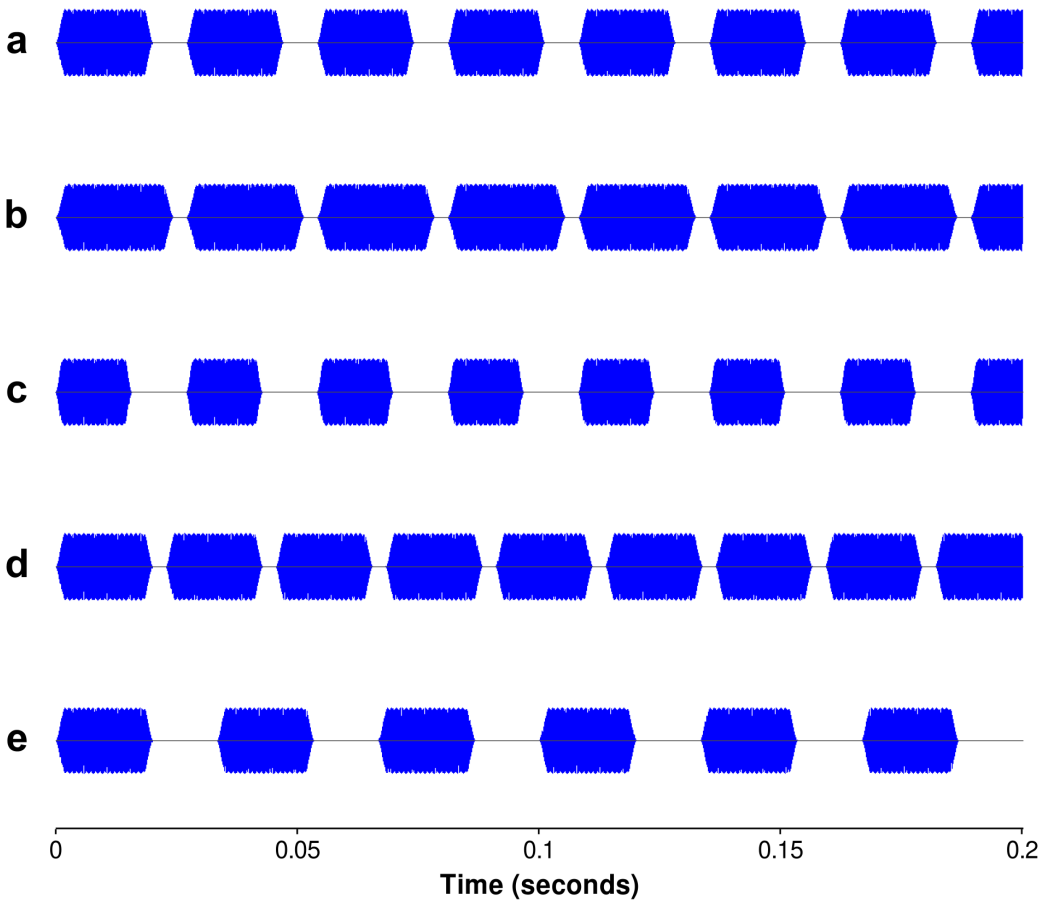


**Figure 4.2** Oscillograms of the first 0.2 seconds of the synthetic test signals: **a**) average call representing mean acoustic parameter values for *N. dorsatus*; **b**) long chirp length; **c**) short chirp length; **d**) high chirp rate; **e**) low chirp rate.

the actual broadcast amplitudes of the varying amplitude test signals approximately 103, 94, and 85 dB.

After completion of one trial, a new set of test signals was randomly chosen from the remaining test sets and the procedure was repeated. No set of signals was used more than once at a given broadcast location. Thus, because flies were released after each trial, the preferences of individual flies could potentially be measured for each of the test signal sets, but no fly could be counted more than once per test set.

Broadcast trials were conducted during August of 2011 at four study sites in Ellsworth, Harvey, McPherson, and Reno counties in central Kansas (sites 7, 8, 9, and 11 in Figure 4.3).

These sites all had qualitatively similar habitat consisting of native midgrass prairie vegetation intermixed with either planted or naturally occurring trees. The mid to late summer species-level cicada diversities at these sites were nearly identical. All four localities had the relatively common species *Neotibicen auriferus* (Say), *N. dealbatus* (Davis), *N. dorsatus*, and *N. pruinosus*, as well as the much less abundant *N. lyricen* (De Geer). Two additional species were documented during this study but were not found at all sites: *Diceroprocta vitripennis* (Say) (Ellsworth County) and *N. superbus* (Fitch) (Harvey, McPherson, and Reno counties). These last two species were rarely heard calling at any of the sites. All broadcasts were conducted during the afternoon when both the cicadas and flies were known to be active (B. Stucky, pers. obs.). Broadcast locations at each field site were separated by approximately 40 m or more to minimize the chances of counting individual flies more than once for a given set of test signals.

The count data for each set of test signals were analyzed separately using log-linear models with the number of flies as the Poisson-distributed response variable (i.e., generalized linear models with log link function and Poisson-distributed response) (Dobson and Barnett 2008). Nested candidate models were compared using the difference of their deviance statistics (i.e., likelihood-ratio tests). Pseudo-$R^2$ values for the fitted models were calculated using the deviance residuals method (Cameron and Windmeijer 1996).

Prior to evaluating the main questions of interest, models that included the variables *signal level* (low, medium, or high), *field site*, and *sex* as main effects along with their pairwise interactions were tested against reduced models that lacked the interaction between *field site* and *signal level* in order to assess whether the effects of signal type varied among the field sites. For all four test signal sets, the interaction between *field site* and *signal level* was not significant (all

*p*-values > 0.05), so field site was ignored and the count data were grouped by signal level for all subsequent analyses.

To test whether the signal preferences of flies differed between females and males, full models with *signal level* and *sex* as main effects along with the interaction between the two were tested against reduced, additive models with only *signal level* and *sex* as main effects. To evaluate whether the different values of the acoustic variables in the test signal sets had an effect on signal attractiveness, additive models with *signal level* and *sex* as main effects were tested against reduced models with only *sex* as the explanatory variable.

Plots of standardized residuals were examined to verify that the final models were appropriate for the data. All statistical analyses were conducted in R version 3.1.0 (R Core Team 2015). The study site location maps were produced with QGIS version 2.2.0 (QGIS Development Team 2014).

### *Host range of* E. erro

The probable host range of *E. erro* was assessed primarily by broadcasting model calls of potential host cicada species in the field and noting whether or not *E. erro* were attracted to the acoustic stimuli. These broadcast surveys were supplemented by observations of *E. erro* responding to calling cicadas in the field and occasional collections of infected cicadas from which parasitoids were reared as described in Stucky (2015).

Model cicada calls for each cicada species were constructed using the same methods described in detail in Stucky (2015) and summarized briefly here. First, calling song recordings from multiple individuals of each cicada species were gathered in the field (16-bit uncompressed PCM audio at a sample rate of 44.1 kHz). For species with calling songs that consisted of repeated chirp or AB elements, recordings were analyzed to determine peak frequency, chirp or

AB length, and chirp or AB rate. Most species with calls that lacked a distinct chirp or AB structure instead had calls with regular frequency modulations, and in these cases, calls were analyzed to determine peak frequency and frequency modulation rate. Species whose calls had neither clear chirp or AG structure nor regular frequency modulations were analyzed using peak frequency alone. A few species had calls with strongly multimodal power spectra, and in these cases two or more peak frequencies were determined. The mean values of all measured acoustic variables were calculated for each cicada species, and a single model signal was constructed that was as close as possible to each species' observed mean.

Model calling songs were constructed for 18 cicada species from 5 genera: *Cacama valvata* (Uhler), *Diceroprocta eugraphica* (Davis), *Hadoa chiricahua* Davis, *H. duryi* Davis, *H. inaudita* Davis, *H. townsendii* (Uhler), *Neocicada hieroglyphica* (Say), *Neotibicen auletes*, *N. auriferus*, *N. dealbatus*, *N. dorsatus*, *N. lyricen*, *N. pronotalis* Davis, *N. pruinosus*, *N. resh* (Haldeman), *N. superbus*, *N. tibicen* (Linnaeus), and *N. tremulus* Cole. Calling songs from a minimum of four different individuals were recorded for each species, with the exception of *N. auletes* (Germar), for which only three individuals could be recorded.

Model calls were broadcast at a total of 30 field sites in Colorado, Kansas, and New Mexico (Figures 4.3 and 4.4) on dates ranging from late May to early September of 2010-2013. To ensure that the assessment of potential hosts was phenologically and biogeographically realistic, the calling song for each cicada species was only tested at field sites where that species naturally occurred, at times of the year that adults of the cicada species were active. Calls were usually broadcast at amplitudes of approximately 102-107 dB at 1 m, and when multiple calls were tested at a single field site, they were always broadcast sequentially from a single speaker, never simultaneously from multiple speakers. Model calls were broadcast continuously for 8 or

more minutes per test, and voucher specimens of attracted flies were collected by hand for identification purposes. Because the primary goal of this portion of the study was to investigate the potential host range of *E. erro* over a large geographic area for as many cicada species as possible, logistical constraints made it impossible to attempt any sort of comparatively meaningful quantification of the relative attractiveness of the various test signals. Instead, the data reported here are limited to presence/absence of *E. erro* at model call broadcasts for each cicada species (presence indicates that 3 or more *E. erro* were attracted by a signal). In order to reduce the possibility that negative broadcast records were due simply to random chance or

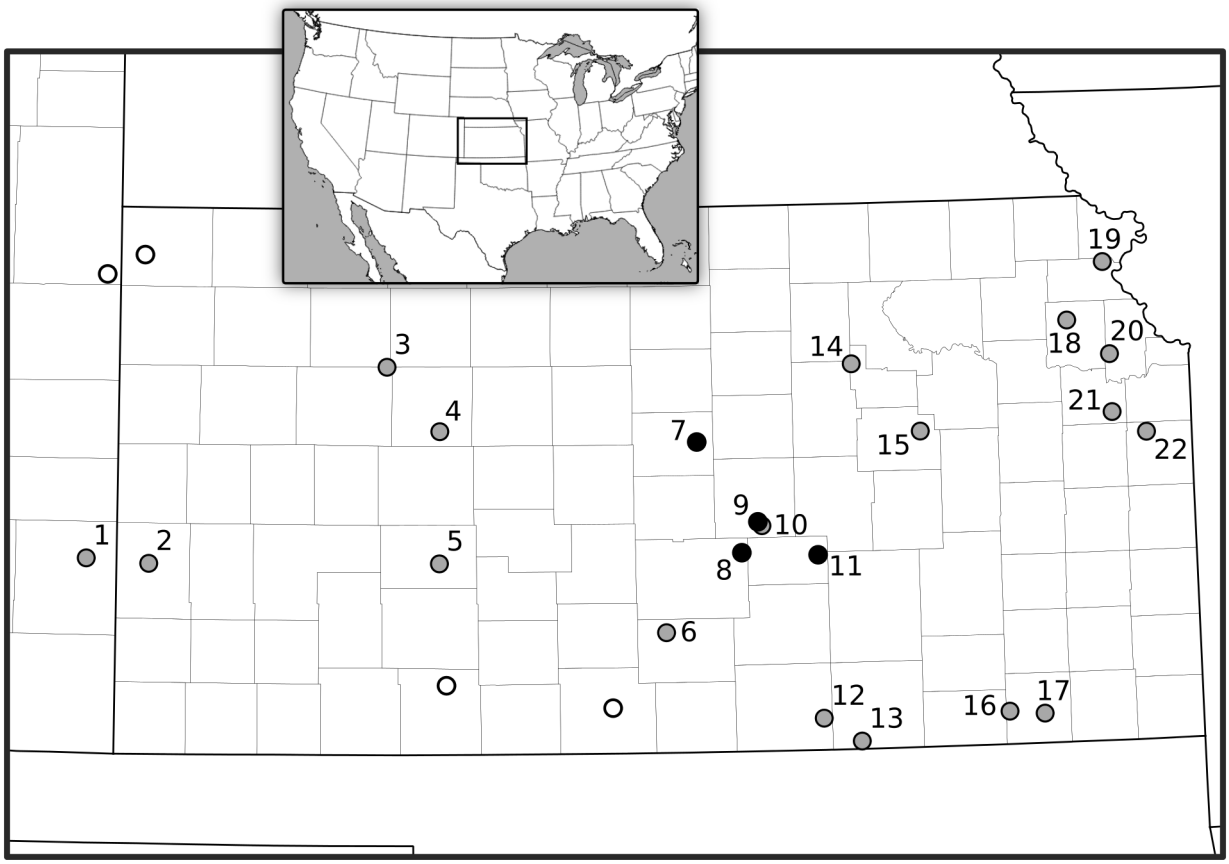


**Figure 4.3** Locations of study sites in Kansas and Colorado. Site numbers are referenced in Table 4.3. Black circles indicate the study sites at which the signal preference choice experiments were conducted, gray circles indicate sites at which *E. erro* were attracted to one or more cicada calling songs, and white circles indicate surveyed sites at which no *E. erro* were collected.

insufficient sampling effort, species with calling songs to which no flies were attracted were always tested multiple times on at least two different days.

***Phonotaxis of individual flies***

The experiments described thus far were designed to assess the aggregate phonotactic behavior of the *Emblemasoma* at a given location. If the results of these experiments suggest that *E. erro* has a broad host range and a generalized acoustic search template, interpretation could be complicated by the possibility that "*E. erro*" is actually a complex of cryptic species, each of which is highly specialized to find only one or a few cicada hosts with very similar mating calls.

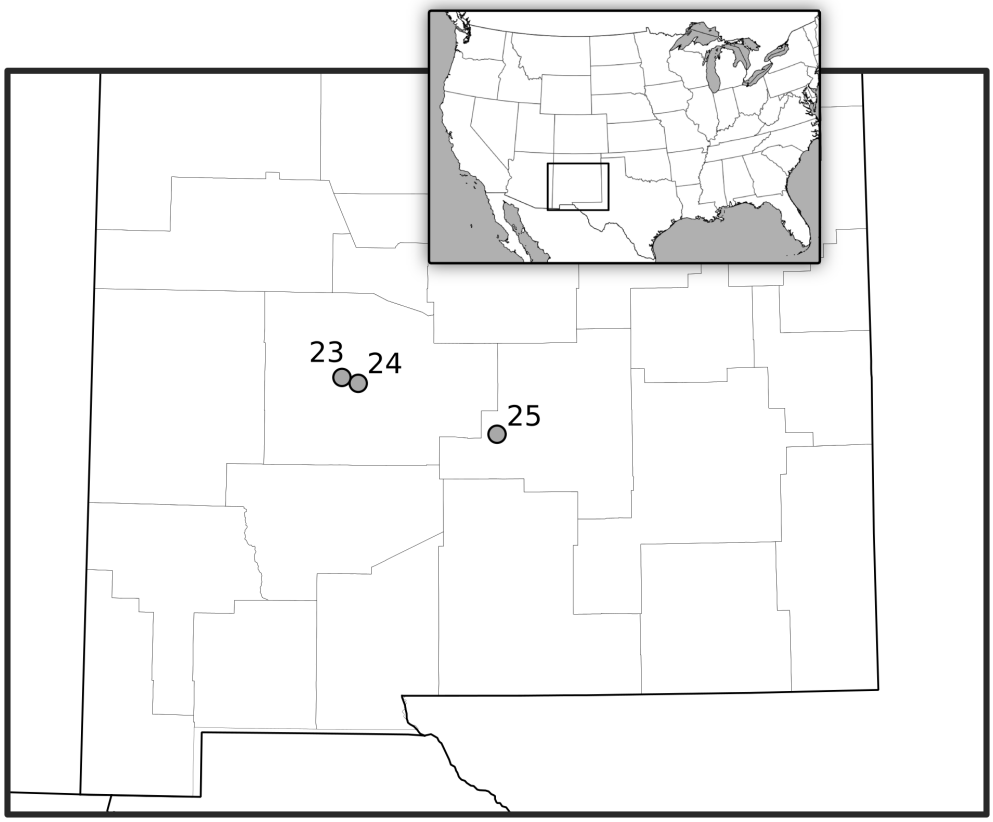


**Figure 4.4** Locations of study sites in New Mexico. Symbology is the same as for Figure 4.3.

To account for this, an additional experiment was conducted to test whether individual *E. erro* are attracted by multiple cicada species with highly divergent mating calls. *E. erro* test subjects were obtained either by broadcasting the model *N. dorsatus* call in the field, as described above, or, in a few cases, by capturing flies that were attracted to calls produced by male *N. dorsatus* inside of a cloth screen cage placed on natural vegetation in the field (the cicadas inside the cage were not visible to the flies). Each tested fly was, therefore, already known to have found the mating call of *N. dorsatus* attractive, so further testing was designed to assess if the call of another cicada species, *N. pruinosus*, was also attractive. *N. pruinosus* was chosen because it was already known that at least some *Emblemasoma* identified as *E. erro* were attracted by its call and use it as a natural host (see the results below), and its calling song is highly divergent from that of *N. dorsatus*: The mating call of *N. dorsatus* has a distinct pulse group structure with no frequency modulations, while the call of *N. pruinosus* is a continuous buzz with no pulse group structure whatsoever but with regular frequency modulations. The only substantial similarities between the calls of these two species is that they are both loud calls produced by large cicadas with broadly overlapping frequency bands of peak acoustic power output.

The behavior of each fly was tested individually using the following procedure. First, the test fly was released into a cylindrical cloth mesh cage approximately 66 cm long and 46 cm in diameter. The cage was placed horizontally on a table outdoors, and all testing was conducted during times of the day and in weather conditions in which cicadas and flies were likely to be naturally active. After release into the cage, the fly was given at least 1 minute to acclimate before testing began. In every case, the fly eventually settled on the top of the cage at one of the circular ends. A small speaker (Samsung Galaxy Player 4.0, Samsung Electronics Co., Ltd.) was

positioned near the cage's bottom, at the end of the cage opposite from where the fly was perched, with the speaker pointing upward into the cage. With this arrangement, phonotaxis to the speaker required the fly to traverse the horizontal length of the cage (i.e., about 60 cm) and descend vertically more than half the height of the cage (i.e., at least 23 cm) to reach the sound source. The model call representing *N. pruinosus* was played from the speaker for 1:00 minute or until the fly completed phonotaxis to the speaker, whichever occurred first. If, after 1:00 minute of playback, the fly appeared to still be engaged in active phonotaxis, the playback was continued until the fly either reached the speaker or lost interest. At the end of the playback, the fly's response was scored as either positive or negative. A positive response was recorded if, at a minimum, the fly traversed the length of the cage to reach the side with the speaker and then arrested its movement near the speaker with its head oriented toward the sound source. If a fly showed no response after the first *N. pruinosus* broadcast, the fly was allowed to rest for several minutes and then the broadcast was repeated once. After testing was completed with the *N. pruinosus* playbacks for each fly, the *N. dorsatus* model call was broadcast using the same procedure as for the *N. pruinosus* broadcasts. Since these flies were already known to have been attracted by the call of *N. dorsatus*, this was intended to serve as a control to verify whether each fly was phonotactically active at the time it was tested.

## 4.3 Results

### *Signal preferences of* E. erro

A total of 172 fly captures were recorded over 78 total trap-hours of broadcasting. The total fly counts for each test signal, as well as the subcounts of males and females, are provided in Table 4.1 and illustrated in Figures 4.5 and 4.6. The results of the statistical analyses are given in Table 4.2.

For all four acoustic variables, there was no detectable difference between the preferences of male and female flies (all $p$-values > 0.1; Table 4.2). Both amplitude and frequency had strong, significant effects on signal attractiveness ($p < 0.001$; Table 4.2, Figure 4.5): The loudest signal caught nearly 10 times as many flies as either of the quieter signals, and the medium and high frequency signals each caught at least 6 times as many flies as the lowest frequency signal. In contrast, neither chirp length nor chirp rate had any effect on fly preference ($p > 0.4$; Table 4.2, Figure 4.6).

**Table 4.1** Total counts of flies captured at each test signal, with subcounts of females and males.

| | Flies captured per test signal (F, M) | | | |
|---|---|---|---|---|
| **Acoustic variable** | **Low** | **Medium** | **High** | **Total** |
| amplitude | 1 (1, 0) | 3 (1, 2) | 29 (20, 9) | 33 (22, 11) |
| frequency | 4 (3, 1) | 28 (19, 9) | 24 (19, 5) | 56 (41, 15) |
| chirp length | 14 (12, 2) | 12 (9, 3) | 19 (12, 7) | 45 (33, 12) |
| chirp rate | 13 (11, 2) | 13 (6, 7) | 12 (8, 4) | 38 (25, 13) |

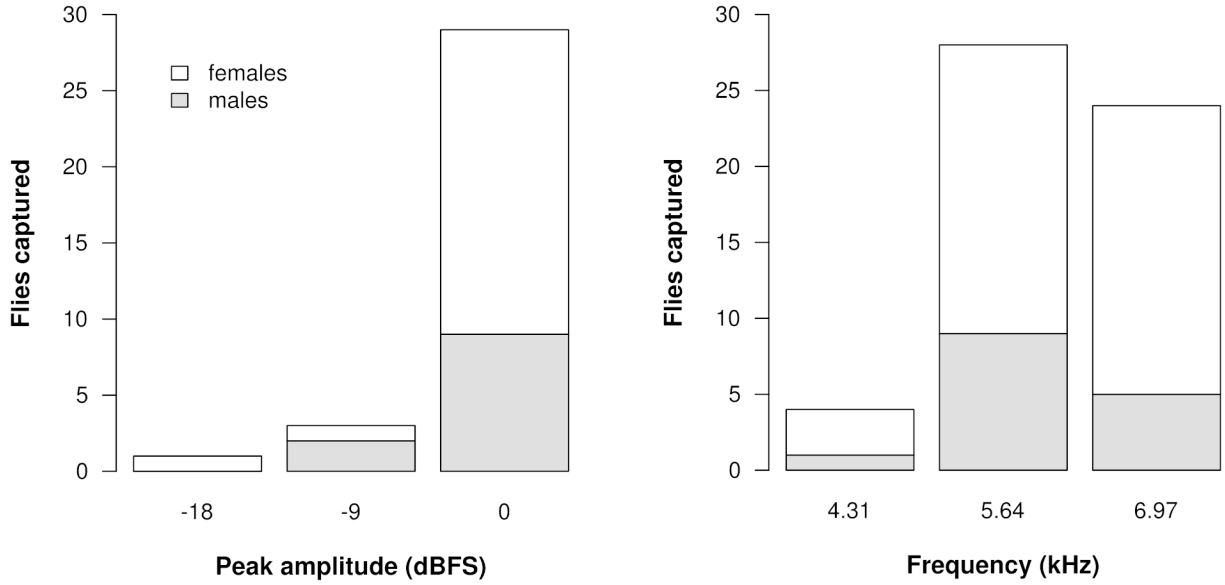


**Figure 4.5** Total numbers of female and male flies attracted to the amplitude and frequency test signals.

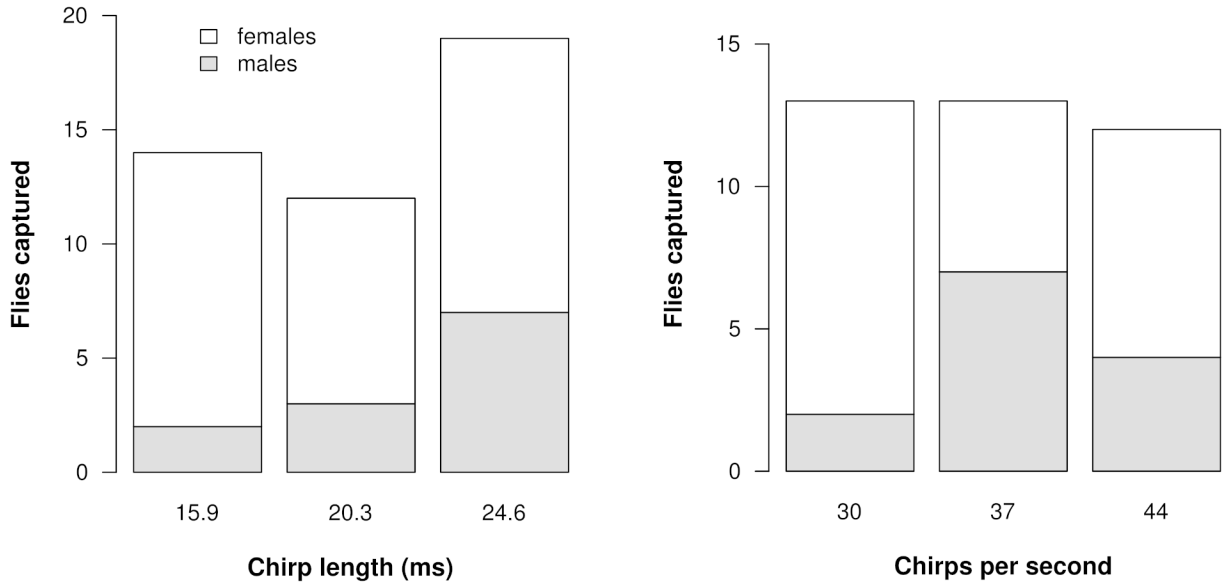


**Figure 4.6** Total numbers of female and male flies attracted to the chirp length and chirp rate test signals.

**Table 4.2** Results of model comparisons for the signal preferences data.

| Acoustic variable | Additive model (sex + signal level) | 1-factor model (sex only) | Model comparisons |
|---|---|---|---|
| amplitude | deviance: 2.27 | deviance: 45.9 | full vs additive: $p = 0.32$ |
| | pseudo-$R^2$: 0.95 | pseudo-$R^2$: 0.075 | additive vs 1-factor: $p < 0.001$ |
| frequency | deviance: 0.86 | deviance: 23.3 | full vs additive: $p = 0.65$ |
| | pseudo-$R^2$: 0.98 | pseudo-$R^2$: 0.35 | additive vs 1-factor: $p < 0.001$ |
| chirp length | deviance: 2.2 | deviance: 3.9 | full vs additive: $p = 0.33$ |
| | pseudo-$R^2$: 0.84 | pseudo-$R^2$: 0.72 | additive vs 1-factor: $p = 0.43$ |
| chirp rate | deviance: 4.44 | deviance: 4.49 | full vs additive: $p = 0.11$ |
| | pseudo-$R^2$: 0.47 | pseudo-$R^2$: 0.46 | additive vs 1-factor: $p = 0.97$ |

### *Host range of* E. erro

Of the 18 model cicada calling songs that were tested, *E. erro* exhibited positive phonotaxis to the calls of 15 species (Table 4.3). In addition, flies were observed performing phonotaxis to or attempting to attack calling individuals of 5 cicada species in the field (Table 4.3). Infected specimens of 6 species were also obtained (Table 4.3). In total, the evidence

suggests that 16 of the 19 cicada species surveyed are likely hosts for *E. erro*. No flies were ever attracted to the model calling songs of *Diceroprocta eugraphica* or *Neocicada hieroglyphica*, and no other evidence was obtained that these cicadas might be hosts for *E. erro*. Table 4.3 also includes the cicada *Quesada gigas* (Olivier), which was reported as a host for *E. erro* in Brazil from a rearing of a single female fly (Lopes 1981).

**Table 4.3** The estimated host range of *E. erro* across all study locations. "*E. erro* attracted to broadcast" indicates whether flies performed phonotaxis to model calling song broadcasts. "*E. erro* attracted to calling cicadas" indicates that flies were observed performing phonotaxis to calling male cicadas. Study site numbers correspond to the maps in Figures 4.3 and 4.4. *The infected *N. resh* is a specimen from the collection of Texas A&M University from which *E. erro* were reared (T. Moore, personal communication). **Literature record from South America recorded by Lopes (1981). *Q. gigas* does not occur in the study area, but is included here for completeness.

| Cicada species | *E. erro* attracted to broadcast | *E. erro* attracted to calling cicada | Infected cicadas collected | Study sites |
|---|---|---|---|---|
| *Cacama valvata* | Y | | | 23,24 |
| *Neotibicen auletes* | Y | | | 14 |
| *N. auriferus* | Y | | Y | 7 |
| *N. dealbatus* | Y | Y | Y | 4,7,8,10,11,13 |
| *N. dorsatus* | Y | Y | Y | 1,2,5,6,7,8,9,10,11,18,20 |
| *N. lyricen* | Y | | | 16,17 |
| *N. pronotalis* | | | Y | 18 |
| *N. pruinosus* | Y | Y | Y | 3,4,6,8,9,10,11,13,14,15,17,19,20, 21,22 |
| *N. resh* | Y | | Y* | 16,17 |
| *N. superbus* | Y | | | 12,13 |
| *N. tibicen* | Y | | | 16 |
| *N. tremulus* | Y | | | 6 |
| *Hadoa chiricahua* | Y | | | 23 |
| *H. duryi* | Y | | | 23 |
| *H. inaudita* | Y | Y | | 25 |
| *H. townsendii* | Y | Y | | 25 |
| *Quesada gigas* | | | Y** | |

***Phonotaxis of individual flies***

A total of 14 *E. erro* were collected in the field at the call of *N. dorsatus* and subsequently tested for their response to the call of *N. pruinosus*. When tested, 6 of the flies showed no response to broadcasts of either *N. pruinosus* or *N. dorsatus*. Of the 8 flies that did perform phonotaxis during the tests, 5 responded to the calls of both *N. pruinosus* and *N. dorsatus*, 2 responded to the call of *N. dorsatus* only, and 1 responded to the call of *N. pruinosus* only. Therefore, of 8 flies that were phonotactically active during the tests, 6 responded to the call of *N. pruinosus*. Flies performed phonotaxis both by making short flights and by walking, and in 10 of the 13 total positive phonotactic responses, the flies eventually traveled all the way to the location of the speaker. Flies never showed any response to the speaker when it was not broadcasting a model cicada call.

## 4.4 Discussion

***Signal preferences of* E. erro**

The results of the field choice experiments were consistent with the hypothesis that *E. erro* locates potential hosts primarily by focusing on the amplitude and frequency components of an acoustic signal, not its temporal structure. Manipulating the chirp length or chirp rate of a model *N. dorsatus* call had little effect on the signal's attractiveness to searching parasitoids, but changes in either amplitude or frequency had a dramatic effect on the numbers of flies that were captured (Tables 4.1 and 4.2, Figures 4.5 and 4.6). Thus, it seems that *E. erro*'s acoustic search template is designed to recognize potential host insects that produce loud, airborne signals with a carrier frequency of about 5 to 6 kHz.

Of course, it is possible that *E. erro* actually does use temporal signal structure to locate hosts and that the tested ranges of these variables were simply too narrow to detect this. This

seems unlikely, though. For both temporal structure variables (chirp length and rate), the minimum and maximum values that were tested exceeded the minima and maxima observed in the sample of 20 recordings of *N. dorsatus* calling songs. If either chirp length or rate were important for *E. erro* in finding *N. dorsatus*, then extreme lengths and rates that rarely or never occur in nature, as were tested in this study, would not be expected to be as attractive to *E. erro* as values that actually match the natural calling song of the host.

The use of amplitude for signal discrimination merits further discussion. Because higher amplitude signals are detectable over a larger area than lower amplitude signals, louder signals would be expected to attract more flies regardless of whether those flies were intentionally choosing them over quieter signals (Forrest and Raspet 1994). Furthermore, perceived loudness decreases with a receiver's distance from a sound source, which means that, without additional spatial information, perceived volume alone is an imperfect indicator of the acoustic power of a calling insect. Nevertheless, if a female fly simultaneously perceives more than one sound source, there is at least one biological reason for her to preferentially choose the signal that sounds the loudest. For cicadas, calling song amplitude scales with body size, such that larger cicadas produce louder calls (Sanborn and Phillips 1995). By choosing the loudest singers, a female fly would be selecting the hosts with the most food resources for her offspring. Determining whether or not female flies are actively choosing louder signal sources requires knowledge of the flies' behavioral response amplitude threshold (Forrest and Raspet 1994), but this information is not yet available for *E. erro*.

There were no detectable differences between the preferences of female and male flies for any of the acoustic variables that were tested, which suggests that both sexes have extremely similar acoustic search templates. This finding is consistent with the hypothesis that male flies

are attracted to host calling songs as a way to find potential mates (see Chapter 3). If male flies use cicadas to find mates, they could maximize their chances of encountering females by preferring the same host signals that females prefer, and this appears to be the strategy of male *E. erro*.

### *Host range of* E. erro

If *E. erro* discriminates potential host signals primarily on the basis of amplitude and frequency, as suggested by the results of the first part of this study, then *E. erro* would be predicted to parasitize cicada species that have calls with relatively similar amplitudes and frequencies but possibly dissimilar temporal structures. Furthermore, insects with acoustic sexual advertisement signals, including cicadas, usually encode most of the species-specific information in the temporal structure of their calls (e.g., Walker 1957, 1973, 2004, Gerhardt and Huber 2002, Walker et al. 2003, Quartau and Simões 2006), so if *E. erro* ignores temporal structure, then its potential host range should be relatively large.

The results of the host range surveys were consistent with these predictions. Over a large geographic area, *E. erro* exhibited positive phonotaxis to the calls of 15 cicada species (Table 4.3). In most cases, the calling songs of these 15 species have very different temporal structures. Some, such as *N. auriferus*, have calls that are more or less continuous trains of pulses with little obvious secondary structure. Some have calls that consist of sequences of pulse groups (e.g., *N. auletes*, *N. dorsatus*) or continuous pulse trains punctuated by amplitude bursts (e.g., *C. valvata*, *H. chiricahua*), with repetition rates that vary widely among the species but with little variation in frequency. Others, such at *N. pruinosus*, have calls with rhythmic changes in frequency but with no temporal pulse group structure. And some species have highly complex calls that

combine rhythmic changes in AB or chirp structure with pulsating frequency and amplitude modulations (e.g., *N. dealbatus*, *H. duryi*).

If a particular temporal feature of an acoustic signal were essential for host location, it seems exceedingly unlikely that the calls of all of these species would have triggered phonotaxis by *E. erro*. They simply have too little in common. Adaptation to the temporal properties of the calling songs of local cicada species is not a convincing explanation, either, because the differences in temporal structure among the calling songs of the cicada species present at a single locality were often just as large as the differences among cicadas that were not sympatric. For example, at three field sites in central Kansas, *E. erro* were attracted to the calling songs of 3 local cicada species with highly divergent temporal call structures: *N. dealbatus*, *N. dorsatus*, and *N. pruinosus* (Table 4.3).

What the calling songs of all of these 15 cicada species do have in common, though, is that they are loud, broad-spectrum signals with their acoustic power spread across a wide range of frequencies that includes those frequencies most attractive to *E. erro*. (i.e., approximately 5.5 – 7 kHz as estimated from the results of the first part of the study). If host finding by *E. erro* does depend primarily on the amplitude and frequency characteristics of an acoustic signal, then all of these species would be readily detectable.

It is not entirely clear why the calling songs of two cicada species, *D. eugraphica* and *N. hieroglyphica*, failed to attract any flies. The power spectra of the calls of these two species show at least some overlap with the frequencies that are attractive to *E. erro*. Farris et al. (2008) also found that the call of *N. hieroglyphica* was not attractive to the unidentified species of *Emblemasoma* that they studied. Perhaps these species truly are unattractive to *E. erro*, but it is

also possible that *E. erro* were simply uncommon or absent at the places and times these cicadas' calling songs were tested.

In any case, *E. erro*'s relatively non-specific signal preferences probably allow it to exploit all, or nearly all, of the large cicada species that are present at any given locality, and they also allow this parasitoid to persist in radically different local cicada communities across a large geographic area. For example, none of the cicada species that were documented as likely hosts in central New Mexico are found at any of the study sites in Kansas and Colorado. Even among the Kansas and Colorado localities, some cicada species that are apparently utilized as hosts at the central and eastern-most study sites, such as *Neotibicen auletes*, *N. lyricen*, *N. resh* and *N. tibicen*, were not found at the field sites located further west.

I have so far presented the results of this study as evidence that *E. erro* hosts on multiple cicada species with calls that meet relatively broad amplitude and frequency requirements, but it is also possible that "*Emblemasoma erro*" is actually a complex of morphologically cryptic species, each of which is a specialist on a different species of cicada. In that case, the apparent host range of *E. erro* would merely be an artifact of incomplete taxonomic knowledge. This scenario is certainly possible, but unlikely. First, the results of the phonotactic behavior tests of individual flies demonstrate unambiguously that at least some individuals of *E. erro* are attracted to the calling songs of multiple cicada species that have calls with highly divergent temporal structures but similar amplitudes and frequency components. At the very least, this suggests that even if *E. erro* really is a complex of species, the conclusions above are still probably valid for at least some of those species. Second, analysis of DNA sequence data for two mitochondrial gene fragments suggests that individuals of *E. erro* from Kansas and Colorado that were collected at the calling song of a particular cicada species were not necessarily more closely related to each

other than they were to *E. erro* collected at the calls of other cicada species (B. Stucky, unpublished), which is not consistent with the hypothesis that *E. erro* is actually a complex of cryptic specialist species.

***When is species-specific signal recognition advantageous?***

As discussed in the introduction, detailed signal preference information is available for only two other species of acoustic parasitoids, *Emblemasoma auditrix* and *Ormia ochracea*, and both of these species have signal preferences that are tuned primarily for detecting a single host species at a given geographic locality. Broader host range seems to be a clear benefit to *E. erro*'s rather generalized signal preferences, so why have *E. auditrix* and *O. ochracea* forgone this strategy in favor of recognizing the call of a single host species? There are no obvious answers to this question, but three (not necessarily mutually exclusive) hypotheses are discussed here. Highly specialized acoustic search templates might be 1) an adaption to improve search efficiency and avoid bad hosts; 2) a consequence of physiological or morphological specialization on a single host; or 3) a response to competition with other parasitoids.

First, single-species acoustic recognition could be an adaptation to maximize the efficiency of host searching and avoid bad hosts. Parasitoids such as *E. auditrix* and *O. ochracea* that listen for species-specific signal information probably have very little risk of wasting time and energy pursuing acoustic cues that do not lead to suitable hosts (assuming a biologist with a loudspeaker is not running any experiments nearby). Whether selection will favor search specialization should depend on how effectively a more general search strategy, such as *E. erro*'s, can distinguish desirable signals from other acoustic noise. In other words, if a parasitoid relies on relatively nonspecific acoustic search criteria, such as carrier frequency, how often will the parasitoid mistakenly follow "false positive" signals that lead to unsuitable hosts?

More thorough acoustic surveys of individual study sites will be needed to answer this question, but some informed speculation is still possible. For parasitoids that attack cicadas, the risk is probably relatively low. Cicadas mostly call during the day, and across much of the North American range of *E. auditrix* and *E. erro*, there are very few or no non-cicada insects that produce acoustic calls of comparable amplitude and frequency during the day. Furthermore, cicada calling song amplitude and frequency are both correlated with body size, such that larger cicadas have higher amplitude, lower frequency calls (Bennet-Clark and Young 1994, Sanborn and Phillips 1995). This means that even though *E. erro* is sympatric with several very small species of cicada that would probably be unsuitable hosts because of their body size, such as *Beameria venosa* (Uhler), *Cicadetta calliope* (Walker), and *Pacarina shoemakeri* Sanborn and Heath, *E. erro* is unlikely to detect these cicadas because their calling songs are of relatively low amplitude and most of their acoustic energy is at frequencies above 10 kHz (Beamer 1928, Sanborn et al. 2009, 2012, B. Stucky, unpublished data). In comparison, nocturnal parasitoids such as *O. ochracea* that attack crickets or other night-calling Orthoptera might require more specialized search strategies. Across its range in North America, *O. ochracea* is sympatric with many species of field crickets (Gryllinae), bush crickets (Eneopterinae), tree crickets (Oecanthinae), and katydids (Tettigoniidae), at least some of which have calls that overlap in frequency with those of *O. ochracea*'s field cricket hosts (Capinera et al. 2004, Elliott and Hershberger 2007). Also, call frequency is not as reliable an indicator of body size for ensiferan Orthoptera as it is for cicadas. For example, crickets commonly have calls that are of much lower frequency than other orthopterans of similar body size (Gerhardt and Huber 2002). All of this suggests that *O. ochracea* must cope with a complex acoustic landscape in which generalized acoustic search criteria, such as signal frequency, might not perform well.

A second hypothesis is that phonotactic specialization on a single host species corresponds with physiological or morphological specialization on that host. *E. auditrix* has evolved morphological and behavioral adaptations that allow it to inject larvae directly into the body of *O. rimosa* by piercing a timbal membrane (Schniederkötter and Lakes-Harlan 2004), and this almost certainly limits its host options. Many other North American cicadas have protective timbal covers that would deter this method of infection, including some cicada species that are sympatric with *E. auditrix*. For *E. auditrix*, then, acoustic recognition of a single host species might be necessitated by specialized infection behaviors that prevent it from exploiting most other potential hosts. In contrast, *E. erro* larviposits directly on the body of its cicada hosts (Stucky 2015), a technique that has no special dependence on the anatomy of any particular cicada species.

For the tachinid *O. ochracea*, a similar explanation of signal specialization seems unlikely. Laboratory rearings indicate that *O. ochracea* larvae can survive on many different species of host cricket besides those it naturally infects, including some that are not closely related to its natural hosts (Mangold 1978, Wineriter and Walker 1990, Walker and Wineriter 1991, S.A. Adamo et al. 1995, but see Thomson et al. 2012), and its method of host infection does not suggest special adaptation to any particular host species (Cade 1975). Furthermore, this fly's ability to exploit different host species across its geographic range (see references in introduction) also suggests that phonotactic specialization is not demanded by fundamental physiological or behavioral constraints on potential host range.

A third hypothesis to explain signal specificity is that competition with other parasitoids has resulted in partitioning of host resources so that *E. auditrix* and *O. ochracea* have mostly specialized on single host species. In general, there is some evidence that interspecific

competition can be an important influence on parasitoid host ranges, but this has been a controversial topic (Godfray 1994). Unfortunately, there is so little information available about the host ranges and biogeography of most *Emblemasoma* that it is difficult to assess possible competition among these insects. Museum collections indicate that at least two other species of *Emblemasoma*, *E. erro* and *E. albicoma* Reinhard, are partially sympatric with *E. auditrix* (B. Stucky, unpublished data). Throughout the geographic area covered by this study, *E. erro* is the only species of *Emblemasoma* that has thus far been collected. However, *E. erro*'s range extends into South America, and the majority of *Emblemasoma* species are found in the Neotropics (Lopes 1988, Pape 1996). It appears, then, that there is at least the possibility of competition for cicada hosts among *Emblemasoma*, but again, so little is known about most of these flies that it cannot even be stated with certainty whether they are parasitoids. As for *O. ochracea*, there are at least six other native species of *Ormia* in North America that might be sympatric with *O. ochracea*, and there is evidence for one or more undescribed species in the southern United States (Sabrosky 1953a, 1953b). Of these six species, at least three are also parasitoids of ensiferan Orthoptera (Nutting 1953, Burk 1982a, Walker et al. 1996). So, compared to *Emblemasoma*, there is stronger evidence for possible competition for hosts among *Ormia*, but much more data will be needed about the host ranges, signal preferences, and biogeography of these species before even tentative conclusions can be drawn.

### 4.5 Conclusions

Previous work on the signal search templates of acoustic eavesdropping parasitoids has suggested that these insects tend to have highly specific signal preferences that depend on a combination of signal amplitude, frequency, and temporal structure for host recognition. The results of this study show, however, that at least some acoustic parasitoids have broad signal

search templates that allow them to detect a large number of potential host species. *E. erro*'s acoustic search strategy explains, at least in part, why it has a much larger host range than its close relative, *E. auditrix*.

This study has taken the first steps toward understanding the acoustic signal preferences and host range of *E. erro*, but many important questions remain. Perhaps most crucially, I only examined the acoustic search template of populations of *E. erro* in central Kansas. How do these populations compare to those in central New Mexico or elsewhere that encounter entirely different cicada communities? Similarly, I documented which cicadas species' calling songs trigger phonotaxis by *E. erro*, but an important next step will be to quantify the relative attractiveness of these hosts. To what extant does *E. erro*, like *O. ochracea*, modify its template for signal recognition to match the local community of potential hosts? In addition, might learning play a role in shaping *E. erro*'s host searching, as has been shown for some other eavesdropping parasitoids (Kaiser et al. 1989, Hedlund et al. 1996, Schöller and Prozell 2002, Huigens et al. 2009)?

Examining the evolutionary malleability of signal recognition in *E. erro* and other acoustic parasitoids, as suggested by several of the preceding questions, could be an especially fruitful direction for future research. When *O. ochracea* was introduced to the Hawaiian islands, where none of its native hosts were available, it was able to quickly shift to a novel host, the cricket *Teleogryllus oceanicus* (Zuk et al. 1993). *O. ochracea* on Hawaii now so completely prefer the call of *T. oceanicus* that they virtually ignore the calling songs of their original host species (Gray et al. 2007). Thus, for at least some species of acoustic parasitoids, natural selection can rapidly alter the criteria for acoustic signal recognition. Because of its expansive geographic range, *E. erro* would be ideal for investigating this phenomenon in *Emblemasoma*.

Detailed analysis of the acoustic search criteria of populations of *E. erro* that exploit different host cicada species could reveal what role, if any, the evolution of signal preference has played in the success of this remarkable parasitoid.

# CHAPTER 5

## EAVESDROPPING PARASITOID FLIES (*EMBLEMASOMA ERRO*) AVOID SUPERPARASITISM BY CHANGING THE COMMUNICATION SIGNALS OF THEIR HOST CICADAS

### 5.1 Introduction

Life is perilous for a larval parasitoid. Even hidden away inside of its host, a parasitoid faces many threats to its survival. Some of these threats originate from the host itself, such as attack by the host's immune system or behavioral responses to impair the parasitoid (Vinson and Iwantsch 1980b, Godfray 1994, Singer et al. 2009). Other dangers are external, such as premature host death due to predation (Brodeur and Boivin 2004). And some threats come from other members of the parasitoid's own species, the most important of which is competition or conflict caused by superparasitism (Salt 1961, Godfray 1994). Superparasitism occurs when a searching female parasitoid adds her eggs or larvae to a host that has already been parasitized by herself or another member of her species.

Because the food resources inside of a host are necessarily finite, superparasitism can have disastrous consequences for juvenile parasitoids. For solitary parasitoids (those species for which only a single parasitoid can develop to the adult stage on a given host), superparasitism is a certain death sentence for all but one of the parasitoids sharing a host (Salt 1961, Godfray 1994). For gregarious parasitoids (those species for which more than one parasitoid can develop to the adult stage on a given host), the consequences of superparasitism may not always be so dire, but parasitoids can still suffer reduced fitness or death due to competition (Salt 1961, Brodeur and Boivin 2004).

Given the severity of superparasitism's consequences, it is not surprising that many parasitoids have evolved mechanisms for avoiding superparasitism. These typically require that

female parasitoids have some way to discriminate between uninfected and infected host animals so that they can refrain from attacking already-parasitized hosts (Salt 1934, 1961, van Lenteren 1981, Godfray 1994, Nufio and Papaj 2001). The exact mechanisms of host discrimination vary among parasitoid species, but host discrimination usually requires that either 1) adult female parasitoids can directly or indirectly detect the presence of a parasitoid inside of a host, such as by sensing parasitoid eggs, larvae, or changes in host physiology due to parasitism; or 2) foraging female parasitoids can leave a marking pheromone on, in, or near parasitized hosts so that these host animals can be identified and avoided by searching female parasitoids (van Lenteren 1981, Godfray 1994, Nufio and Papaj 2001). In some cases, host discrimination mechanisms have become quite elaborate, such as *Cotesia* wasps that use differences in plant volatiles to detect whether potential host caterpillars are already parasitized (Fatouros van Loon et al. 2005).

Regardless of the mechanistic details, all of these host discrimination strategies share a key feature: In every case, the adult female parasitoid is responsible for finding a potential host, determining whether it is already parasitized, and then deciding whether to attack it or ignore it. However, host discrimination by adult females might not be the only option for avoiding superparasitism. Many parasitoids find their hosts by eavesdropping on the intraspecifc communication signals of other insects (Zuk and Kolluru 1998, Haynes and Yeargan 1999, see also Chapter 1), so for these parasitoids, the probability of finding a potential host insect is directly related to that insect's communication signals and signaling behavior. If larval eavesdropping parasitoids could modify their host's communication signals in a way that made their host less attractive or less apparent to foraging female parasitoids, then the larvae could reduce their risk of superparasitism independently of the actions of adult females. This

hypothesis has been discussed in the context of suggestive results from earlier studies (e.g., Soper et al. 1976, Beckers and Wagner 2011, see discussion in section 6.4), but it has yet to be thoroughly investigated for any eavesdropping parasitoid. There is, however, abundant evidence that parasitoids are able to manipulate their hosts for a variety of other purposes (Vinson and Iwantsch 1980a, Godfray 1994, Brodeur and Boivin 2004), and there is no a priori reason to expect that parasitoids should not be able to manipulate their hosts' communication systems, too.

In this study, I test the hypothesis that larvae of the eavesdropping parasitoid *Emblemasoma erro* Aldrich (Diptera: Sarcophagidae) alter the communication signals of their hosts as a means of avoiding superparasitism. *E. erro* is a parasitoid of the cicada *Neotibicen dorsatus* (Say), as well as other *Neotibicen* species, in North America (Stucky 2015, see also Chapter 4). Female *E. erro* locate hosts for their offspring by homing in on the loud, airborne, acoustic mating calls of male cicadas, which they subsequently parasitize by depositing first-instar larvae on the cicada's body. The larvae spend about 4 days developing inside of their host, then leave the host to pupariate once their growth is complete (Stucky 2015).

To test this hypothesis, I answered three main questions. First, does superparasitism have negative fitness consequences for *E. erro*? Second, do the mating calls of male *N. dorsatus* cicadas change after they are parasitized? And third, if the calls of cicadas change after being parasitized, do those changes decrease the cicadas' attractiveness to searching female *E. erro* parasitoids? In addition to answering these questions, I also investigated the physical activity of *E. erro* larvae inside of their host cicadas after infection.

Using a combination of laboratory and field-based methods, I demonstrate that all of these questions may be answered in the affirmative, and that all available evidence indicates that *E. erro* larvae manipulate the communication systems of their hosts to avoid superparasitism. I

then discuss 1) the possible role of signal manipulation in predator avoidance; 2) alternative explanations for host signal changes; 3) the evolution of host signal manipulation; and 3) the evidence for signal manipulation in other species of eavesdropping parasitoids.

## 5.2 Methods

### *Effects of host parasitoid load on parasitoid fitness*

To evaluate the effects of parasitoid load on parasitoid fitness, parasitoid larvae were reared from infected *N. dorsatus* host cicadas with varying parasitoid loads and then allowed to pupariate. The sizes of the resulting puparia were measured and the effects of parasitoid load on puparial size were estimated. Although organismal fitness is notoriously difficult to quantify, for sarcophagids, other dipterans, and insects in general, intraspecific variation in body size is usually strongly related to intraspecific variation in female fecundity and male reproductive fitness, with larger individuals generally being more fit (Kamal 1958, Thornhill and Alcock 1983, So and Dudgeon 1989, Tanaka et al. 1990, Berrigan and Locke 1991, Honěk 1993, Kingsolver and Pfennig 2004, Harrison et al. 2012). Therefore, for this study, parasitoid body size (i.e., puparial size) was used as a proxy for parasitoid reproductive fitness.

The host *N. dorsatus* used for this part of the study included naturally parasitized cicadas captured directly in the field, cicadas voluntarily parasitized by female *E. erro* in captivity, or cicadas that were artificially parasitized by dissecting live first-instar larvae from female flies and applying them to the bodies of healthy, unparasitized cicadas. Adult *N. dorsatus* (both parasitized and unparasitized) were mostly collected in central Kansas, with additional collection sites in western Kansas and eastern Colorado (Figure 5.1). Cicadas were maintained in outdoor cages with live green ash (*Fraxinis pennsylvanica*) tree limbs as a food source. Female *E. erro* were captured at the central Kansas field sites by broadcasting audio signals representing the call

of a male *N. dorsatus*. Captured flies were kept in small mesh cages in the laboratory and provided with sucrose and water *ad libitum*. Parasitized host cicadas were kept in the outdoor cages until they became moribund or died, at which point they were moved indoors to small plastic "emergence" containers to capture all emerging parasitoid larvae. The parasitoid larvae were then kept in the emergence containers until they pupariated. Further details of the methods for capturing *E. erro*, obtaining parasitoid infections in captivity, artificially infecting cicadas in the laboratory, and rearing host cicadas and parasitoids are provided in Stucky (2015).

Once the parasitoid puparia were fully sclerotized, they were placed on a white background with a millimeter-scale ruler and photographed from directly overhead using a 90 mm fixed focal length macro lens. The length and width of the puparia were measured from the

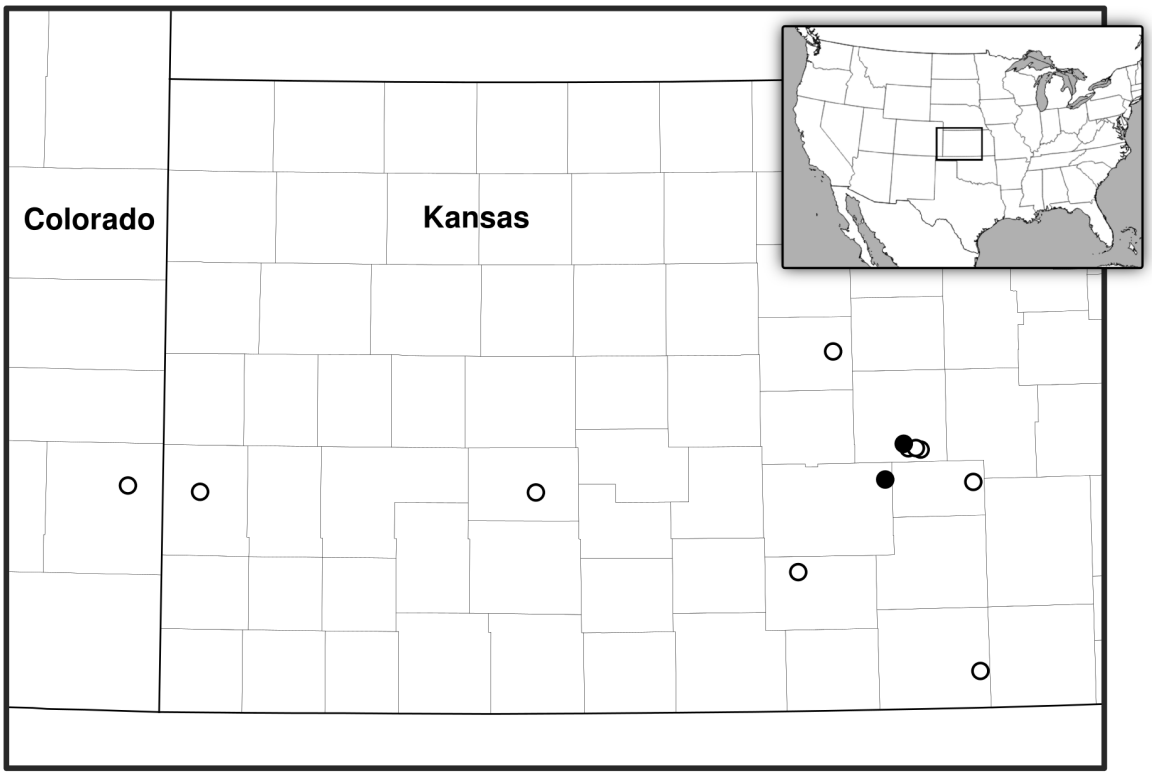


**Figure 5.1** Field sites for collecting *N. dorsatus* (both open and filled circles) and for running the broadcast choice experiments to assay the phonotactic behavior of female flies (filled circles).

photographs using ImageJ (Schneider et al. 2012), and the volume of each puparium was estimated by treating it as a simple cylinder with spherical ends, using the formula $volume = \pi\left(\frac{width}{2}\right)^2\left(length - \frac{width}{3}\right)$. The parasitoid load of each host was taken as the total number of larvae that emerged from the host. Although it is possible that this approach underestimated the parasitoid loads of some hosts by not accounting for larvae that failed to emerge, occasional dissections of host cicadas to check for dead, unemerged larvae suggested that few, if any, larvae were overlooked.

Maximum-likelihood (ML) estimation was used to fit candidate models of the relationship between host parasitoid load and puparial size to the data. To avoid problems with data non-independence for parasitoids that shared the same host, mean puparial size was calculated for each host and was used as the response variable for all models. Because the "true" relationship between parasitoid load and puparial size was not immediately obvious from a scatterplot of the data, a variety of mathematical relationships were modeled, including simple linear functions, various curvilinear functions, and piecewise linear functions. All likelihood calculations assumed that the errors were normal, independent, and identically distributed. For the best-fitting models, diagnostic plots of the residuals were used to assess whether this assumption was reasonable. The relative fits of the candidate models were compared using Akaike's information criterion (AIC; Akaike 1973, 1974) and the Bayesian (or Schwarz) information criterion (BIC; Schwarz 1978) . In addition, the predictive power of the models was assessed by calculating $R^2_{PP}$, the square of the Pearson correlation coefficient between the observed mean puparial volumes and the model-predicted puparial volumes (i.e., the fitted values) (Zheng and Agresti 2000). For ordinary least squares linear regression, $R^2_{PP}$ is

equivalent to the standard coefficient of determination, $R^2$. All statistical analyses for this study were done with R version 3.1.3 (R Core Team 2015).

### *Changes in host calling song after infection*

To assess whether the calling songs of male *N. dorsatus* change after being parasitized by larval *E. erro*, unparasitized *N. dorsatus* were infected with *E. erro* in captivity and the calling songs of the cicadas were acoustically monitored both before and after infection. Methods for capturing *N. dorsatus* and *E. erro* and maintaining both species in captivity are briefly summarized above and described in detail in Stucky (2015); methods for infecting *T. dorsatus* with *E. erro* in captivity are summarized below.

Previous work on the signal preferences of *E. erro* revealed that these flies discriminate audio signals primarily by differences in signal amplitude and frequency (see Chapter 4). Consequently, acoustic monitoring of cicada calls in the present study focused on the amplitude and frequency structure of the calls. The methods for monitoring these acoustic features differed, so they are here presented separately, with the methods for amplitude discussed first.

#### *Amplitude measurements*

Although male *N. dorsatus* will readily produce their mating calls when confined in suitable outdoor cages, accurately measuring the amplitude of the mating calls is challenging for at least two reasons. First, perceived amplitude (e.g., the amplitude detected by a microphone) depends on the distance of the receiver from the sound source, which means that changing the distance between a calling cicada and the microphone will cause the measured amplitude to change, and second, the sound field around a calling cicada is not of uniform intensity, which means that the perceived amplitude can vary depending on how the cicada's body is oriented toward the receiver (Aidley 1969, MacNally and Young 1981, Young 1990, Bennet-Clark and

Young 1998, Michelsen and Fonseca 2000, but see Sanborn et al. 2009). The most straightforward way to avoid these problems is to ensure that the microphone is always the same distance from calling cicadas and that the same part of the cicadas' bodies always faces the microphone each time the amplitude is measured. Unfortunately, if amplitude measurements are to be taken on caged but otherwise unrestrained cicadas, it is very difficult to guarantee that these two conditions are always met. In addition, *N. dorsatus* are only acoustically active for part of the day and then only in suitable weather conditions (Cole 2008, B. Stucky, personal obs.), and even in the best of circumstances, calling activity can be sporadic and unpredictable. Thus, if amplitude measurements depended on voluntary calling behavior, temporally fine-grained monitoring of call changes might be impossible.

One possible solution to all of these problems is to use the cicada's "alarm call" to monitor call changes rather than the mating call. The alarm call (also referred to in the literature as the distress, protest, or stress call/sound/song) is a loud, harsh, sustained shriek that is emitted when cicadas are startled, captured, or annoyed (Myers 1929, Smith and Langley 1978, Sanborn and Phillips 1995). Unlike mating calls, which are only produced when cicadas voluntarily choose to call, cicadas can usually be induced to produce an alarm call whenever they are restrained. Therefore, by monitoring alarm calls instead of mating calls, acoustic call properties could be measured whenever desired and the microphone distance and cicada orientation could be easily controlled.

The chief difficulty with this approach, of course, is that female *E. erro* do not use the alarm calls of cicadas to find their hosts. Therefore, the method is only useful if alarm call amplitude serves as a good proxy measurement for mating call amplitude. To test this, mating

call and alarm call amplitudes were measured for multiple male *N. dorsatus* and analyzed to determine whether alarm call amplitude was a good predictor of mating call amplitude.

All amplitude measurements were recorded in dB SPL (decibels of sound pressure level) (reference 20 μPa) using a RadioShack® model 33-2055 SPL meter. The SPL meter was set to C-weighting and was operated in maximum/minimum measurement mode so that the maximum dB SPL observed during a cicada call could be recorded. A thin, wooden dowel rod was mounted to the SPL meter such that the rod extended 50 cm beyond the meter's microphone. The tip of the dowel rod was positioned next to the calling cicadas, which ensured that all amplitude measurements were made at a uniform distance.

To measure the amplitude of mating calls, male *N. dorsatus* were confined in cylindrical, cloth mesh cages, and the cages were placed outdoors during the usual time of acoustic activity for *N. dorsatus*. Each cicada was observed until it voluntarily produced a mating call while perched on the outer wall of the cage with its ventral side facing the experimenter. While the cicada was calling, the SPL meter was used to measure the maximum amplitude of the call at 50 cm. To measure the amplitude of alarm calls, the SPL meter/dowel rod assembly was mounted to a tripod. Each cicada was held between the experimenter's thumb and index finger at the wing bases and positioned at the end of the dowel rod so that the ventral side of the cicada was facing the SPL meter's microphone. The cicada's body was then gently tapped to induce the cicada to produce its alarm call, and the maximum amplitude was recorded over a period of about 10 seconds or more. Some attempts to induce an alarm call were more successful than others, so this procedure was repeated three times for each cicada and the maximum dB SPL observed during the three attempts was taken as the cicada's alarm call amplitude.

For both mating and alarm calls, amplitude measurements were only kept if the cicada produced a "full" call, which was judged by whether the cicada raised its abdomen to open the opercular gap and expose the tympana, which maximizes the sound output (Young 1990). Also, while measuring the amplitude of a call, either the cicada (for alarm calls) or the SPL meter (for mating calls) was slowly rotated in space to vary the angle between the cicada's ventral surface and the SPL meter microphone (while maintaining a distance of 50 cm). This procedure was intended to compensate for possible non-uniformity of the sound field around the calling insect.

Mating call and alarm call amplitude measurements were taken for 18 male *N. dorsatus*. These data were analyzed using simple linear regression with alarm call amplitude as the predictor variable and mating call amplitude as the response. Diagnostic plots of the standardized residuals were used to verify that the linear regression model was appropriate for the data. The relationship between these variables was highly significant ($p < 0.0001$), and alarm call amplitude was an excellent predictor of mating call amplitude ($R^2 = 0.941$). The slope of the relationship was effectively 1 ($b = 0.920$, 95% confidence interval: 0.798 – 1.043). Furthermore, the data included both parasitized and unparasitized cicadas, so the relationship between mating call and alarm call amplitude did not change once a cicada was infected. Thus, alarm call amplitude is a good proxy for mating call amplitude.

Having established that a cicada's mating call amplitude could be tracked by monitoring the cicada's alarm call amplitude, amplitude changes following infection with *E. erro* were observed as follows. First, the alarm call amplitude of an unparasitized male *N. dorsatus* was measured as described above. Next, the cicada was infected with *E. erro* larvae either by allowing a female fly to voluntarily larviposit on it in captivity or by artificially infecting it in the lab with first-instar *E. erro* larvae that had been dissected from a gravid female fly. Further

details of these infection methods are provided in Stucky (2015). After infection, the cicada's alarm call amplitude was measured at regular time intervals until the cicada either died or ceased to produce an alarm call. With a few exceptions, alarm call amplitude measurements were taken every 6 hours after infection. In addition, most cicadas were also measured more frequently during the first 6 hours after infection to better characterize the amplitude trend early in the infection process. For amplitude measurements that were taken at night when the outside air temperature had cooled, cicadas were first moved into a warmer environment (around 26° C) for several minutes before testing in order to minimize any effects of low temperature on call amplitude. In addition to the experimental group of parasitized cicadas, a negative control group of cicadas was also kept in the same conditions to determine whether call changes detected in the experimental group were due to infection with *E. erro* or some other cause, such as natural call changes as cicadas aged or the effects of living in captivity. The alarm calls of the negative control cicadas were monitored approximately every 24 hours, and all amplitude measurements were taken during times of peak acoustic activity for *N. dorsatus*.

*Frequency measurements*

Frequency measurements were taken by recording cicada calls with a Sennheiser ME67 shotgun microphone with an MZW 67-PRO windscreen (Sennheiser Electronic GmbH & Co. KG, Hannover, Germany) and a Sony PCM-M10 digital audio recorder (Sony Corporation, Tokyo, Japan). All audio was recorded as uncompressed, 16-bit PCM data at a sampling rate of 44.1 kHz. Recordings were made with the microphone between 0.5 and 1 meter from the calling cicada in order to minimize background noise while also avoiding near-field acoustic effects (Michelsen and Nocke 1974, Peterson 1980). Each raw recording was imported into Audacity®

(Audacity Team 2014) and the portion of the call at or near peak amplitude was extracted and run through Audacity's "normalize" filter to standardize the signal strength.

Like most cicadas, *N. dorsatus* has a broad-spectrum mating call with peak acoustic power output across a relatively wide frequency range. Thus, although the overall peak frequency of *N. dorsatus*' mating call is at about 4.3 kHz (Stucky 2015), similar acoustic power output is found in a frequency band from about 3 kHz to beyond 7 kHz (Cole 2008). Because female *E. erro* pay more attention to frequencies in the upper half of this band (see Chapter 4), three frequency-related measurements were taken for each audio recording: the overall peak frequency, the acoustic power at 5.6 kHz, and the acoustic power at 7.0 kHz. (In addition to approximately covering the upper half of the peak frequency band of the *N. dorsatus* mating call, 5.6 kHz and 7.0 kHz also correspond with the test signals used for the female preference experiments in Chapter 4.)

For each call recording, the call's power spectral density was estimated using Welch's method with 512-sample fast Fourier transforms and the Hann window function with 50% window overlap (Welch 1967, Harris 1978). Power values were converted to dB with the maximum power as the reference value; that is, the maximum was placed at 0 dB. The frequency of maximum power (i.e., the maximum peak of the spectral density plot) was used as the estimate of the overall peak frequency, unless additional peaks were within 0.5 dB of the maximum, in which case the frequencies of the maximum and all peaks within 0.5 dB of the maximum were averaged to estimate the overall peak frequency. To estimate the acoustic power at 5.6 and 7.0 kHz, the maximum dB within 500 Hz of each target frequency (i.e., within a band from 5.1-6.1 kHz and 6.5-7.5 kHz) was estimated using linear interpolation of the discrete power spectral density.

As discussed above for measuring cicada call amplitudes, there are multiple difficulties with monitoring the mating calls of cicadas voluntarily calling from inside of cages. Although frequency measurements of cicada calls are certainly less sensitive to measurement distance and microphone orientation than are amplitude measurements, there is still some evidence that the frequency structure of cicada calls might not be uniform at all points around a cicada's body (Michelsen and Fonseca 2000). Therefore, it was hoped that, as with amplitude, the alarm call could be used to monitor the spectral structures of cicada mating calls.

To test this, an experimental procedure similar to that for amplitude was followed. Male *N. dorsatus* cicadas were placed in outdoor, cylindrical, cloth mesh cages during times of normal acoustic activity for *N. dorsatus*. When a cicada voluntarily produced its mating call, the call was recorded and analyzed as described above. Recordings were made with the microphone aimed at, or as close as possible to, each cicada's ventral side. Alarm call production was induced in the same manner as for the amplitude study, with the cicada held about 0.5 to 1 meter in front of the microphone with its ventral side facing the microphone. The alarm calls were recorded and analyzed in the same way as the mating calls. As with the amplitude study, both unparasitized and parasitized cicadas were used.

Mating call and alarm call measurements of all three frequency-related variables – peak frequency, acoustic power around 5.6 kHz, and acoustic power around 7.0 kHz – were taken for 16 male *N. dorsatus*. The relationship between mating call and alarm call was analyzed for each variable using simple linear regression with alarm call value as the predictor and mating call value as the response. Unfortunately, the relationship between alarm call value and mating call value was weak for all three variables. All $p$-values were greater than 0.08, and all $R^2$ values were less than 0.2. The spectral structure of the alarm call of *N. dorsatus* is apparently not a

good proxy for the spectral structure of its mating call, at least not for the variables measured in this study.

Because alarm calls could not be used for the frequency measurements, monitoring the spectral structures of cicada calls before and after infection instead relied on direct measurement of mating calls. Otherwise, the procedure was similar to that used for amplitude. First, the values of all three frequency-related variables were measured for the mating calls of unparasitized cicadas, as described above. Next, the cicadas were infected with *E. erro* larvae either by allowing female *E. erro* to voluntarily infect the cicadas in captivity or by artificially infecting them in the laboratory. After infection, frequency measurements of the mating calls of the parasitized cicadas were usually taken at least once a day, more often if possible, until the cicadas either died or ceased calling. To minimize any variation caused by non-uniform sound fields around the calling cicadas, all mating call recordings were made with the microphone aimed directly at, or as close as possible to, the cicadas' ventral sides. Because the frequency measurements relied on voluntary calling behavior, measurements could only be taken during the day and it was not possible to enforce any regular sampling interval. Consequently, in comparison to the amplitude data, the frequency data for individual cicadas were necessarily much more sparse and irregularly spaced in time. As with amplitude, a negative control group of unparasitized cicadas was also kept in the same conditions and the calling songs of these cicadas were monitored in the same way as for the parasitized cicadas.

*Statistical analyses*

The final data sets included a few pairs of host cicadas that had been infected by larvae from the same female *E. erro*. To avoid possible bias and pseudoreplication, the data sets were pruned so that each host cicada was infected by a different female fly.

Values for two additional explanatory variables, effective clutch size and ambient temperature, were also added to the datasets for inclusion in the statistical analyses. Temperature is well known to influence the development and growth of insects in general (Harrison et al. 2012) and it therefore might be expected to affect how rapidly *E. erro* larvae can induce changes in their hosts. Effective clutch size was included because the total number of larvae inside of a host cicada might influence the overall effect of parasitization on host mating calls. For this study, the effective clutch size was the total number of *E. erro* larvae that emerged from a host. If a host died prematurely before parasitoid larval development was complete, then the effective clutch size was instead estimated by dissecting the host and counting the remaining larvae. Temperature data were taken from the Daymet 1-km daily surface weather dataset (Thornton et al. 1997, 2014). For each amplitude or frequency observation of a cicada's call, ambient temperature was calculated as the cumulative mean of the daily minimum and maximum temperatures for each day the host cicada was infected up to, and including, the day of the amplitude or frequency observation.

All host cicadas that survived long enough for their parasitoids to complete larval development eventually ceased to produce any sound at all, and the loss of calling ability was often abrupt (see section 5.3, Results, below). Because of this, calling song changes were statistically modeled as two separate processes: First, the changes in mating call amplitude and frequency structure that occur prior to the loss of calling ability, and second, the time required for complete loss of calling ability.

Calling song changes prior to the loss of calling ability were analyzed using univariate mixed effects (i.e., multilevel) models with normally distributed random effects and normally distributed errors, with *amplitude*, *peak frequency*, acoustic *power at 5.6 kHz*, or acoustic *power*

*at 7.0 kHz* as the response variable. Elapsed *time* since infection, *effective clutch size*, and ambient *temperature* were used as predictor variables. To account for the repeated acoustic measurements of individual host cicadas, *host cicada* was included as a random effect with random intercepts and, in some models, random coefficients for the effect of elapsed time since infection. All models were fitted using maximum likelihood estimation. Linear models were fitted directly by the lme4 package (Bates et al. 2015); nonlinear models were fitted using an iterative combination of parameter estimation by lme4 for the linear components and direct optimization of likelihood values for the nonlinear components. Candidate models were evaluated using AIC scores, BIC scores, and $R^2_{PP}$ values. Selected likelihood-ratio tests were also used as an additional criterion for model comparison. Confidence intervals for model estimates were determined using a parametric bootstrap of the random effects and residual errors of the fitted model, as described by Thai et al. (2013), with 1,000 replicates. For the best models, diagnostic plots of the residuals were examined to evaluate whether the models' assumptions were reasonable.

Loss of calling ability was analyzed using multiple linear regression with *time* to call loss as the response variable and ambient *temperature* and *clutch size* as the predictors. For each host cicada, the elapsed time to call loss after infection with *E. erro* was estimated by taking the mean of the elapsed times of the last amplitude measurement for which the host still had an audible call and the first measurement for which no call could be detected. Candidate models and predictor variables were evaluated using the standard inference techniques for general linear models (e.g., *F* tests to determine the significance of individual predictors), and diagnostic plots of the standardized residuals were examined to verify that the model assumptions were not violated.

### *Effects of calling song changes on* **E. erro***'s host finding behavior*

Field choice experiments were used to assess whether parasitized male *N. dorsatus* cicadas are less attractive to *E. erro* than are unparasitized cicadas. To evaluate *E. erro*'s preferences, flies were presented with three simultaneously broadcast synthetic acoustic signals that were based on the results of the previous experiments: one that represented the call of an uninfected cicada, one that represented a cicada 6 hours after infection, and one that represented a cicada 12 hours after infection. The numbers of flies attracted to each of these signals were analyzed to determine whether the infection status of potential hosts had an effect on *E. erro*'s host finding behavior.

#### *Acoustic test signals*

The test signals for evaluating the phonotactic behavior of *E. erro* were constructed from a mix of amplitude-modulated sine waves at three frequencies: 4.308 kHz, 5.6 kHz, and 7.0 kHz. 4.308 kHz represented the mean peak frequency of of the *N. dorsatus* mating call (Stucky 2015), and together, these three frequencies approximately spanned the frequency band of overall peak acoustic output for *N. dorsatus*. These frequencies also directly corresponded to the frequency-related components of the *N. dorsatus* mating call that were monitored pre- and post-infection, as described above. The mix of sine waves was amplitude-modulated to produce discrete "chirps" of sound 20.3 ms long, repeated at a rate of 37 chirps per second. This temporal structure matched the mean pulse group structure of the natural mating call of *N. dorsatus* (Stucky 2015). The beginning and end of each chirp was smoothed to 0 amplitude using the Tukey window function with a 20% taper (Tukey 1967). All test signals were generated using custom-written software. The components of this basic test signal were modified as appropriate to replicate the amplitude and frequency structure of the mating call of

*N. dorsatus* in three distinct states of parasitization: uninfected, 6 hours after infection, and 12 hours after infection.

For amplitude, most infected cicadas in the study of post-infection call changes were regularly monitored at 6-hour intervals immediately after infection, so it was possible to directly calculate the observed mean amplitudes of the cicadas' calls before infection and at 6 and 12 hours after infection. These observed mean amplitudes were used to calibrate the amplitudes of the three test signals for the broadcast experiments. The peak sample value for the synthetic signal that represented an uninfected cicada was placed at 0 dBFS (decibels relative to full scale), then, the amplitudes of the signals representing infected cicadas at 6 and 12 hours after infection were adjusted to match the mean observed amplitude change for infected cicadas at 6 and 12 hours after infection.

Although the call amplitudes of infected cicadas could be monitored at regular intervals, the frequency-related variables could only be measured when cicadas voluntarily chose to sing (see the methods description above). It was therefore impossible to calculate observed mean values of the frequency-related variables at 6 and 12 hours after infection. Instead, these values were taken as the mean at the desired time after infection as estimated by the best statistical model for each frequency variable. In other words, the relative amplitude of each sine wave component of the test signals was adjusted to match the relative acoustic power of *N. dorsatus* calls at the peak frequency, 5.6 kHz, and 7.0 kHz, as estimated for cicadas before infection and at 6 and 12 hours after infection. These frequency-related estimates were combined with the observed mean amplitude changes to produce the three test signals for the field choice experiments.

*Broadcast choice experiments*

The acoustic broadcast choice experiments were conducted in 2014 and 2015 at two field sites in central Kansas (Figure 5.1). The test signals were each broadcast from one of three live traps designed for capturing *E. erro*; see Appendix A for a detailed description of the trap construction and design. The output volume of each trap was calibrated using an SPL meter (RadioShack® model 33-2055) mounted to a tripod and positioned directly above the trap's loudspeaker, with the SPL meter's microphone at a distance of 0.5 meters from the speaker. The signal used for calibration was generated from a recording of the natural call of *N. dorsatus* and was normalized so that the peak sample value was at 0 dBFS, as in the synthetic test signal representing an uninfected cicada. The amplifier gains were adjusted so that this test signal registered at about 101-102 dB SPL, which made the volume approximately comparable to that of the loudest natural *N. dorsatus* calling songs, as measured with the same SPL meter.

For each choice trial, the three traps were placed in the field with each trap at a vertex of an equilateral triangle with 10 meter sides; that is, each trap was separated from the other two by a distance of 10 meters. Each of the three test signals was randomly assigned to one of the traps, and the three signals were broadcast simultaneously for 10 minutes. After the initial 10 minutes of broadcasting, the signals were rotated among the traps and broadcast for another 10 minutes, then rotated a second time and broadcast for another 10 minutes. Thus, for each trial, the traps were simultaneously operated for a total of 30 minutes of broadcast time (or 90 total trap-minutes, accounting for all three traps), and each of the three test signals was broadcast from each of the three traps for 10 minutes at each trap. At the end of the trial, the total number of flies trapped at each of the test signals was counted and the flies were sexed. To ensure that no individual fly could be counted more than once in the results, all flies captured at a given field

site were kept in live cages in the laboratory and maintained on sucrose and water *ad libitum* until all broadcast experiments were concluded at the field site. After the broadcast trials were concluded for the season, the flies were released back into the wild at the field sites where they had been captured.

*Statistical analyses*

The counts of flies attracted to the test signals were analyzed using log-linear models with number of flies as the Poisson-distributed response variable (i.e., generalized linear models with log link function and Poisson-distributed response) (Dobson and Barnett 2008). The test *signal type*, fly *sex*, *field site*, and *year* were considered as possible predictor variables. Broadcast trials in which no flies were captured were not included in the final dataset. Model parameters were estimated using maximum likelihood estimation, and candidate models were compared using AIC scores, BIC scores, $R^2_{PP}$ values, and selected likelihood-ratio tests (i.e., the difference of the model deviances). Diagnostic plots of the standardized residuals from the best models were examined to evaluate model assumptions and whether the models were appropriate for the data.

### *Activity of* E. erro *larvae inside of their host*

To further explore the role of *E. erro* larvae in the call changes of their cicada hosts, a simple experiment was used to document within-host larval movement and activity. Unparasitized male *N. dorsatus* were artificially infected in the laboratory with live, first-instar *E. erro* larvae that had been dissected from gravid female flies, as described in detail in Stucky (2015). To mimic the natural infection behavior of female *E. erro* (Stucky 2015), most cicadas were infected by placing the larvae at the base of the wings, but a few cicadas were infected by placing the larvae at the junction of the mesothorax and metathorax or at the junction of the

metathorax and the first abdominal tergite. After infection, the cicadas were housed in outdoor live cages, as described above. Each infected cicada was euthanized by freezing from between 0.5 to 3 hours after it had been infected. After freezing, the cicadas were thawed, dissected, and the locations of all *E. erro* larvae, as well as the condition of the cicada's sound-producing structures, were noted. A few cicadas were kept alive until they became mute, at which point they were frozen and dissected in the same manner as the others. The results of these experiments were supplemented by dissections of a few hosts that were naturally or artificially infected in captivity but died prematurely (that is, before the *E. erro* larvae were mature and ready to leave their host), and by dissections of a few mute hosts that were already infected when they were collected in the field.

## 5.3 Results

### *Effects of host parasitoid load on parasitoid fitness*

To estimate the effect of host parasitoid load on parasitoid fitness, puparial length and width measurements were taken from 393 *E. erro* puparia reared from 82 *N. dorsatus* host cicadas. The vast majority of the hosts (68 hosts, 83%) were cicadas that had been naturally parasitized in the field. Of the remainder, 13 were voluntarily parasitized by female flies in captivity, and 1 was artificially infected in the laboratory (16% and 1%, respectively).

The parasitoid loads of the host cicadas ranged from 1 to 19 larvae per host (mean = 4.8, median = 3.5). Hosts with very large parasitoid loads were relatively infrequent, however; about 90% of the host cicadas had parasitoid loads of 9 or less.

Both the body size data and the results of the statistical analysis were consistent with the hypothesis that large parasitoid loads are costly for developing parasitoids. A scatterplot showing the mean puparial volume for each host is provided in Figure 5.2. These data clearly

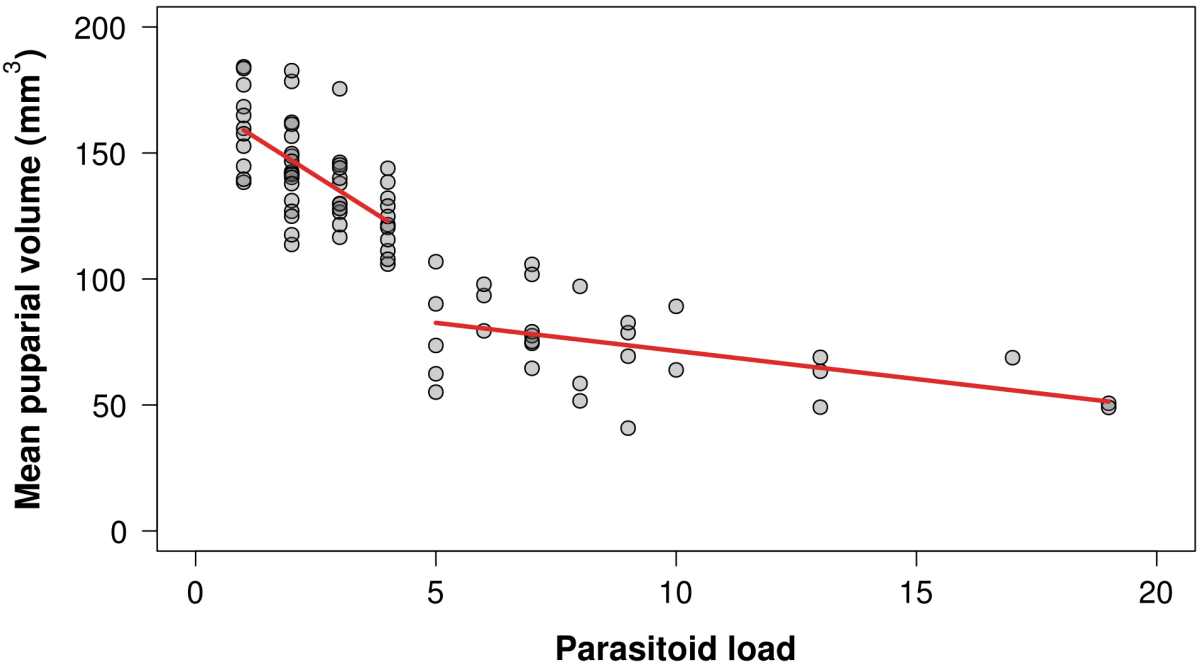


**Figure 5.2** The puparial volumes of *E. erro* from *N. dorsatus* hosts with varying parasitoid loads. Each point represents the mean volume of all puparia from a single host. The red line represents the best model of the relationship between parasitoid load and mean puparium size.

show a general trend of decreasing puparial size with increasing parasitoid load. For example, the mean observed puparial volume (averaged per host) for hosts with parasitoid loads of 1, 4, 7, 13, and 19 were as follows: 161.0 $mm^3$ (n = 11), 122.8 $mm^3$ (n = 11), 82.6 $mm^3$ (n = 7), 60.5 $mm^3$ (n = 3), and 49.8 $mm^3$ (n = 2).

The BIC scores, AIC scores, and the $R^2_{PP}$ values of the models, given in Table 5.1, indicated that the best model for these data was a piecewise linear model with a breakpoint between 4 and 5 larvae per host (depicted graphically in Figure 5.2). This model explained the data very well, with $R^2_{PP} = 0.828$. The model suggests that, on average, mean puparial volume always decreases with increasing parasitoid load, and puparial volume declines most sharply when parasitoid loads increase from 4 to 5 larvae per host. According to this model, parasitoids emerging from hosts with parasitoid loads of 19 are expected to be only about 27% as large as parasitoids emerging from hosts with parasitoid loads of 1, and parasitoids from hosts with

**Table 5.1** Comparison of statistical models for the relationship between the parasitoid load of host *N. dorsatus* and the size of *E. erro* puparia.

| Model | $R^2_{PP}$ | BIC | ΔBIC | AIC | ΔAIC |
|---|---|---|---|---|---|
| piecewise linear, separate slopes | 0.828 | 711.21 | 0.00 | 696.77 | 0.00 |
| sigmoid: rational | 0.811 | 714.50 | 3.29 | 702.46 | 5.70 |
| sigmoid: logistic | 0.803 | 717.94 | 6.74 | 705.91 | 9.15 |
| sigmoid: Gompertz | 0.803 | 718.06 | 6.86 | 706.03 | 9.26 |
| exponential | 0.789 | 719.04 | 7.83 | 709.41 | 12.65 |
| hyperbolic | 0.781 | 722.05 | 10.84 | 712.42 | 15.66 |
| piecewise linear, single slope | 0.786 | 724.52 | 13.31 | 712.48 | 15.72 |
| logarithmic | 0.772 | 725.50 | 14.30 | 715.88 | 19.11 |
| linear | 0.651 | 756.05 | 44.85 | 748.83 | 52.07 |
| intercept only (null model) | n/a | 837.90 | 126.69 | 833.08 | 136.32 |

parasitoid loads of 5 are expected to be only about 64% as large as parasitoids emerging from hosts with parasitoid loads of 4 (Figure 5.2). It should also be noted that the sigmoid rational function model also fit the data reasonably well, and the relationship between puparial volume and parasitoid load estimated by this model was roughly in agreement with that of the piecewise linear model.

### *Changes in host calling song after infection*

After being parasitized by larval *E. erro*, male *N. dorsatus* cicadas exhibited rapid changes to both the amplitude and spectral structure of their calls (Figures 5.3 - 5.5). The effect of parasitization on call amplitude was especially striking (Figure 5.3). The call amplitudes of all host cicadas decreased after infection, and amplitude loss was often detectable within an hour or less after a host was infected. By 6 hours after infection, most cicadas had experienced a marked decrease in call amplitude that was followed by a more gradual, continued decline. Specifically, the observed mean call amplitude for all cicadas prior to infection was 98.5 dB SPL, but the mean amplitude dropped to 89.7 dB SPL 6 hours after infection (a change of -8.6 dB SPL) and to

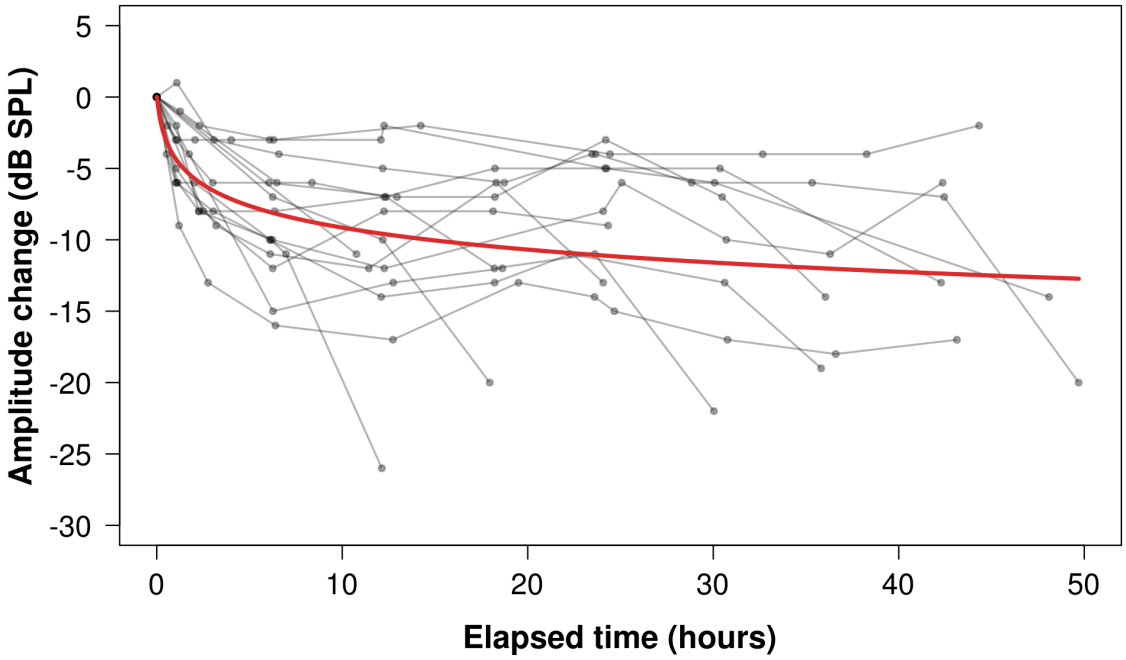


**Figure 5.3** Changes in the amplitude of the mating calls of male *N. dorsatus* after infection with *E. erro* larvae. Each point represents a single amplitude measurement, and the gray lines connect measurements from the same host cicadas. The red line represents the fixed effects of the best model of the relationship between elapsed time since infection and mating call amplitude. To make the overall trend easier to see, the data are plotted as change in amplitude since infection rather than raw amplitude, but the model was fitted to the raw amplitude data.

88.2 dB SPL 12 hours after infection (a change of -10.3 dB SPL). Because the decibel scale is logarithmic, a decrease of 10.3 dB SPL corresponds to an approximately 10-*fold* (i.e., an order of magnitude) decrease in acoustic power.

Statistical analyses of the amplitude data confirmed that there was a strong effect of elapsed time since infection on the amplitudes of the cicadas' calls, with all three model comparison criteria (AIC, BIC, and likelihood ratio tests) decisively favoring models that included a fixed effect of elapsed time post-infection (Table 5.2). Neither effective clutch size nor ambient temperature appeared to have a substantial effect on call amplitude, either as main effects or by interacting with the effect of elapsed time (Table 5.2). The final, "best" model of amplitude change was: $amplitude = -2.25 \log(elapsed\_time + 0.175) + 94.51$, with random

intercepts and random slopes. This model explained the data quite well, with $R^2_{PP} = 0.73$, and the observed mean amplitudes after infection were well within the 95% confidence intervals of the model estimates (Table 5.6). As expected from the observed pattern of amplitude change, this model indicates a rapid initial drop in call amplitude during the first few hours immediately after infection, followed by a more gradual, continued decline in amplitude as the infection progresses (Figure 5.3).

The results of the model analyses for the call frequency-related variables are given in Tables 5.3 - 5.5, and an example of the overall effect of parasitization on the frequency structure of a cicada's call is illustrated in Figure 5.4. Parasitization by *E. erro* did not appear to have an effect on the overall peak frequency of *N. dorsatus*'s mating call, as the null, intercept-only

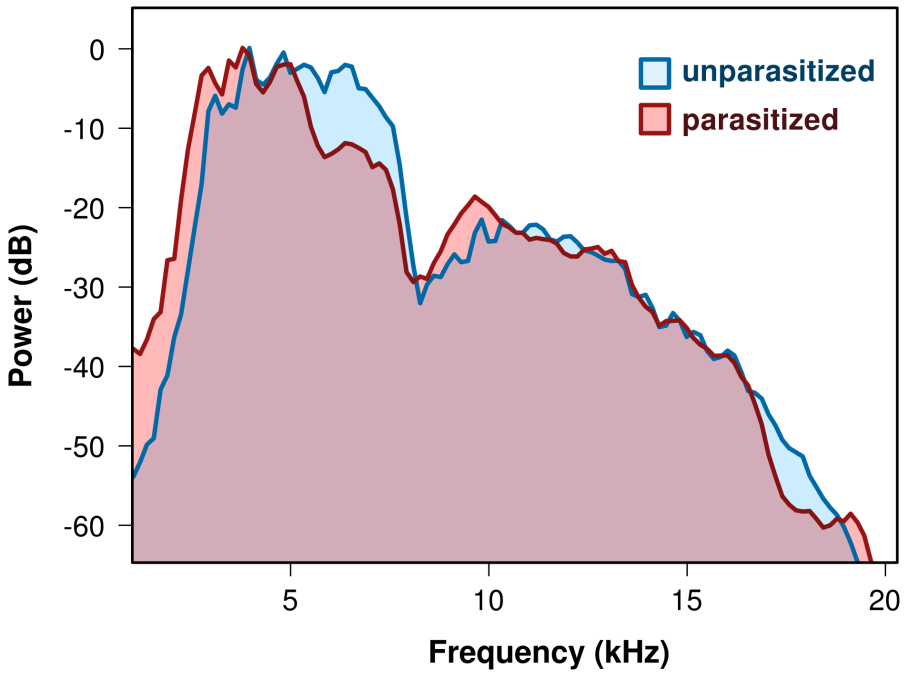


**Figure 5.4** Representative power spectral densities of the mating call of a male *N. dorsatus* cicada before and approximately 43 hours after infection with *E. erro* larvae. There was considerable variation in spectral density changes among host cicadas, so this should not be interpreted as a depiction of the overall trend. However, the relative acoustic powers at 5.6 and 7.0 kHz of this cicada's call after infection were similar to the expected values predicted by the statistical models.

model fit the data as well as, or better than, models that included an effect of elapsed time on peak frequency (Table 5.3).

However, parasitization did appear to have an effect on the relative acoustic power of the calls at 5.6 kHz and 7.0 kHz. The effect was most pronounced for acoustic power at 7.0 kHz (Figure 5.5), for which all three model comparison criteria indicated a strong, negative fixed effect of elapsed time since infection on acoustic power (Table 5.5). As with amplitude, neither ambient temperature nor effective clutch size appeared to be of much importance. The best model was $power = -0.57 \log(elapsed\_time + 0.000271) - 9.36$ ( $R^2_{PP} = 0.74$), and even though the model selection criteria suggested that random effects for elapsed time might not be needed in the model, both random intercepts and random slopes were retained in the final model as a conservative measure to properly account for the experimental design. This model estimates that the average acoustic power at 7.0 kHz for an uninfected cicada is -4.7 dB, which agrees very well with the observed mean of -4.67 dB. At 6 hours after infection, the mean estimated acoustic power is -10.4 dB (a change of -5.7 dB), with further decline to -10.8 dB by 12 hours after infection (a change of -6.1 dB) (Table 5.6).

For the acoustic power of the mating calls at 5.6 kHz, the model comparisons suggested that there was a weak, negative, fixed effect of elapsed time since infection (Table 5.4). Again, there was no detectable effect of either ambient temperature or effective clutch size. Although the model selection criteria for the fixed effects suggested that the linear model was better than the logarithmic model, the raw likelihood values for the two models were virtually the same, with the extra parameter of the log model accounting for its comparatively poorer scores. Furthermore, because the analyses of the data for amplitude and acoustic power at 7.0 kHz both strongly suggested that the pattern of call change was nonlinear, it seemed likely that the changes

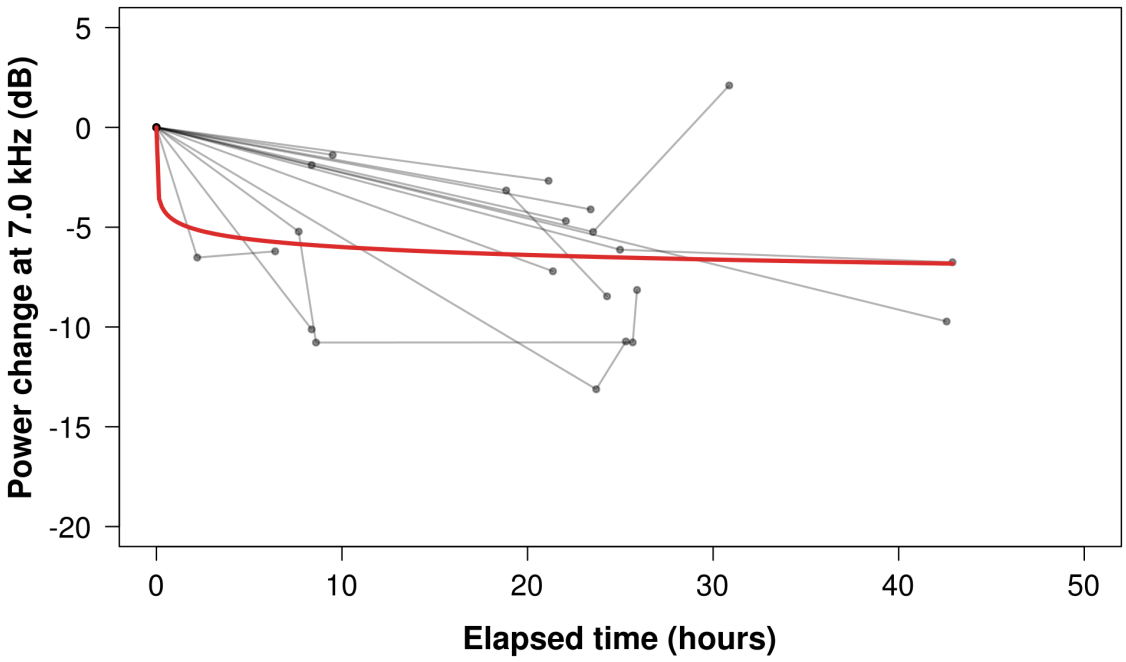


**Figure 5.5** Changes in the frequency structure of the mating calls of male *N. dorsatus* after infection with *E. erro* larvae, measured as relative acoustic power at 7.0 kHz. Each point represents a single power measurement, and the gray lines connect measurements from the same host cicadas. The red line represents the fixed effects of the best model of the relationship between elapsed time since infection and acoustic power at 7.0 kHz. To make the overall trend easier to see, the data are plotted as change in power since infection rather than the raw power measurements, but the model was fitted to the raw power data.

at 5.6 kHz were also nonlinear, but that the effect was simply too small to be able to detect a difference between the linear and log models with the available data set. Consequently, the log model was retained as the presumptive "best" model of acoustic power at 5.6 kHz. The final model was $power = -2.07 \log(elapsed\_time + 19.27) - 3.74$ ( $R^2_{PP} = 0.82$). As can be seen from the model comparisons (Table 5.4), most of the predictive power of this model is due to the random effects. Thus, although the data suggest a trend of declining acoustic power at 5.6 kHz following infection with *E. erro*, the effect is small and there is considerable variation from host to host. The mean estimated acoustic power at 5.6 kHz prior to infection is -2.4 dB, which agrees well with the observed mean of -2.5 dB, and at 6 and 12 hours after infection, the acoustic

**Table 5.2** Comparison of statistical models for the relationship of the amplitude of male *N. dorsatus* mating calls with elapsed *time* since parasitization by *E. erro*, parasitoid *clutch* size, and ambient *temp*erature. The column "*p*" gives the *p*-values from likelihood ratio tests comparing two nested models. The number in parentheses indicates the comparison model for a given likelihood ratio test. Note that exponential and hyperbolic (i.e., rational function) relationships were also considered; they resulted in models that were qualitatively very similar to the logarithmic model but of slightly poorer fit. For simplicity, those models are excluded from the table.

| Model num. | Model form | Fixed covariate effects | Random effects, grouped by host | $R^2_{PP}$ | BIC | ΔBIC | AIC | ΔAIC | *p* |
|---|---|---|---|---|---|---|---|---|---|
| 3 | logarithmic | log(*time*+α) | intercept, log(*time*+α) | 0.733 | 664.9 | 0.00 | 645.5 | 0.00 | (2) <0.001 |
| | logarithmic | log(*time*+α), *clutch*, log(*time*+α)**clutch* | intercept, log(*time*+α) | 0.734 | 670.5 | 5.60 | 645.6 | 0.06 | (3) 0.14 |
| | logarithmic | log(*time*+α), *temp*, log(*time*+α)**temp* | intercept, log(*time*+α) | 0.742 | 671.3 | 6.44 | 646.4 | 0.90 | (3) 0.21 |
| | logarithmic | log(*time*+α), *clutch* | intercept, log(*time*+α) | 0.729 | 668.9 | 4.00 | 646.7 | 1.23 | (3) 0.38 |
| | logarithmic | log(*time*+α), *temp* | intercept, log(*time*+α) | 0.733 | 669.3 | 4.42 | 647.1 | 1.64 | (3) 0.55 |
| 2 | logarithmic | *log(time+α)* | intercept | 0.671 | 672.5 | 7.65 | 658.7 | 13.19 | (0) <0.001 |
| 1 | linear | *time* | intercept | 0.538 | 709.9 | 44.96 | 696.0 | 50.50 | (0) <0.001 |
| 0 | intercept only (null) | none (intercept only) | intercept | 0.266 | 744.4 | 79.53 | 736.1 | 90.61 | – |

**Table 5.3** Comparison of statistical models for the relationship of the peak frequency of male *N. dorsatus* mating calls with elapsed *time* since parasitization by *E. erro*. The column "*p*" gives the *p*-values from likelihood ratio tests comparing the target model to the null model.

| Model form | Fixed covariate effects | Random effects, grouped by host | $R^2_{PP}$ | BIC | ΔBIC | AIC | ΔAIC | *p* |
|---|---|---|---|---|---|---|---|---|
| intercept only (null) | none (intercept only) | intercept | 0.596 | 542.3 | 0.00 | 537.6 | 0.00 | – |
| linear | *time* | intercept | 0.582 | 544.3 | 2.01 | 538.1 | 0.45 | 0.21 |
| logarithmic | log(*time*+α) | intercept | 0.583 | 547.9 | 5.56 | 540.1 | 2.45 | 0.46 |

**Table 5.4** Comparison of statistical models for the relationship of the relative power of male *N. dorsatus* mating calls at 5.6 kHz with elapsed *time* since parasitization by *E. erro*, parasitoid *clutch* size, and ambient *temp*erature. The column "*p*" gives the *p*-values from likelihood ratio tests comparing two nested models. The number in parentheses indicates the comparison model for a given likelihood ratio test.

| Model num. | Model form | Fixed covariate effects | Random effects, grouped by host | $R^2_{PP}$ | BIC | ΔBIC | AIC | ΔAIC | *p* |
|---|---|---|---|---|---|---|---|---|---|
| 3 | logarithmic | log(*time*+α) | intercept, log(*time*+α) | 0.817 | 199.6 | 0.00 | 188.7 | 0.00 | (2) 0.001 |
| | logarithmic | log(*time*+α), *temp* | intercept, log(*time*+α) | 0.828 | 201.8 | 2.22 | 189.4 | 0.66 | (3) 0.25 |
| | logarithmic | log(*time*+α), *clutch* | intercept, log(*time*+α) | 0.823 | 202.6 | 3.03 | 190.2 | 1.47 | (3) 0.47 |
| | logarithmic | log(*time*+α), *temp*, log(*time*+α)**temp* | intercept, log(*time*+α) | 0.826 | 204.7 | 5.11 | 190.7 | 2.00 | (3) 0.37 |
| | logarithmic | log(*time*+α), *clutch*, log(*time*+α)**clutch* | intercept, log(*time*+α) | 0.827 | 205.7 | 6.14 | 191.7 | 3.03 | (3) 0.62 |
| 2 | logarithmic | log(*time*+α) | intercept | 0.644 | 205.8 | 6.27 | 198.1 | 9.38 | (0) 0.16 |
| 1 | linear | *time* | intercept | 0.642 | 202.3 | 2.76 | 196.1 | 7.42 | (0) 0.058 |
| 0 | intercept only (null) | none (intercept only) | intercept | 0.606 | 202.4 | 2.78 | 197.7 | 9.01 | – |

**Table 5.5** Comparison of statistical models for the relationship of the relative power of male *N. dorsatus* mating calls at 7.0 kHz with elapsed *time* since parasitization by *E. erro*, parasitoid *clutch* size, and ambient *temp*erature. The column "*p*" gives the *p*-values from likelihood ratio tests comparing two nested models. The number in parentheses indicates the comparison model for a given likelihood ratio test.

| Model num. | Model form | Fixed covariate effects | Random effects, grouped by host | $R^2_{PP}$ | BIC | ΔBIC | AIC | ΔAIC | *p* |
|---|---|---|---|---|---|---|---|---|---|
| 3 | logarithmic | log(*time*+α) | intercept, log(*time*+α) | 0.741 | 189.0 | 5.17 | 178.1 | 2.06 | (2) 0.38 |
| | logarithmic | log(*time*+α), *temp* | intercept, log(*time*+α) | 0.745 | 192.4 | 8.57 | 180.0 | 3.90 | (3) 0.69 |
| | logarithmic | log(*time*+α), *clutch* | intercept, log(*time*+α) | 0.737 | 192.5 | 8.67 | 180.1 | 4.01 | (3) 0.82 |
| | logarithmic | log(*time*+α), *clutch*, log(*time*+α)**clutch* | intercept, log(*time*+α) | 0.738 | 195.6 | 11.71 | 181.6 | 5.49 | (3) 0.75 |
| | logarithmic | log(*time*+α), *temp*, log(*time*+α)**temp* | intercept, log(*time*+α) | 0.744 | 195.8 | 11.94 | 181.8 | 5.72 | (3) 0.85 |
| 2 | logarithmic | log(*time*+α) | intercept | 0.626 | 183.9 | 0.00 | 176.1 | 0.00 | (0) <0.001 |
| 1 | linear | *time* | intercept | 0.404 | 192.7 | 8.81 | 186.5 | 10.37 | (0) <0.001 |
| 0 | intercept only (null) | none (intercept only) | intercept | N/A | 207.2 | 23.37 | 202.6 | 26.48 | – |

**Table 5.6** Expected amplitudes and relative acoustic powers at 5.6 and 7.0 kHz of the mating call of male *N. dorsatus* at 0, 2, 6, and 12 hours after infection by *E. erro*, as estimated by the best statistical model for each variable. The numerical ranges given in parentheses are 95% confidence intervals for the estimates.

| | | Estimated mean at time *t* hours after infection | | | |
|---|---|---|---|---|---|
| Variable | Best model | *t* = 0 | 2 | 6 | 12 |
| amplitude | amplitude = -2.25 * log(*time* + 0.175) + 94.51 | 98.44 dB SPL (97.0 – 99.9) | 92.76 dB SPL (91.3 – 94.2) | 90.41 dB SPL (88.8 – 92.1) | 88.89 dB SPL (87.0 – 90.8) |
| acoustic power around 5.6 kHz | power = -2.07 * log(*time* + 19.27) + 3.74 | -2.38 dB (-3.5 – -1.2) | -2.59 dB (-4.1 – -1.6) | -2.94 dB (-4.5 – -1.7) | -3.38 dB (-5.3 – -1.7) |
| acoustic power around 7.0 kHz | power = -0.57 * log(*time* + 0.000271) – 9.36 | -4.68 dB (-5.8 – -3.4) | -9.75 dB (-11.4 – -6.5) | -10.38 dB (-11.7 – -8.1) | -10.77 dB (-11.9 – -9.3) |

power is expected to drop to -2.9 dB (a change of -0.6 dB) and -3.4 dB (a change of -1.0 dB), respectively (Table 5.6).

After being parasitized by *E. erro*, all male *N. dorsatus* cicadas eventually lost the ability to produce any sound at all. These "mute" cicadas were never observed producing an audible mating call, and all efforts to induce alarm call production also failed. On average, host cicadas became mute 37.4 hours after infection, but there was considerable variation in muting times among the hosts (s.d. = 10.8 hours, range: 17.0 – 50.2 hours). The model analyses indicated that temperature, but not effective clutch size, had a strong effect on muting times (the *p*-values for *temperature* and *effective clutch size* as predictors were 0.0024 and 0.15, respectively). The best model was *muting time* = –1.85 *temperature* + 84.45, which explained the data fairly well ($R^2$ = 0.59). This model estimates that, on average, each °C increase in ambient temperature decreases the time until a parasitized cicada becomes mute by about 1.9 hours.

However, examination of the standardized residuals from the muting times model suggested that there actually was a trend of decreasing muting time with increasing effective clutch size, but that the effect might have been "masked" by the presence of a single outlier host cicada that had both a large effective clutch size and a long time to muting. Removing this host from the dataset dramatically changed the model analysis results. Without the outlier, the best model was *muting time* = –1.66 *temperature* – 3.41 *clutch_size* + 87.92, and the *p*-values for ambient temperature and effective clutch size as predictors were < 0.001 and 0.0051, respectively. This model had excellent explanatory power, with $R^2$ = 0.85. The estimated effect of temperature for this model is comparable to that of the previous model with the full dataset, but the new model also predicts that, for a given temperature, increasing the effective clutch size

of a host cicada by 1 parasitoid will decrease the host's muting time by 3.4 hours, on average (Figure 5.6).

Choosing between the model with the full dataset or the model without the outlier host is somewhat arbitrary, but there was at least one reason to suspect that the muting time of the outlier host was truly anomalous. This host had been artificially infected in the laboratory with larvae dissected from a female *E. erro*, but the larvae appeared to have just hatched and were noticeably smaller than those typically deposited voluntarily by female flies. Thus, these larvae might have been less mature than those infecting cicadas naturally, and their effect on host calling might have therefore also followed a slightly different trajectory. Either way, there can be very little doubt that temperature has a strong effect on muting time, and the weight of the evidence indicates that effective clutch size also has a negative effect on muting time.

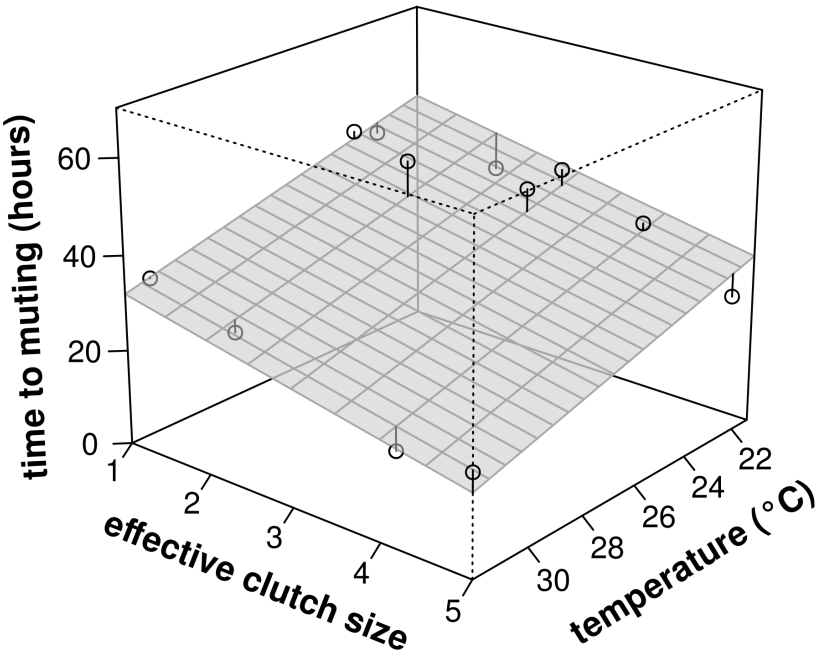


**Figure 5.6** The estimated effects of ambient temperature and effective parasitoid clutch size on the time required for a parasitized male *N. dorsatus* to lose its calling ability. The relationship depicted here is with a probable outlier host excluded; see text for details.

Finally, analyses of the acoustic monitoring data for the negative control cicadas indicated that all of the call changes described above were a consequence of parasitization by *E. erro*. All model comparisons for the negative control data suggested that, on average, there were no changes to the uninfected cicadas' calls over time for any of the acoustic variables.

***Effects of calling song changes on* E. erro*'s host finding behavior***

Based on the results of the analysis of *N. dorsatus* mating call changes before and after infection with *E. erro*, reported above, the amplitudes and frequency structures of the three test signals used for the broadcast choice experiments were as follows. *Amplitude*: The amplitudes of the 6 hour post-infection and 12 hour post-infection test signals differed by -8.6 dB and -10.3 dB, respectively, from the amplitude of the uninfected test signal. *Peak frequency*: Peak call frequency did not change substantially after infection, so the peak frequency of all three test signals was the same. *Relative power at 5.6 and 7.0 kHz*: For the uninfected test signal, the 5.6 and 7.0 kHz components were attenuated to -2.38 dB and -4.67 dB relative to the peak frequency, respectively. For the 6 hour post-infection signal, these components were attenuated to -2.94 dB and -10.38 dB, and for the 12 hour post-infection signal, they were attenuated to -3.38 dB and -10.78 dB.

A total of 76 *E. erro* (61 females and 15 males) were captured during 18 successful field broadcast trials with these three test signals. The numbers of flies trapped at each of the test signals strongly suggested that the signal representing an uninfected male *N. dorsatus* was far more attractive to *E. erro* than were the two signals representing infected cicadas (Figure 5.7, Table 5.7). In total, the traps with the uninfected test signal captured more than 10 times as many flies as the traps with either of the two infected test signals.

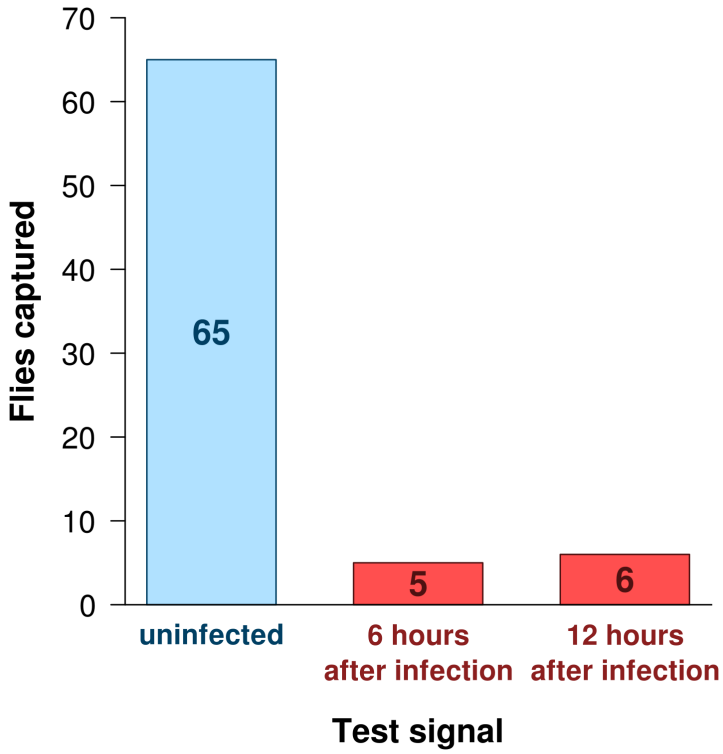


**Figure 5.7** Total numbers of *E. erro* captured at each of the three test signals representing the mating calls of an uninfected male *N. dorsatus* and infected cicadas 6 and 12 hours after parasitization.

**Table 5.7** Results of the broadcast choice experiments to assay the phonotactic behavior of *E. erro* in response to the calls of uninfected and infected male *N. dorsatus*.

| | Number of flies | | |
|---|---|---|---|
| **Test signal** | **Female** | **Male** | **Total** |
| uninfected | 50 | 15 | 65 |
| 6 hours post-infection | 5 | 0 | 5 |
| 12 hours post-infection | 6 | 0 | 6 |
| **total:** | 61 | 15 | 76 |

Statistical analysis of the data confirmed that there was a strong effect of test signal type on the number of flies captured. Initial evaluation of models including the interaction of *field site* and *signal type* and the interaction of *year* and *signal type* indicated that were no differences in the relative attractiveness of the test signal types between field sites (i.e., the interaction

between *field site* and *signal type*) or between years (i.e., the interaction between *year* and *signal type*): All ΔAIC and ΔBIC values favored models that excluded these interactions, and all *p*-values from likelihood ratio tests that compared models with and without the interactions were > 0.13. Consequently, the effects of *field site* and *year* were not considered further and the data were aggregated by *signal type* and fly *sex* for all subsequent analyses.

The results of the model analyses examining *signal type* and *sex* as predictors are given in Table 5.8. Both *signal type* and *sex* had an effect on the number of flies captured, but the effect of *signal type* was by far the most important. All model comparison criteria indicated that there was very little difference between the model including the interaction term *signal type*sex* and the additive model with only the main effects of *signal type* and *sex*, which suggested that the signal preferences of female and male flies were essentially the same. The final, "best" model was log(E(*flies captured*)) = 3.95 – 2.56 *six-hour signal* – 2.38 *twelve-hour signal* – 1.40 *male*, where the predictor variables are binary indicator (i.e., "dummy") variables. The 95% confidence intervals of the estimates of the intercept (which in this model is the effect of the uninfected signal for female flies) and of the effects of the 6-hour and 12-hour signals (which, again, are the effects for female flies) were 3.68 – 4.21, -3.61 – -1.76, and -3.33 – -1.63, respectively. These confidence intervals suggest that there is probably little meaningful difference in the attractiveness of the 6-hour and 12-hour test signals, while the uninfected test signal was clearly far more attractive than the other two. More precisely, this model estimates that, on average, the uninfected test signal will attract at least 10 times as many *E. erro* (either female or male) as either of the infected test signals.

**Table 5.8** Comparison of statistical models for the number of flies captured in the acoustic traps in relation to the test *signal type* and the *sex* of the fly. The column "*p*" gives the *p*-values from likelihood ratio tests comparing two nested models. The number in parentheses indicates the comparison model for a given likelihood ratio test.

| Model num. | Model terms | $R^2_{PP}$ | BIC | ΔBIC | AIC | ΔAIC | *p* |
|---|---|---|---|---|---|---|---|
| 4 | *signal type + sex + signal type*sex* | 1.0 | 28.2 | 0.00 | 29.4 | 0.00 | (3) 0.071 |
| 3 | *signal type + sex* | 0.994 | 29.9 | 1.69 | 30.7 | 1.28 | (0) <0.001 |
| 2 | *signal type* | 0.647 | 58.0 | 29.76 | 58.6 | 29.13 | (0) <0.001 |
| 1 | *sex* | 0.193 | 115.3 | 87.09 | 115.7 | 86.26 | (0) <0.001 |
| 0 | none (intercept only) | n/a | 143.4 | 115.16 | 143.6 | 114.12 | – |

### *Activity of* E. erro *larvae inside of the host*

A total of 10 male *N. dorsatus* were artificially infected in the lab with *E. erro* larvae, frozen, and then dissected to record the locations of the parasitoid larvae and the conditions of the sound-producing structures. Of these, eight were frozen from between 0.5 to 3 hours after they were infected (actual times: 0.5, 0.85, 1.0, 1.0, 1.17, 1.3, 2.0, and 3.0 hours), and the remaining two were frozen after they became mute (at 42.1 and 50.6 hours after infection). These results were supplemented by dissections of four host cicadas that had been infected in captivity but died prematurely (i.e., before the larvae were mature enough to leave the hosts) and two host cicadas that had been infected in the field prior to capture. All four of the captive-infected cicadas were dissected after they had been infected for at least 18 hours, but before they became mute; the two field-infected cicadas were dissected after they became mute.

In total, 33 *E. erro* larvae were recovered from the eight host cicadas that were frozen after they had been infected for three hours or less (range: 3 – 6 larvae per host). All but one of these 33 larvae (97%) were found in the host cicada's abdomen on or near the sound-producing structures in the large interior abdominal cavity that houses the tymbal muscles, tymbal

apodemes, tymbals, and tympana (hereafter referred to as the "acoustic cavity"). The only larva not found in the acoustic cavity was located in the thoracic musculature and was from a host that had been frozen 2 hours after infection. Of the 32 larvae found in the acoustic cavities of their hosts, 13 (41%) were located on the tymbal apodemes or the interior surfaces of the tymbals (Figure 5.8, b), 5 (16%) were located on the tymbal muscles, 4 (13%) were located on or near the tympana, and the remainder (31%) were located on the walls of the acoustic cavity. One of the larvae located on the tymbal muscles appeared to be digging into the muscle with its mouth hooks at 1.17 hours after infection. Otherwise, there generally were no obvious signs of mechanical damage to any of the sound-producing structures. All of the host cicadas but one (which could not be induced to produce a full alarm call at the time it was euthanized) had a measurable drop in alarm call amplitude at the time they were frozen.

Sixteen larvae were recovered from the four host cicadas that were dissected after they had been infected for 18 hours or more but before they became mute. All of these larvae were found in the acoustic cavities of their hosts. In three of the four host cicadas, one or both tymbal muscles showed clear signs of physical trauma.

Finally, 20 larvae were recovered from the four host *N. dorsatus* that were frozen shortly after they became mute. Again, all of these larvae were located in the acoustic cavities of their hosts. Several of the larvae were found with their heads buried in holes that had been bored into the tymbal muscles. The tymbal muscles of all four host cicadas were heavily damaged, and in three of the hosts, one or both tymbal muscles were nearly or entirely detached from their point of origin on the ventral wall of the abdomen (Figure 5.8, c). In one host, at least some of the tymbal muscle tissue appeared to be necrotic as well (Figure 5.8, c).

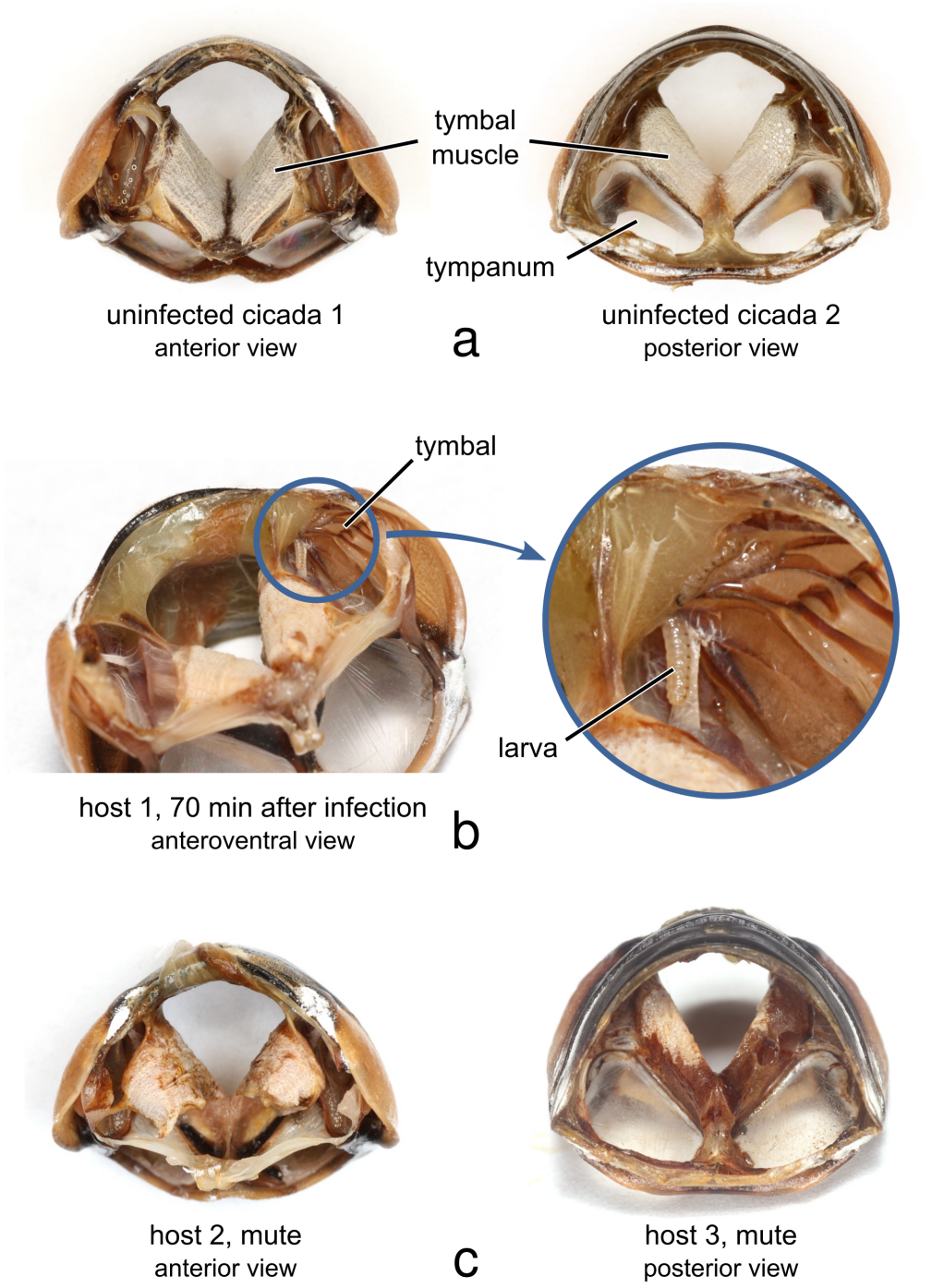


**Figure 5.8** Activity of *E. erro* larvae inside of their host cicadas. At top (**a**) are anterior and posterior views of the first and second abdominal segments of unparasitized male *N. dorsatus* cicadas, with the large tymbal muscles visible in the middle of the segments. Approximately 1 hour after infection (**b**), three first-instar *E. erro* larvae have moved to one of the tymbals and tymbal apodemes of their host. Once a host cicada loses its ability to produce sound, the tymbal muscles are rendered inoperable by *E. erro*, as seen in host 2 and host 3 (**c**), both of which were frozen immediately after they became mute.

## 5.4 Discussion

Recent studies have shown how thoroughly dependent the reproductive behaviors of adult *Emblemasoma erro* are on exploiting the acoustic mating calls of male cicadas. Both male and female *E. erro* use calling cicadas as meeting places to locate mates (see Chapter 3), and female *E. erro* rely on cicada calls to help them find potential hosts for their offspring. The results of the present study tell a different story of host signal exploitation, with the parasitoid larvae, not the adult flies, playing the central role. Rather than merely responding to the calls of their hosts in a particular way, as do the adult flies, *E. erro* larvae modify the communication systems of their hosts, and in so doing, they gain protection from potentially harmful intraspecific competition. The three main sets of results from this work each reveal a part of this story.

*First, superparasitism has real, substantial fitness consequences for larval* E. erro. Fly body size (measured as puparial volume) consistently decreases with increasing host parasitoid load, with an especially large drop between parasitoid loads of 4 and 5 (Figure 5.2). This is significant, because the mean clutch size of larvipositing female *E. erro* is about 2.5 larvae per host (Stucky 2015), and a reanalysis of the clutch size data reported in Stucky (2015) revealed that about 90% of the time, female flies deposited 4 or fewer larvae per host. Considering that at least some larvae often do not survive the initial infection attempt (that is, the effective clutch size is often less than the actual clutch size; B. Stucky, unpublished data), the larviposition habits of female flies appear to be tuned to produce big-bodied offspring, which means that most of the large parasitoid loads – and small puparia – observed in this study were likely a result of superparasitism.

There can be little doubt, then, that flies from superparasitized hosts will often be smaller than those from singly parasitized hosts, and, as a consequence of reduced body size, they will probably also be less fit (Honěk 1993, Kingsolver and Pfennig 2004). In fact, the data presented here probably *underestimate* the true fitness consequences of superparasitism, because some larvae that emerged from very heavily parasitized hosts were extremely small and failed to successfully pupariate. Even small larvae that manage to pupariate are likely to suffer higher mortality in the pupal stage compared to those with larger body sizes (Kamal 1958, S. A. Adamo et al. 1995, Allen and Hunt 2001). In addition to decreased body size, superparasitism probably results in fewer flies ever reaching adulthood at all.

It must be emphasized that superparasitism is not just a theoretical risk for *E. erro* larvae. In addition to the evidence from this study, there is strong circumstantial evidence that superparasitism by *E. erro* does occur in the field (Stucky 2015). Thus, superparasitism poses a realistic threat to the survival and fitness of *E. erro* larvae. In this context, larvae that are able to avoid superparasitism should have a definite advantage over those that cannot.

*Second, infection with* E. erro *larvae causes predictable changes to the mating calls of male* N. dorsatus *cicadas.* Immediately after infection, there is a rapid, precipitous drop in the amplitude of a male cicada's mating call. Amplitude change is usually detectable within just a few hours after infection, and by 12 hours after infection, a cicada's call amplitude might have declined by more than 10 dB SPL (a 10-fold or more decrease in acoustic power). These amplitude changes are also accompanied by changes to the spectral structure of the call. Although the absolute peak frequency of the call does not change (on average), there is a decline in acoustic power at the upper end of the broader peak frequency band, from about 5.6 kHz to 7.0 kHz, with a stronger negative effect as frequencies increase to 7.0 kHz. These changes are

primarily governed by the elapsed time since infection, as neither the ambient temperature nor the effective clutch size have a significant effect on call amplitude or spectral structure after infection.

Eventually, all parasitized cicadas completely lose their ability to produce any sound at all. On average, call loss occurs about 37 hours after infection, but the timing is strongly dependent on ambient temperature and possibly also on the effective clutch size. With relatively low ambient temperatures and small clutch sizes, it can take more than 50 hours before a host cicada becomes mute.

The mating calls of male *N. dorsatus* before and after parasitization (but before muting) are different enough that they are readily distinguishable even to human ears, especially the decrease in amplitude. With a little practice, it is possible to identify parasitized cicadas in the field merely by hearing them call, as I have confirmed on many occasions while collecting *N. dorsatus* for my studies of *E. erro*.

*Third, the calls of parasitized male* N. dorsatus *are much less attractive to* E. erro *than are the calls of unparasitized cicadas.* Earlier work on the signal preferences of *E. erro* determined that these flies prefer high amplitude, relatively high frequency acoustic signals (see Chapter 4), so given the amplitude and frequency-related changes to the call of *N. dorsatus* after infection, it was not surprising that the calls of infected cicadas were less attractive. Indeed, the traps with the signal representing an uninfected cicada captured more than 10 times as many flies as either of the traps with signals representing infected cicadas. The results also show that the effect is about as strong at 6 hours after infection as at 12 hours after infection, so the change in signal attractiveness happens swiftly once a cicada is parasitized. On average, then, infected cicadas have a much smaller chance of being discovered by *E. erro* than do uninfected cicadas,

and the parasitoid larvae living inside of infected cicadas should benefit from a greatly reduced risk of superparasitism.

These conclusions are only valid if the broadcast choice experiments were a reasonable facsimile of the conditions likely to be encountered by female *E. erro* searching for hosts in the field. Of course, some aspects of the experimental design were necessarily artificial, such as the use of the traps, continuous broadcasts for 10 minutes at a time, and perfect rotation of the test signals at each trap position. However, in their essential details, the choice experiments were analogous to biological reality. The test signals were carefully constructed to provide accurate approximations of the average mating calls of *N. dorsatus* cicadas, the trap spacing (ten meters) was a reasonable representation of the spacing between calling male *N. dorsatus* in the field (B. Stucky, unpublished data), and the simultaneous broadcast of the signal choices was also realistic because male *N. dorsatus* within "earshot" of each other in the field often partially synchronize their calling behavior and sing simultaneously (Cole 2008, B. Stucky, pers. obs.). In addition, all broadcast trials were conducted during times of the day, year, and in weather conditions in which adult *N. dorsatus* were naturally active. Therefore, it seems reasonable to conclude that the choices faced by *E. erro* during the broadcast trials were similar to the choices they face when hunting for hosts in the field, and that the changes to *N. dorsatus*'s mating call after parasitization really do help protect *E. erro* larvae from superparasitism.

Taken together, these three key sets of results tell a compelling story of host communication exploitation by *E. erro* larvae, and they offer substantial evidence to support the hypothesis that *E. erro* larvae modify the acoustic signals of their hosts for their own benefit.

***Active manipulation, accidental side effect, or host response?***

There can be little doubt that the mating calls of *N. dorsatus* change after they are parasitized by larval *E. erro*, but the causative mechanisms behind those changes are still not entirely clear. Although it is tempting to conclude that the *E. erro* larvae are actively manipulating the communication systems of their hosts, host manipulation is not the only plausible explanation, and it is often difficult to determine whether physiological, developmental, or behavioral changes to hosts after parasitization are truly a result of direct manipulation by parasitoids (Godfray 1994, Brodeur and Boivin 2004). In the case of *E. erro* and *N. dorsatus*, there are at least three possible (and not strictly mutually exclusive) explanations for why the host cicadas' mating calls change. Either 1) *E. erro* larvae directly and deliberately modify their host's communication signals as an adaptive manipulation of the host; 2) the signal changes are merely a passive, pathological consequence of the trauma of parasitization; or 3) the signal changes are an adaptive response by the host cicadas that also happens to benefit the parasitoids. Although the evidence at present is insufficient to discriminate among these hypotheses with complete certainty, the weight of the evidence suggests that *E. erro* larvae are actively manipulating their hosts and that the alternative explanations are unlikely, for at least four reasons.

First, and perhaps most important, the post-parasitization changes to host cicadas' mating calls are almost certainly beneficial for *E. erro* larvae, and this is, by itself, strong evidence in favor of active host manipulation. Assuming that, as my results suggest, *E. erro* larvae can increase their fitness by modifying their host's mating calls, then any larval behaviors that contribute to those call changes should be selectively advantageous. Given the results of this

study, it seems unlikely that the changes to cicadas' mating calls after parasitization are not at least partially an outcome of adaptive evolution by the parasitoids.

Second, the activities of *E. erro* larvae inside of their hosts are also indicative of deliberate host manipulation by the parasitoids. As the results of the host dissections reveal, after entering their host's body, first-instar *E. erro* larvae immediately migrate to the sound-producing structures in the cicada's abdomen. This occurred regardless of where the larvae entered the host. Thus, the swift drop in amplitude of the mating call of a newly infected cicada corresponds with the movement of *E. erro* larvae to the sound-producing organs.

How *E. erro* larvae actually induce changes to their host's mating call is not known. Considering that at least one larva in the dissected hosts was directly attacking the tymbal muscles at just over an hour after infection, a simple explanation is that physical attack is at least partially responsible for the initial sound changes. It seems somewhat improbable, though, that the minute first-instar larvae would be able to inflict enough damage in such a short time to fully account for the observed changes, and the absence of obvious damage to the tymbal muscles of most of the dissected cicadas in the early hours after infection casts further doubt on this explanation. Alternatively, because the majority of recovered larvae were located on the tymbals, tymbal apodemes, or tymbal muscles, it might be that the larvae's physical presence on these structures alters the acoustic properties of the sound-producing system (e.g., by changing the mass of vibrational components). Other explanations are possible too, of course, such as larval chemical secretions that affect the tymbal muscles in some way. In any case, although the exact mechanism by which the first-instar larvae change their host's signals is unknown, the precision, consistency, and rapidity of their movements in the host certainly suggest that the changes are more than an accidental side effect of parasitization.

By the time a host becomes mute, the causative mechanism is much more clear. In every mute host that was examined, the tymbal muscles were severely damaged. In most cases, the muscles were partially or completely detached from the floor of the abdomen. These changes were certainly due to the *E. erro* larvae, because in uninfected cicadas, the tymbal muscles usually remain intact even after death, and calling ability often persists in a dying cicada even after the loss of coordinated locomotory abilities (B. Stucky, pers. obs.). Thus, when a host *N. dorsatus* finally loses the ability to call, it is because its tymbal muscles have been completely disabled by its parasitoid larvae. As with the activities of first-instar *E. erro* larvae early in infection, the eventual assault by later instars on their host's tymbal muscles is both precise and predictable, and it suggests deliberate larval behavior.

Third, if a host's mating call changes were merely a symptom of an overall decline in the host's condition, then one would expect to see other behavioral changes that were also indicative of host decline, such as increased lethargy and inactivity. This was not the case. Although other host behaviors were not quantified in this study, no obvious behavioral changes in infected cicadas were observed until near the very end of their lives, long after they were already mute, when they lost their ability to fly and became relatively inactive. To the contrary, parasitized cicadas were typically just as wary and difficult to capture in the field as unparasitized cicadas, and in captivity, they attempted to engage in the usual male behaviors of calling and mating, even after they were completely muted. For example, when male *N. dorsatus* call, they perform a stereotyped, visually distinctive sequence of behaviors in which they partially spread and lower their wings while simultaneously raising and extending their abdomens. On numerous occasions, I observed mute, male, host cicadas go through this complete calling behavior sequence as they attempted to join in the chorus of other male *N. dorsatus* calling nearby. Given

these observations, along with the known activity of the larvae inside of their hosts, it is unlikely that the alteration and eventual loss of the mating calls of parasitized male *N. dorsatus* are due merely to a general loss of host vitality.

Fourth, while there are certainly examples of host changes after infection that benefit the parasitoid and could also be adaptive for the host (Brodeur and Boivin 2004), it is doubtful that this is the case for *N. dorsatus* and *E. erro*. Once parasitized, a male *N. dorsatus* has only a few days left in which to mate before he dies. In this context, an impaired mating call could only be a detriment because it would make it difficult or impossible for the cicada to acquire any last mating opportunities. Even if the signal changes make it less likely that the parasitized cicada will attract any more parasitoids or generalist predators (see the next section), these outcomes primarily benefit the parasitoid larvae, not the host. After all, the host cicada's fate is already sealed, and if the mating call changes mean that the cicada's probability of further reproductive success is nearly zero, then protection against predators and parasitoids is of little or no fitness consequence. In short, it is difficult to imagine any sort of adaptive advantage of the mating call changes for the host cicadas themselves.

***Do host call changes also help* E. erro *escape from predation?***

This study has focused on the adaptive advantage of host signal modification to avoid superparasitism, but a complementary hypothesis is that host signal modification also benefits larval *E. erro* in another way, by reducing their risk of predation. The idea is that calling behavior is risky for a male *N. dorsatus* (and any parasitoid larvae inside of it) because the acoustic calls are conspicuous and might attract generalist predators. By decreasing the amplitude of their host's mating call and eventually eliminating it altogether, larval parasitoids could reduce the chance that a predator will find their host.

Indeed, cicadas' loud, persistent mating calls should be detectable by a wide variety of vertebrate predators, yet there seems to be remarkably little published, empirical evidence that any generalist predators actually hunt cicadas by ear (ignoring the fact that acoustic orientation is a favored strategy of human cicada collectors). There are a few reports of birds possibly locating cicadas by their calls (Swinton 1880, Brues 1950, Doolan and MacNally 1981, Gosper 1999), as well as a cat (Myers 1929), but the records are mostly anecdotal or speculative. Myers (1929) considered Swinton's (1880) report of birds following cicada songs to be doubtful. There are also many records of avian predators congregating to feed at the dense, noisy choruses of periodical cicadas (*Magicicada* sp.) (e.g., Forbush 1924, Jones 1934, Howard 1937, Strehl and White 1986, Koenig and Liebhold 2005), but it is not clear what role sound played in attracting the birds or if individual cicadas were actually hunted by sound. Some authors have even suggested that loud cicada choruses might actually repel avian predators (Simmons et al. 1971, Moulds 1990).

Despite the limited evidence that cicadas are hunted acoustically, it is clear that an assortment of avian, mammalian, lizard, and possibly amphibian predators are able to hunt for crickets or katydids by homing in on their mating calls (Walker 1964b, 1979, Bell 1979, Buchler and Childs 1981, Sakaluk and Belwood 1984, Tuttle et al. 1985, Belwood and Morris 1987, Hosken et al. 1994, Bailey and Haythornthwaite 1998, Jones et al. 2011), so it seems likely that cicadas at least occasionally fall victim to similar tactics. Nevertheless, it is currently unknown if, or how often, male *N. dorsatus* are attacked by acoustically orienting predators. If they are, then predator avoidance is undoubtedly an additional benefit of host signal modification for *E. erro*, but more research is needed to test this hypothesis.

***The evolution of host signal manipulation***

As mentioned briefly above, natural selection operating on the larval parasitoid offers a straightforward explanation for how host signal modification could have evolved in the *E. erro*/*N. dorsatus* system. Larval *E. erro* that can acoustically "hide" their hosts from searching female parasitoids (and possibly also from predators) are expected to have a competitive advantage over those that cannot, and this would provide the selective pressure needed to drive the evolution of direct manipulation of the host's communication system. One might imagine that the path toward host manipulation began with ancestral parasitoids that incidentally attacked their host's sound-producing structures while feeding on the host, and that these early behaviors were elaborated and refined by natural selection to result in the pattern of mating call amplitude and spectral changes, and eventual host muting, that we see today.

In addition to the obvious role of the larvae in the evolution of host signal manipulation, it is likely that selection on adult female flies has also shaped the dynamics of this system. Specifically, female flies with the ability to accurately discriminate between the signals of uninfected and infected cicadas should have an advantage over those that cannot, because they will be able to reliably choose uninfected, competition-free hosts for their offspring. Natural selection should therefore favor female flies that can accurately determine the parasitization state of a potential host from its mating call.

However, because larval *E. erro* change the amplitude of their host's calls, both the larvae and female flies will benefit even if female flies *do not* actively choose the calls of uninfected cicadas. This is simply because the detectable range of high amplitude calls is larger than that of low amplitude calls, so quiet mating calls will automatically attract fewer female *E. erro* regardless of whether female flies actively choose the signals of uninfected cicadas (see Forrest

and Raspet (1994) for a thorough discussion of this phenomenon). In other words, larval *E. erro* could simply be hiding their hosts from searching female flies in the absence of any real female signal preference. It can be difficult to determine whether differential attractiveness of signals that vary in amplitude is a result of true choice or merely due to the passive effects of signal visibility. Distinguishing between these possibilities requires knowing the behavioral threshold of hearing for the signal receiver (Forrest and Raspet 1994), but such data are not currently available for *E. erro*.

In the case of *N. dorsatus*, mating call amplitude changes after infection are also accompanied by changes to the frequency structure of the calls, which could provide another source of information for female flies to use in signal discrimination. Earlier research on the signal preferences of female *E. erro* (see Chapter 4) indicates that the frequency changes to the calls of host cicadas at least roughly correspond with the frequency preferences of *E. erro*, but more work will be needed to establish whether call frequency changes after parasitization actually play a role in signal attractiveness.

In the absence of conclusive evidence of active female choice, though, it does not seem too great a leap to at least conclude that some degree of active signal preference by *E. erro* is likely, even considering nothing more than the fitness benefits of being able to avoid host cicadas that are already parasitized. If this is the case, then female signal preferences have probably coevolved with host signal manipulation by the larvae: As ancestral *E. erro* larvae began to modify the signals of their hosts, natural selection would have favored signal changes that played to the existing preferences of female flies. Female flies, in turn, would have been selected for the ability to detect the signal changes caused by the larvae, which could have led to further refinement of signal modification on the part of the larvae, and so on.

If the avoidance of superparasitism and acoustically orienting predators has driven the evolution of host signal manipulation by *E. erro*, then the timing of host muting poses a problem. If the larva's goal is to acoustically hide its host, then why wait to completely silence the host until several days after infection? From the perspective of the larval parasitoid, this strategy is clearly suboptimal. As the results of the broadcast choice experiments demonstrate, female *E. erro* can and do respond to the calls of infected cicadas, so larval parasitoids undoubtedly are exposed to greater risk of superparasitism before their host is silenced than after. Larval *E. erro* seem to only lose by allowing their hosts to continue to call.

The simplest answer to this problem is that larval *E. erro* actually are silencing their hosts as soon as they can, and that physical constraints or host defenses preclude them from muting their hosts more quickly. If muting requires physical destruction of the tymbal muscles, than it might be that the larvae are simply incapable of inflicting enough damage until they grow to a certain size or have had enough time inside of the host. Furthermore, as is discussed in more detail below, host cicadas might actively resist the parasitoid larvae, which could also impose limits on how rapidly larvae can eliminate their host's calling abilities.

From the perspective of the adult female fly, though, immediate host muting might not be the optimal strategy for host signal manipulation, because female flies might not always want to strictly avoid superparasitism. Indeed, both theoretical predictions and experimental evidence indicate that under certain conditions, such as when unparasitized host animals are rare, female parasitoids should *choose* to superparasitize (van Alphen and Visser 1990, Godfray 1994, Dorn and Beckage 2007). In some populations of *N. dorsatus*, *E. erro* parasitism rates of male cicadas can exceed 75% (Stucky 2015). When parasitism rates are that high, uninfected male cicadas are probably difficult to find, but if infected cicadas are still detectable by *E. erro*, then female flies

can still locate hosts for their larvae, even if they have no choice but to superparasitize. In such situations, risking lower fitness for offspring because of superparasitism is a better proposition than leaving no offspring at all. There is anecdotal evidence to suggest this happens with *E. erro*: At field sites with extreme parasitism rates, I have observed female *E. erro* acoustically locate and attack host cicadas that were, judging from their mating calls, already parasitized.

For female flies, being able to detect already-parasitized hosts will sometimes be advantageous. From the point of view of the individual *E. erro* larva that is already inside of a host, superparasitism seems to never be advantageous. This suggests that there could be conflict between females and their offspring with regards to the optimal host manipulation strategy. Whether this potential conflict has been important in the evolution of host signal manipulation is not clear. A key question is whether selection on female flies could lead to host signal modification in which infected hosts remain detectable for some period of time. The problem is that even if a female fly might sometimes want to superparasitize, she would never want other female flies to superparasitize the hosts of *her* offspring. Thus, it might still be in the best interests of a female for her offspring to mute their hosts as quickly as possible.

As a final comment on the evolution of host signal modification, it is important to remember that mating call modification undoubtedly affects the fitness of the host cicadas, too. As was noted earlier, parasitization is always fatal for *N. dorsatus*, so once a male cicada becomes parasitized, there is little he can do except try to seek as many additional mating opportunities as possible before he dies. The actions of *E. erro* larvae are in direct conflict with this goal, because reducing the amplitude of and then eliminating the male's mating call will make it much more difficult, and eventually nearly impossible, for him to attract any further mates. In this context, selection should favor male cicadas that are able to resist *E. erro*'s efforts

to modify their calls, because these males would, on average, be more likely to find mates even after they are parasitized.

If male cicadas that can resist *E. erro*'s manipulative efforts do have a fitness advantage, then there is likely an ongoing evolutionary tug-of-war between larval *E. erro* and their hosts, as *E. erro* larvae attempt to commandeer their hosts' communication systems and male *N. dorsatus* try to maintain a functional mating call as long as possible. In the results of this study, there was considerable variation in the effect of signal modification after infection among the acoustically monitored host cicadas. Considering amplitude, for example, some hosts suffered severe amplitude losses of 10 dB SPL or more by as little as 6 hours after infection, while others exhibited comparatively minor amplitude loss all the way up to the point when they were silenced. It could be that this variability in effect size is at least in part a reflection of the struggle between parasitoids and their hosts for control of the host's communications system.

***Host signal modification by other eavesdropping parasitoids***

This study is the first to demonstrate that the larvae of an acoustically orienting parasitoid can cause their hosts to produce an altered mating call, that the mating call changes can manifest rapidly after infection, and that they cause the host to become much less attractive to female parasitoids, thus substantially reducing the risk of superparasitism for parasitoid larvae. If *E. erro* has discovered this strategy, it seems likely that other acoustic parasitoids might use it, too. Indeed, investigations of other acoustic eavesdropping parasitoids have provided some intriguing clues that this might be the case.

*Emblemasoma auditrix* (Shewell) is the only other species of sarcophagid acoustic parasitoid for which any detailed behavioral or life history information has been published. Like *E. erro*, it is an acoustically orienting parasitoid of cicadas, but *E. auditrix* is a specialist

parasitoid of the cicada *Okanagana rimosa* (Say) (Soper et al. 1976, Lakes-Harlan et al. 2000). At some point after infection, parasitized male *O. rimosa* no longer produce an alarm call (Soper et al. 1976). Soper et al. did not report the effects of parasitization on *O. rimosa*'s mating call, although it seems likely that the mating call is also lost. Neither the timing nor mechanism of the alarm call change were reported, but Soper et al. (1976) concluded that, based on the developmental stages of parasitoid larvae dissected from field-collected hosts, call loss must occur early in the infection process. However, unlike *E. erro*, adult female *E. auditrix* rupture the tymbal membranes of host cicadas as part of their infection behavior (Schniederkötter and Lakes-Harlan 2004), so any post-infection changes to the host's mating call might actually be due to the actions of the female fly, not the larvae inside of the host. If this is correct, then the host call changes caused by *E. auditrix* could in some ways be considered a highly specialized form of host marking: As with the pheromonal host marking practiced by some species of parasitoid wasps (Nufio and Papaj 2001), female *E. auditrix* might be modifying their offspring's' hosts in a way that reduces their chances of being superparasitized.

In general, tachinid acoustic parasitoids of the tribe Ormiini have been far better studied than sarcophagid acoustic parasitoids, and there is evidence from studies of several ormiine species that these flies can induce changes in the calling behaviors of their hosts. As far as is known, all ormiines are acoustically orienting parasitoids of nocturnal crickets and katydids (Lehmann 2003). The hosts of three ormiine parasitoids – *Homotrixa alleni* Barraclough, *Ormia ochracea* (Bigot), and *Therobia leonidei* Mesnil – all exhibit a decline in total time spent calling after they are infected (Cade 1984, Allen 1995, Zuk et al. 1995, Kolluru et al. 2002, Orozco and Bertram 2004, Lehmann and Lehmann 2006, Beckers and Wagner 2011, but see Kolluru 1999). Additionally, Burk (1982a) stated that the katydid hosts of *Ormia lineifrons* Sabrosky cease

calling approximately 5 days after infection, but he provided no further details. Although the total calling time of the hosts of these parasitoids can change, the acoustic properties of the calls themselves apparently do not change, at least not in the case of *O. ochracea* (Beckers and Wagner 2011).

Whether the post-infection decreases in host calling times are due to host manipulation by the larvae or are instead merely a symptom of overall host decline is not known, and the extent to which the decreased calling times might reduce the parasitoid larvae's chances of superparasitism is also poorly known. Adamo et al. (1995) found that female *O. ochracea* were just as likely to superparasitize crickets (*Gryllus* sp.) that had already been parasitized for 3 days as they were to attack unparasitized crickets. However, the host choices in their study were presented serially rather than as a single, simultaneous choice, so it is possible that female *O. ochracea* behave differently when they have options. Further research is needed to test whether or not reduced host calling activity is of adaptive benefit to ormiine parasitoids.

Regardless, the mechanisms of orthopteran sound production probably limit ormiine parasitoids' options for host manipulation. Unlike cicadas, which have dedicated sound-producing organs, katydids and crickets produce their calls by stridulating with their wings. Sound production is therefore dependent on the wings and flight musculature (Bailey 1991), and if flight is important to the hosts' survival, then any actions by the parasitoids that impair these structures could end up being detrimental to both the host and the parasitoids. This might at least partially explain why the post-infection signaling changes of ormiine hosts are, so far as currently known, limited to reductions in total time spent calling rather than changes to the calls themselves.

Finally, there is no reason why eavesdropping parasitoids that exploit pheromone communication signals could not also modify their hosts' signals to avoid superparasitism. I am unaware of any study that has investigated this possibility, and this could be a fruitful direction for future research.

## 5.5 Conclusions

For many parasitoid species, competition or conflict caused by superparasitism can take a heavy toll on a developing parasitoid's reproductive fitness by causing either reduced body size or death. Data from field observations and hundreds of rearings confirm that *E. erro* larvae are not exempt from this danger. My results strongly suggest that larval *E. erro* mitigate the threat of superparasitism by altering the communication signals of their host cicadas to make them far less detectable, and possibly also less enticing, to female parasitoids foraging for hosts. Eventually, larval parasitoids rob their hosts of the ability to produce any sound at all, effectively rendering them invisible to host-searching female parasitoids.

The extent of *E. erro*'s exploitation of cicada communication is remarkable. These flies use the communication system of *N. dorsatus* cicadas to find hosts, to find mates, and, as this study has shown, to avoid superparasitism. I am unaware of any other eavesdropping parasitoids that have achieved such multifaceted integration of host communication into their life histories. It would be interesting to know if other species of *Emblemasoma* exhibit this same suite of signal exploitation behaviors, or if it has evolved in other, unrelated lineages of eavesdropping parasitoids.

I will close by suggesting that this study also points to a new direction for theoretical research on the problem of superparasitism. For the last several decades, superparasitism has been a topic of major importance in parasitoid research (see, e.g., reviews by van Lenteren 1981,

van Alphen and Visser 1990, Godfray 1994, Dorn and Beckage 2007). Both theoretical and empirical work on this topic has focused on the role of adult female parasitoids, and rightly so: There is voluminous evidence that female parasitoids of many species are able to discriminate between parasitized and unparasitized hosts and therefore avoid superparasitism, if desired (Salt 1961, van Lenteren 1981, Godfray 1994, Nufio and Papaj 2001). For at least some eavesdropping parasitoids, though, the actions of the female are clearly only part of the story. This study suggests that larval host manipulation to reduce superparasitism can operate independently of decision making by female parasitoids, which raises intriguing new questions about the evolutionary ecology of superparasitism. For example, under what circumstances should host signal manipulation evolve? What role might selection on the larva and selection on the adult female play in its evolution? Could host marking by adult females and host manipulation by larvae coexist as complementary strategies? These and related questions should prove to be fertile ground for developing new theory recognizing that both female parasitoids and their offspring can play a part in managing the risk of superparasitism.

# CHAPTER 6

## THE EVOLUTION OF HEARING AND ACOUSTIC EAVESDROPPING IN SARCOPHAGIDAE

### 6.1 Introduction

Female parasitoids of most parasitoid species face the challenge of finding suitable host animals for their offspring. Host animals, which are likely to lose their life if they are discovered and attacked by a parasitoid, must do their best to ensure that parasitoids cannot find them. Natural selection acting in these two opposing directions has led to a common pattern in parasitoid/host relationships: Host animals are often well hidden and difficult to detect, and, as a consequence, female parasitoids must rely on indirect sources of information, such as volatiles emanating from their host's food plants, to help them find their hosts (Vinson 1976, Godfray 1994). Such host-finding strategies have been remarkably successful, but they suffer from the basic problem that the key long-range information sources that parasitoids use are indirect and inherently unreliable.

As an alternative strategy, some parasitoids have discovered that their hosts' long-range communication signals can allow for highly efficient, accurate host location that obviates the need for indirect sources of information (Zuk and Kolluru 1998, Haynes and Yeargan 1999, see also Chapter 1). Even host insects that are normally cryptic and inconspicuous might still need to communicate with conspecifics, and when they do, they may have little choice but to advertise their presence to any parasitoids that can eavesdrop on the conversation. Sexual advertisement signals have proven especially useful in this regard (Zuk and Kolluru 1998, see also Chapter 1), because they are often selected for long-distance detectability (Burk 1988, Greenfield 2002).

Eavesdropping is not without its own challenges, however. Most obviously, a parasitoid cannot eavesdrop if it cannot detect its hosts' communication signals. This is not a trivial

problem, because long-distance insect sexual communication signals are usually species-specific (Alexander 1967, West-Eberhard 1984), and detecting them requires sophisticated, finely-tuned sensory structures (Haynes and Yeargan 1999). Furthermore, the host communications intercepted by eavesdropping parasitoids often have little in common with the parasitoids' own intraspecific communication systems (see Chapter 1), which means a parasitoid's communications repertoire might be of little help in becoming an eavesdropper. How, then, do parasitoids acquire the sensory structures required for eavesdropping?

There are two basic answers to this question (which are not strictly mutually exclusive). The first is that host finding itself is the selective pressure that leads to the evolution of the new sensory structures required for eavesdropping (Robert et al. 1992, 1996, Lakes-Harlan et al. 1999) (hereafter referred to as the "host-finding hypothesis"). An alternative hypothesis is that these sensory structures are exaptations (Gould and Vrba 1982) that originally arise for some other purpose and are later co-opted for eavesdropping. The host-finding hypothesis has been widely discussed (e.g., Robert et al. 1992, 1996, Lakes-Harlan et al. 1999, Yager 1999, Lehmann 2003, Rosen et al. 2009), but strong evidence for it remains elusive. As with many complex evolutionary hypotheses, testing the host-finding hypothesis is challenging. However, the most convincing evidence in support of this hypothesis will come from parasitoid lineages that meet three conditions: 1) the parasitoid lifestyle was already established before eavesdropping evolved, thus ensuring that the need to find hosts was in place as a selective pressure to promote eavesdropping; 2) eavesdropping required the acquisition of an entirely new sensory modality (new to the parasitoid lineage, that is), so that the required sensory structures are synapomorphic for the parasitoid lineage; and 3) the sensory structures and sensory modality are not ancestrally

associated with any uses besides eavesdropping on hosts. So far, there are no eavesdropping parasitoids that are known to meet all three of these criteria.

The best evidence so far for the host-finding hypothesis comes from tachinid flies of the tribe Ormiini, which use hearing to home in on the nocturnal mating calls of their cricket and katydid (Orthoptera: Ensifera) hosts (Lehmann 2003). Although several authors seem to have accepted the host-finding hypothesis as correct in this case (e.g., Lehmann 2003, Rosen et al. 2009), the evidence is inconclusive. These flies certainly meet the first criterion because all tachinids with known life histories are parasitoids (Wood 1987, Stireman et al. 2006), which means that the parasitoid lifestyle undoubtedly preceded the evolution of eavesdropping. These flies also meet criterion two, because "ears" are not found anywhere in Tachinidae besides Ormiini. The third criterion is the problem. Ormiines' nocturnal habits probably expose them to bat predation, so it could be that these flies initially evolved hearing to escape bats, a phenomenon that has certainly occurred in other insect orders (Yager 1999, Stumpner and von Helversen 2001). In fact, at least two species of ormiine, *Ormia ochracea* (Bigot) and *Therobia leonidei* (Mesnil), have ears that are sensitive to the ultrasonic frequencies used by bat sonar (Robert et al. 1992, Stumpner and Lakes-Harlan 1996), and *O. ochracea* also exhibits an evasive behavioral response to ultrasound (Rosen et al. 2009). Furthermore, at least some species of the probable sister group of Ormiini, the non-hearing tribe Glaurocarini, are apparently also nocturnal parasitoids of orthopterans (Crosskey 1965, Lehmann 2003). This suggests that nocturnality preceded the evolution of eavesdropping in Ormiini, which lends further support to the hypothesis that bat predation was an important factor in the evolution of ormiine hearing. Also, despite claims to the contrary (Rosen et al. 2009), the fossil record is unclear about the origins of tachinid flies, and it is quite plausible that echolocating bats were already a part of the

nocturnal soundscape by the time ancestral ormiines began to evolve hearing (Robert et al. 1996, Lehmann 2003, Grimaldi and Engel 2005, Stireman et al. 2006).

A better test of the host-finding hypothesis might be provided by a different group of acoustic eavesdropping parasitoids: sarcophagid flies of the genus *Emblemasoma*, which use cicadas (Hemiptera: Cicadidae) as their hosts (Soper et al. 1976, Stucky 2015). Like the ormiines, *Emblemasoma* have ears for long-range host finding that are not found anywhere else in their family (Lakes-Harlan et al. 1999). Unlike the ormiines, *Emblemasoma* species and their hosts are known to be diurnal (Lakes-Harlan et al. 1999, 2000), which avoids the major problem with interpreting the evolution of hearing in Ormiini. However, in contrast to tachinids, which are all parasitoids, sarcophagids encompass a great diversity of lifestyles, including scavengers, predators, parasites, and parasitoids (Ferrar 1987, Shewell 1987, Pape 1996, Povolný and Verves 1997, Pape and Dahlem 2010). Therefore, phylogenetic information about *Emblemasoma* is needed to assess whether the parasitoid lifestyle was established prior to the evolution of eavesdropping, but no such information is currently available. To further complicate matters, the species currently placed in *Emblemasoma* were originally classified in three separate genera (*Colcondamyia*, *Emblemasoma*, and *Pessoamyia*) until Pape (1990) synonomized all three genera. Despite being synonymized, these genera are morphologically distinct and their evolutionary histories are unknown.

In this study, I analyzed whether the host-finding hypothesis provides the best explanation for the evolution of hearing in *Emblemasoma* (*sensu lato*). I first generated new mitochondrial and nuclear DNA sequence data from representatives of all three genera currently synonomized with *Emblemasoma* as well as representatives of several other sarcophagid genera, including the parasitoid-rich genus *Blaesoxipha* (Pape 1994), some species of which exhibit

behavioral similarities to *Emblemasoma* parasitoids (Stucky 2015). Using these data, along with sequence data harvested from GenBank (http://www.ncbi.nlm.nih.gov/genbank/), I completed a new phylogenetic analysis of Sarcophagidae, then used the results to infer the evolutionary history of larval feeding strategies within the sarcophagid subfamily Sarcophaginae (which includes *Emblemasoma*). After presenting these results, I discuss 1) their implications for our understanding of sarcophagid phylogeny and taxonomy; 2) the evolution of larval feeding strategies in the subfamily Sarcophaginae; and 3) the evolution of hearing and acoustic eavesdropping in Sarcophagidae. I argue that *Emblemasoma* provides the best evidence yet in support of the host-finding hypothesis.

## 6.2 Methods

### *Phylogeny of Sarcophagidae*

*Specimens for DNA sequencing*

New specimens representing a variety of sarcophagid genera and species were collected for DNA extraction and sequencing. Collecting efforts were primarily focused on obtaining representatives of the three genera currently synonymized with *Emblemasoma* (*Colcondamyia*, *Emblemasoma* (*sensu stricto*), and *Pessoamyia*) from a variety of geographic localities. Additional specimen collecting was limited to the sarcophagid subfamily Sarcophaginae, which includes *Emblemasoma* along with the vast majority of sarcophagid species, accounting for about three fourths of the total species diversity in the family (Piwczyński et al. 2014). Representatives of the genus *Blaesoxipha* were especially sought because *Blaesoxipha* includes a large number of parasitoid species (Pape 1994), at least some of which have behavioral similarities to some species of *Emblemasoma* (Stucky 2015), and morphological evidence has

previously suggested that the species of *Emblemasoma* formerly placed in *Colcondamyia* might be closely related to *Blaesoxipha* (Pape 1987, 1990).

Most specimens of *Emblemasoma* were collected by capturing adult flies at the broadcasts of acoustic signals representing the calling songs of known or potential host cicadas. The methods for constructing these acoustic signals are described in detail in Stucky (2015). Briefly: Audio recordings of known or potential host cicada species were gathered in the field, these recordings were analyzed to estimate the mean values of multiple acoustic parameters (e.g., peak frequency, various measures of temporal structure), and model acoustic signals were constructed to match, as closely as possible, the mean calling song for each cicada species. For the cicada *Okanagana rimosa* (Say), host of the parasitoid *Emblemasoma auditrix* (Shewell), the calling song analysis results of Lakes-Harlan et al. (2000) were used. Model calling song signals were constructed either from components of the field audio recordings or by generating synthetic signals from amplitude-modulated sine waves. The model calling songs were broadcast in the field using a loudspeaker, and attracted flies were captured either by hand or using a custom-built live trap (B. Stucky, in prep.). Some specimens of *Emblemasoma* were obtained by rearing adult flies from parasitized cicadas following the rearing methods described in Stucky (2015).

Specimens of other, non-*Emblemasoma*, sarcophagid species were opportunistically collected by hand in the field or by attracting flies to carrion baits. For bait, fresh carcasses of channel catfish (*Ictalurus punctatus*) or black bullhead catfish (*Ameiurus melas*) were placed in the field for 24-48 hours, and adult sarcophagids that were attracted to the dead fish were captured by hand.

Most specimens captured for DNA sequencing were collected directly into, and subsequently stored in, 95% ethanol. In some cases, two legs were removed and stored in

ethanol so that the remainder of the specimen could be pinned and spread for identification purposes. A few specimens were pinned without removing any appendages and stored dry until used for DNA extraction.

Collecting localities for all specimens are given in Table 6.1. In total, 41 specimens, representing 21 species or putative species, were newly collected and sequenced for this study (Table 6.1).

**Table 6.1** New specimens collected and sequenced for this study.

| Species | Authority | Year | Locality |
|---|---|---|---|
| *Blaesoxipha* (*Acanthodotheca*) sp. | n/a | 2014 | Juab Co., UT, USA |
| *B.* (*Gigantotheca*) *impar* | (Aldrich 1916) | 2011 | McPherson Co., KS, USA |
| *B.* (*Gigantotheca*) *plinthopyga* | (Wiedemann 1830) | 2014 | Millard Co., UT, USA |
| *B.* (*Kellymyia*) *kellyi* | (Aldrich 1914) | 2012 | Hamilton Co., KS, USA |
| *B.* (*Kellymyia*) *kellyi* | (Aldrich 1914) | 2013 | Sherman Co., KS, USA |
| *Boettcheria cimbicus* | (Townsend 1892) | 2014 | McPherson Co., KS, USA |
| *Emblemasoma* (*Colcondamyia*) *auditrix* | (Shewell 1976) | 2012 | Crawford Co., MI, USA |
| *E.* (*Colcondamyia*) *auditrix* | (Shewell 1976) | 2012 | Emmet Co., MI, USA |
| *E.* (*Colcondamyia*) *auditrix* | (Shewell 1976) | 2012 | Mackinac Co., MI, USA |
| *E.* (*Colcondamyia*) *auditrix* | (Shewell 1976) | 2012 | Mackinac Co., MI, USA |
| *E.* (*Emblemasoma*) *erro* | Aldrich 1916 | 2012 | Socorro Co., NM, USA |
| *E.* (*Emblemasoma*) *erro* | Aldrich 1916 | 2012 | Lincoln Co., NM, USA |
| *E.* (*Emblemasoma*) *erro* | Aldrich 1916 | 2012 | Prowers Co., CO, USA |
| *E.* (*Emblemasoma*) *erro* | Aldrich 1916 | 2011 | Kingman Co., KS, USA |
| *E.* (*Emblemasoma*) *erro* | Aldrich 1916 | 2012 | Harvey Co., KS, USA |
| *E.* (*Emblemasoma*) *erro* | Aldrich 1916 | 2012 | Reno Co., KS, USA |
| *E.* (*Emblemasoma*) *erro* | Aldrich 1916 | 2012 | Ellsworth Co., KS, USA |
| E. (Emblemasoma) nr. *erro* | n/a | 2011 | Marion Co., FL, USA |
| E. (Emblemasoma) nr. *erro* | n/a | 2011 | Marion Co., FL, USA |
| *E.* (*Emblemasoma*) *faciale* | Aldrich 1916 | 2011 | Berrien Co., GA, USA |
| *E.* (*Emblemasoma*) *faciale* | Aldrich 1916 | 2011 | Berrien Co., GA, USA |
| *E.* (*Emblemasoma*) *faciale* | Aldrich 1916 | 2011 | Marion Co., FL, USA |
| *E.* (*Emblemasoma*) *faciale* | Aldrich 1916 | 2011 | Baker Co., FL, USA |
| *E.* (*Emblemasoma)* sp. | n/a | 2011 | Mesa Co., CO, USA |
| *E.* (*Emblemasoma)* sp. | n/a | 2014 | Juab Co., UT, USA |
| *E.* (*Emblemasoma)* sp. | n/a | 2014 | Juab Co., UT, USA |
| *E.* (*Emblemasoma)* sp. | n/a | 2014 | Juab Co., UT, USA |

| Species | Authority | Year | Locality |
|---|---|---|---|
| *E.* (*Pessoamyia*) *emblemasoma* | (Dodge 1968) | 2013 | Barro Col. Is., Panama |
| *E.* (*Pessoamyia*) *emblemasoma* | (Dodge 1968) | 2013 | Barro Col. Is., Panama |
| *E.* (*Pessoamyia*) *emblemasoma* | (Dodge 1968) | 2013 | Barro Col. Is., Panama |
| *E.* (*Pessoamyia*) *emblemasoma* | (Dodge 1968) | 2013 | Barro Col. Is., Panama |
| *Oxysarcodexia ventricosa* | (van der Wulp 1895) | 2013 | McPherson Co., KS, USA |
| *Ravinia derelicta* | (Walker 1953) | 2014 | McPherson Co., KS, USA |
| *R. lherminieri* | (Robineau-Desvoidy 1830) | 2014 | McPherson Co., KS, USA |
| *R. vagabunda* | (van der Wulp 1895) | 2014 | McPherson Co., KS, USA |
| *Sarcophaga* (*Bercaea*) *africa* | (Wiedemann 1824) | 2014 | McPherson Co., KS, USA |
| *S.* (*Liopygia*) *crassipalpis* | Macquart 1839 | 2012 | Boulder Co., CO, USA |
| *S.* (*Liosarcophaga*) *sarracenioides* | Aldrich 1916 | 2014 | McPherson Co., KS, USA |
| *S.* (*Neobellieria*) *bullata* | Parker 1916 | 2014 | McPherson Co., KS, USA |
| *S.* (*Neobellieria*) *cooleyi* | Parker 1914 | 2012 | Boulder Co., CO, USA |
| *S.* (*Wohlfahrtiopsis*) *arizonica* | (Townsend 1919) | 2014 | Millard Co., UT, USA |

*DNA extraction, amplification, and sequencing*

For DNA extraction, two legs (a fore and middle leg) were removed from each fly specimen, placed in a 1.5 ml microcentrifuge tube with PBS (phosphate-buffered saline), and ground using a microtube pestle. For the smallest fly specimens, three legs were used. Total DNA was extracted and purified from the ground fly legs using the Qiagen DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany), following the Qiagen supplementary protocol for insects. The only difference from the published protocol was that the final elution step was performed as two separate 90 µl elutions, for a final extraction volume of 180 µl.

For each fly specimen, fragments of 4 mitochondrial and 3 nuclear genes were amplified by PCR and then sequenced. The mitochondrial genes were the protein-coding genes cytochrome oxidase subunit 1 (CO1) and cytochrome B (CytB), and the ribosomal RNA-coding genes 12S and 16S. The nuclear genes were the protein-coding gene elongation factor 1-alpha (EF1-α) and the ribosomal RNA-coding genes 18S and 28S. These genes were selected to

provide a mix of nuclear, mitochondrial, protein-coding, and non-protein-coding genes, as well as to align with existing sarcophagid sequence data in GenBank (http://www.ncbi.nlm.nih.gov/genbank/), especially the sequencing efforts of Kutty et. al (2010).

The primers used for PCR amplification are given in Table 6.2. Candidate calyptrate fly primer sequences from the literature (primarily Simon et al. (1994), Kutty et al. (2007), and Feng-Yi Su et al. (2008)) and from S.N. Kutty (pers. comm.) were aligned with known sarcophagid sequences from GenBank and modified, if appropriate, to better match sarcophagid

**Table 6.2** Primers used for PCR amplifications. "(F)" and "(R)" after the primer names indicate forward and reverse primers, respectively.

| Gene | Primer name | Primer sequence (5' to 3') | References |
|---|---|---|---|
| 12S | 12Sr_cal (F) | CCCTGATACACAAGGTA | Feng-Yi Su et al. (2008), S.J. Kutty, pers. comm. |
| | 12Sai (R) | AAACTAGGATTAGATACCCTATTA | Kutty et al. (2007) |
| 16S | LR-J-12887 (F) | CCGGTTTGAACTCAGATCATGT | Simon et al. (1994) |
| | LR-N-13398 (R) | CGCCTGTTTAACAAAAACAT | Simon et al. (1994) |
| CO1, first half | TY-J-1460 (F) | TACAGTCTATTGCCTAAATTTCAGCC | modified from Simon et al. (1994) |
| | C1-N-2191 (R) | CCWGGTAAAATTAAAATATAAACTTC | modified from Simon et al. (1994) |
| CO1, second half | C1-J-2183 (F) | CAACATTTATTTTGATTYTTYGG | modified from Simon et al. (1994) |
| | TL2-N-3014 (R) | TCCATTGCACTAATCTGCCATATTA | Simon et al. (1994) |
| CytB | CB-J-10933 (F) | TATGTATTACCATGAGGGCAAATATC | modified from Simon et al. (1994) |
| | TS1-N-11683 (R) | AATTTCTATCTTATGTTTTCAAAAC | modified from Simon et al. (1994) |
| EF1-α | M-441 (F) | CAGGAAACAGCTATGACCGCTGAGCGY GARCGTGGTATCAC | Kutty et al. (2007) |
| | rcM4 (R) | ACAGCVACKGTYTGYCTCATRTC | Kutty et al. (2007) |
| 18S | 18sf (F) | CATATCCGAGGCCCTGTAAT | S.N. Kutty, pers. comm. |
| | 18sr (R) | AGTTTTCCCGTGTTGAGTCA | S.N. Kutty, pers. comm. |
| 28S | rc28A (F) | AGCGGAGGAAAAGAAAC | Kutty et al. (2007) |
| | 28C (R) | GCTATCCTGAGGGAAACTTCGG | Kutty et al. (2007) |
| 28S | 28BJSf (F) | GGCATTTCCAAAGAGTCGTG | this chapter |
| | 28BJSr (R) | CGGTCTTCCATCAGGGTTTC | this chapter |

DNA. In the case of the nuclear gene 28S, the primers rc28A and 28C (Kutty et al. 2007) did not always cleanly amplify sarcophagid DNA, so a new primer pair for 28S was created ("28BJSf" and "28BJSr" in Table 6.2). These primers were designed using the 28S sequence of *Sarcophaga crassipalpis* Macquart from Singh and Wells (2013) (which is probably from *S. bullata* Parker, not *S. crassipalpis*; see the section "*Additional DNA sequence data*", below), with the aid of the software Primer3Plus (Untergasser et al. 2007) and Integrated DNA Technologies' OligoAnalyzer 3.1 (https://www.idtdna.com/calc/analyzer). GenBank's nucleotide BLAST (Basic Local Alignment Search Tool) service (Altschul et al. 1990, Johnson et al. 2008) was used to verify, as best as possible, that the new primer sequences were conserved among sarcophagids in general. All primers were purchased from Eurofins MWG Operon (Eurofins MWG Operon LLC, Huntsville, AL, USA).

The PCR protocols for 12S, 16S, the second half of CO1, CytB, EF1-α, and 28S comprised the following steps: 1) an initial denaturation at 94-95° C for 2:00-3:00 min; 2) 30-34 cycles of denaturation at 94-95° C for 0:30-1:00 min, annealing at 46-59° C for 0:15-1:00 min, and extension at 72° for 1:00-1:30 min; and 3) a final extension at 72° for 5:00 min. The annealing times and temperatures were experimentally optimized for each gene and primer pair. Occasionally, up to 38 cycles were needed to produce sufficient PCR product. For the first half of CO1, a "touchup" PCR protocol was used, where the basic reaction steps were the same as for the other genes, but with the annealing temperature gradually incremented as the reaction progressed. The annealing temperatures were 44° (first 4 cycles), 45° (next 10 cycles), and 46° (final 18 cycles). For 18S, a "touchdown" PCR protocol was used, such that for the first 10 cycles, the annealing temperature was decremented by 1° C each cycle from an initial annealing temperature of 61° C to a final annealing temperature of 52° C, which was then used for all

remaining cycles. All PCR reagents except for the primers were purchased from Promega Corporation (Madison, WI, USA). All PCR reactions consisted of 20 µl of GoTaq® Green Master Mix, 0.8-5.6 µl of each primer (at 10 µM), 0.75-5 µl of template DNA, and enough nuclease-free water to produce a total reaction volume of 40 µl. All PCRs were run on Eppendorf Mastercycler® gradient thermal cyclers (Eppendorf AG, Hamburg, Germany). PCR products were visualized using agarose gel electrophoresis with ethidium bromide staining.

All PCR products were sent to Beckman Coulter Genomics (Danvers, MA, USA) for purification and DNA sequencing. Cycle sequencing reactions used BigDye® Terminator v3.1 reagents (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with the same primers used for PCR amplification. DNA sequences were determined using Applied Biosystems® 3730xl DNA Analyzers (Thermo Fisher Scientific, Inc.). All PCR products were sequenced using both the forward and reverse PCR primers.

All sequencing trace files (i.e., the sequencing chromatogram data files) were processed with SeqTrace 0.9.0 (Stucky 2012) to generate high-quality DNA sequences for phylogenetic analysis. Each matching pair of forward and reverse reads was aligned in SeqTrace to produce a single consensus sequence that was then trimmed to remove low-quality beginning and ending bases. A minimum consensus quality score of 30 was required for a base call to be accepted. Finally, all chromatograms and their corresponding trimmed consensus sequences were manually inspected in SeqTrace to correct any remaining base-calling problems. The finished sequences for the first and second halves of CO1 did not overlap, leaving a small gap in sequencing coverage in the middle of the gene. To join the sequences for the two halves of CO1, the sequences for each half were aligned to the mitochondrial genome of *Sarcophaga impatiens* Walker (Nelson Cameron et al. 2012) using MUSCLE 3.8.31 (Edgar 2004). The alignment was

inspected in Mesquite 3.02 (Maddison and Maddison 2015), the sequencing coverage gaps were filled in using 'N' (i.e., unknown) characters, and the final CO1 sequences for each specimen were exported to FASTA text files.

*Additional DNA sequence data*

To increase the species diversity of the phylogenetic analysis, GenBank was searched for additional sarcophagid sequence data that matched the gene fragments sequenced for this study. For each gene, an exemplary DNA sequence (i.e., both long and complete) was selected from among this study's new sequence data and used as the query sequence for a search of all sarcophagid sequences in GenBank using GenBank's BLAST Web API. All search requests used the program "blastn" with the nucleotide database ("nr") and included an Entrez query to limit the search to known sarcophagid sequences.

Matching sequences returned by the BLAST searches were initially filtered by the length of their corresponding "high-scoring segment pair" (HSP) alignments with the query sequence and the HSP *E*-values (see Altschul et al. (1997) for more about HSPs and *E*-values). Sequences with HSP alignment lengths of less than 50 bp or with *E*-values greater than or equal to 0.001 were excluded from further consideration. For each HSP that met these initial criteria, the matching sequence's accession number, taxonomic metadata, and HSP details were stored in a local SQLite database (http://www.sqlite.org/), along with a "coverage" score for the HSP, which was calculated as the proportion of the total length of the query sequence that was covered by the HSP alignment (e.g., a coverage score of 1.0 indicated that the entire query sequence was included in the HSP alignment). This produced a database of "candidate" sequences from which the final set of GenBank sequences used for the phylogenetic analysis was chosen. The sequence data in the database represented 256 unique species-group names.

Approximately two thirds of those species-group names had DNA sequence data for only a single gene (usually CO1), so including those species in the phylogenetic analysis would have resulted in a sparse character matrix with substantial missing data. In general, taxa with extensive missing data can have a negative effect on the accuracy and precision of phylogenetic inference, but this is not always so and the impact is often negligible (e.g., Lemmon et al. 2009, Wiens and Morrill 2011, Simmons 2012, 2014, Roure et al. 2013, Shavit Grievink et al. 2013). Nevertheless, recent work using Bayesian phylogenetic inference for sarcophagids suggests that GenBank-derived datasets with large numbers of species but relatively little sequence data for many of those species might result in Bayesian analyses that fail to converge (Piwczyński et al. 2014). Consequently, selection of GenBank sequences for phylogenetic inference focused on choosing sarcophagid species for which a relatively large amount of relevant DNA sequence data was available, rather than maximizing the total number of species in the analysis by including many species with limited sequence data.

To accomplish this goal, the following steps were used to choose the final set of sequences. First, for each unique species represented in the database of candidate sequences, the combinations of GenBank sequences that maximized the mean coverage score across all seven genes (i.e., the arithmetic mean of the coverage scores for all seven genes) were determined. All species with maximum mean coverage scores less than 0.5 were excluded from further consideration. Next, publication metadata were examined for the remaining sequences, and any sequences not associated with a peer-reviewed publication were eliminated. If possible, alternative sequences were substituted in their place and the mean coverage scores were recalculated. Once more, any species with mean coverage scores less than 0.5 were eliminated. Next, for species with multiple combinations of sequences that achieved the maximum mean

coverage score, a single sequence was chosen for each included gene such that the total number of data sources (i.e., the number of source specimens) was minimized. After this step, most species were represented by sequence data from a single study, but a few species included multiple gene sequences from Kutty et al. (2010) and sequences for one or more genes (usually just CO1) from a different study or studies. In such cases, there was a risk that if one (or more) of the source specimens was misidentified, the combined sequence data for the "species" might contain conflicting phylogenetic signal. As a final step to mitigate this risk, for each multi-study species, each sequence not from Kutty et al. was compared to other sarcophagid sequences in GenBank using the online BLAST tool in an attempt to confirm the species identification. In every case, it was possible to compare the sequence either to a shorter fragment of the same gene and species from Kutty et al. (2010) or to sequences of the same gene and species from other studies. This analysis revealed one likely misidentification: The highest-coverage sequences for 28S and EF1-α for *Sarcophaga crassipalpis* were from Singh and Wells (2013), but comparison of this 28S sequence to other GenBank sequences indicated that the source specimen was almost certainly *S. bullata* Parker or a close relative, not *S. crassipalpis*. Consequently, these two sequences were removed from the sequence set and replaced with alternatives. The final set of GenBank sequence data included 44 unique sarcophagid species, each of which was represented by at least four of the seven genes included in this study. The complete, final set of GenBank sequences, with all accession numbers, is provided in Appendix B.

The same procedure was used to choose outgroup species and sequences from GenBank sequence data. The GenBank search for outgroup sequences was limited to the families Calliphoridae and Tachinidae because, even though relationships within the superfamily Oestroidea are still not fully resolved, there is little doubt that the members of both of these

families are closely related to, yet clearly distinct from, Sarcophagidae (e.g., McAlpine 1989, Pape 1992, Rognes 1997, Kutty et al. 2010, Marinho et al. 2012), and so should provide reasonable choices for outgroup species. From the final set of potential outgroup sequences, four species were selected that included data for all seven genes and had mean coverage scores greater than 0.96 (Appendix B). These outgroup species were the calliphorids *Calliphora vomitoria* (Linnaeus), *Chrysomya megacephala* (Fabricius), *Lucilia sericata* (Meigen), and *Pollenia rudis* (Fabricius).

*DNA sequence alignment*

For each gene, all new sequences from this study and all sequences selected from GenBank were combined in a single FASTA-format text file and aligned using MAFFT 7.215 (Katoh and Standley 2013). MAFFT was chosen because of its superior performance in a variety of benchmarking studies (e.g., Wilm et al. 2006, Carroll et al. 2007, Dessimoz and Gil 2010). All MAFFT alignments used the "L-INS-i" method, which was specified with the command-line options "`--localpair --maxiterate 1000`".

The 12S primers that were used for this study cover a region of the mitochondrial genome that extends beyond the 12S gene to include the valine tRNA gene and a short fragment (approximately 180 bp) of the 16S gene. Visual inspection of the initial alignment of the resulting "12S" sequences suggested that the 12S and valine tRNA portions were more conserved within the sequence set than was the 16S portion. Consequently, the 16S portions of these "12S" sequences were removed and aligned separately. The alignment of these short 16S fragments was then concatenated with the alignment for the main 16S fragments to produce a single alignment for all of the 16S data. End gaps between the two 16S alignments were replaced with "N" (i.e., unknown) characters prior to concatenation.

DNA sequences that code for structural RNAs can be challenging to align because the presence of highly variable regions often makes it difficult to identify homologous bases. In these cases, alignment techniques that make use of RNA secondary structure can improve the accuracy of both the initial alignment and subsequent phylogenetic analyses (Gardner 2005, Wilm et al. 2006, Letsch et al. 2010). However, alignment algorithms that incorporate secondary structure prediction are generally much more computationally demanding than nucleotide-only alignment, and if the structural RNA-coding DNA sequences are sufficiently similar, structure-aware alignment might not be needed (Gardner 2005, Wilm et al. 2006). For this study, the methodological recommendations of Wilm et al. (2006) were followed. Based on an extensive performance benchmarking study that included a variety of multiple alignment software programs, these authors recommended first aligning a sequence set with a standard nucleotide-only alignment program (i.e., software that is not aware of secondary structure), then calculating the average pairwise sequence identity (APSI) for the resulting alignment. If the APSI score is less than or equal to 55%, structure-aware alignment should be considered; otherwise, nucleotide-only alignment should be satisfactory. After aligning all rDNA sequence data with MAFFT's nucleotide-only L-INS-i method, the APSI scores for the alignments were 86% for 16S and >95% for the remaining genes (12S, 18S, and 28S). These results strongly suggested that the L-INS-i alignments were of high quality and that structure-based alignment was not necessary. However, because MAFFT also provides an alignment method, "X-INS-i", for producing alignments that consider secondary structure (Katoh and Toh 2008), I also aligned all of the rDNA sequences using the X-INS-i method. I then calculated overlap scores to compare the L-INS-i and X-INS-i alignments for each gene (overlap scores range from 0 to 1.0, with a score of 1.0 indicating that two alignments are identical; see Lassmann and Sonnhammer

(2002, 2005) for the formal definition). The overlap score for the two 16S alignments was >0.97, and for all remaining genes, the overlap scores were >0.99. Thus, there was very little difference between the L-INS-i and X-INS-i alignments. Furthermore, visual inspection of the X-INS-i alignments indicated some problems with fragment placement at the ends of the alignments, so the L-INS-i alignments were used for phylogenetic inference.

After generating all of the initial alignments with MAFFT, each alignment was manually inspected in Mesquite and/or AliView (Larsson 2014) to check for and correct any obvious alignment errors. The alignments for the protein-coding genes (CO1, CytB, and EF1-α) were all completely gap free and required no further adjustments. A few very minor adjustments were made to the 12S and 16S alignments; no adjustments were made to the 18S and 28S alignments.

Next, the ends of each alignment for the protein-coding genes were trimmed up to the first and last column with data for more than one sequence. End trimming was more aggressive for the rDNA alignments because it was often very difficult to assess the alignment quality where data from only a few sequences were present. In cases where the final sequences on either end of an alignment consisted only of outgroup taxa, the alignments were further trimmed until at least 2 or more ingroup taxa were represented.

For each protein-coding gene, AliView's "count stop codons" feature was used to determine the correct reading frame. For the mitchondrial genes CO1 and CytB, the inferred reading frame was confirmed by comparison to reference mitochondrial genomes. For convenience in defining data partitions for the phylogenetic analysis, the alignment for each protein-coding gene was trimmed, if necessary, so that the reading frame began with the first column of the alignment.

Finally, the alignments for all seven genes were concatenated to produce a single data matrix that included all seven genes. Prior to concatenation, the end gaps between alignments were replaced with "'N" characters. The final, combined alignment matrix was just over 6.9 kb in length and included 89 rows representing 66 species or putative species.

*Phylogenetic inference*

The basic workflow for the phylogenetic analysis was as follows. First, PartitionFinder 1.1.1 (Lanfear et al. 2012) was used to determine an optimal data-partitioning strategy for the full alignment and the most suitable model of molecular evolution for each partition. Next, a preliminary maximum likelihood (ML) analysis with RAxML 8.1.16 (Stamatakis 2014) was used along with RogueNaRok 1.0 (Aberer et al. 2013) to identify "rogue taxa," which were then removed from the alignment. The resulting alignment was analyzed again with PartitionFinder to infer the optimal data-partitioning strategy and select the best models of evolution for the Bayesian analysis, which was completed using MrBayes 3.2.4 (Ronquist et al. 2012). All phylogenetic analyses were executed on a 12-CPU Dell PowerEdge server with AMD Opteron 4228 processors running Xubuntu GNU/Linux (http://xubuntu.org/). Each of the basic steps outlined above is next described in more detail.

First, PartitionFinder was used to determine an optimal (or nearly so) data-partitioning strategy for the full alignment, along with the most suitable model of molecular evolution for each data partition. Identifying an appropriate partitioning scheme is important for successful phylogenetic inference because it allows the analysis to account for differences in evolution among the positions in an alignment (Lanfear et al. 2012). For the PartitionFinder analyses, 13 starting data blocks were defined: one for each of the four rRNA-coding genes, and one for each codon position of each of the three protein-coding genes. Because of the relatively large number

of data blocks, the search for an optimal partitioning scheme used PartitionFinder's greedy algorithm rather than an exhaustive search of the full partition scheme space. PhyML (Guindon et al. 2010) was used for all likelihood calculations. The models of molecular evolution that were considered in each analysis were limited to those supported by the target phylogenetic inference program (i.e., RAxML or MRBayes). The best partitioning scheme was selected by comparing the Bayesian information criterion scores (BIC; Schwarz 1978, Sullivan and Joyce 2005) for all candidate partitioning schemes.

Next, the results from PartitionFinder were used in a maximum likelihood phylogenetic analysis with RAxML. To minimize run time, RAxML was compiled with support for pthreads, thread pinning, and AVX (Advanced Vector Extensions) instructions. For the RAxML analysis, PartitionFinder determined that the optimal partitioning scheme was to group CO1 and CytB together and place each codon position in a separate partition (3 partitions total), group 12S and 16S together in one partition, group 18S and the first two codon positions of EF1-α together in one partition, use a single partition for 28S, and use a single partition for the third codon position of EF1-α, for a total of seven data partitions. PartitionFinder identified the GTR+I+G (general time reversible with a proportion of invariant sites and gamma-distributed rate heterogeneity) model of molecular evolution as the best model for all partitions except for the third codon position of EF1-α, for which the GTR+G (same as GTR+I+G except without a proportion of invariant sites) model was selected. However, RAxML does not allow different models of rate heterogeneity to be used in a single analysis, and the RAxML manual cautions against use of the GTR+I+G model, so the GTR+G model was used for all partitions. To estimate branch support for the ML tree and generate starting trees for the ML search, all analyses used RAxML's rapid

bootstrap algorithm (Stamatakis et al. 2008) with the extended majority-rule consensus automatic stopping criterion (Pattengale et al. 2009).

Following the initial ML analysis, inspection of the estimated ML tree indicated that the outgroup was not consistently recovered in the bootstrap samples (bootstrap support = 0.96). A closer look at the bootstrap samples revealed that this was because a single outgroup member, *Pollenia rudis*, occasionally was placed within the ingroup. On the ML tree, *P. rudis* was connected to the rest of the outgroup by a very long branch, and on the occasions when *P. rudis* was placed within the ingroup, it was paired with ingroup taxa that also had long terminal branches. The occasions where *P. rudis* was misplaced therefore seemed to be cases of the so-called "long-branch attraction" artifact, which is known in both theory and practice to sometimes result in incorrect tree topologies with problematic outgroup taxa (e.g., Wheeler 1990, Huelsenbeck et al. 2002, Bergsten 2005, Luo et al. 2010, Kirchberger et al. 2014). Consequently, *P. rudis* was dropped from the alignment and the analyses with PartitionFinder and RAxML were repeated on the new alignment with the reduced outgroup.

One potential problem for phylogenetic inference is that substitutionally saturated or highly length-variable sequence segments might be impossible to align unambiguously, leading to regions of an alignment that lack meaningful phylogenetic signal (Talavera and Castresana 2007, Dress et al. 2008, Misof and Misof 2009). To deal with this issue, alignment "masking" algorithms have been proposed to objectively identify and remove ambiguously aligned portions of an alignment, and these methods have been shown to improve phylogenetic resolution for at least some real datasets (e.g., Lake 1991, Gatesy et al. 1993, Castresana 2000, Talavera and Castresana 2007, Dress et al. 2008, Misof and Misof 2009, Kück et al. 2010). To investigate whether alignment masking might be beneficial for the present study, the alignment for each

gene was analyzed using Aliscore 2.0 (Misof and Misof 2009). Aliscore was run with the default window size of 6 and all possible sequence pairs were compared at each window position. All ambiguously aligned regions identified by Aliscore were removed from the alignments. In order to preserve the reading frames for the protein-coding genes, one or two columns adjacent to the masked portions were also removed, if necessary. The masked individual alignments were concatenated together and the PartitionFinder analysis was repeated on the combined, masked alignment matrix. The resulting optimal partitioning scheme and suggested models of molecular evolution were exactly the same as for the unmasked alignment. Next, three independent RAxML ML analyses, as described above, were run on the full masked alignment, and three independent ML analyses were run on the full unmasked alignment. To assess whether alignment masking improved tree resolution, the minimum and mean bootstrap branch support values were calculated for each ML tree, and the overall average minimum and mean branch support values were then calculated for all three trees from each of the masked and unmasked alignments. The overall average minimum and mean branch support values were 0.16 and 0.753, respectively, for the masked alignment and 0.13 and 0.757 for the unmasked alignment. Thus, there did not appear to be strong evidence that alignment masking improved tree resolution, and the full unmasked alignment matrix was retained for all subsequent analyses.

As a final step before Bayesian phylogenetic analysis, RogueNaRok was used to identify so-called "rogue taxa" in the dataset. Rogue taxa are taxa whose positions in the tree topology are uncertain and unstable (Wilkinson 1996, Sanderson and Shaffer 2002), and the inclusion of rogues in a phylogenetic analysis can severely degrade tree resolution and support (Thorley and Wilkinson 1999, Thomson and Shaffer 2010, Aberer et al. 2013, Hinchliff and Roalson 2013). Rogues are most likely caused by weak or contradictory phylogenetic signal, which can result

from including sequences from misidentified specimens in an analysis, so rogues might be an especially common problem in studies that combine data from many different sources (Thomson and Shaffer 2010, Pattengale et al. 2011, Hinchliff and Roalson 2013). For the present study, RogueNaRok was run with its default settings, which optimize the support values of branches on the majority-rule consensus tree for a sample of bootstrap trees. Three independent RAxML ML analyses with final bootstrap sample sizes of from 300 to 400 trees were analyzed, and all taxa identified as rogues in at least two of the three analyses were accepted as rogue taxa and dropped from the data set, with one exception. One of the *E. auditrix* specimens sequenced for this study was identified as a rogue, but after inspecting the bootstrap trees, it was obvious that the only uncertainty was in how this specimen should be placed relative to the other *E. auditrix* specimens. As a group, the *E. auditrix* specimens formed a clade with 100% bootstrap support in all three analyses, so for the purposes of this study, none of the *E. auditrix* specimens could be considered rogues in any meaningful sense and all were retained in the dataset. All taxa accepted as rogues were from GenBank sequence data. In total, eight taxa were removed from the dataset (*Argoravinia rufiventris*, *Dexosarcophaga transita*, *Peckia intermutans*, *Sarcophaga fertoni*, *S. forma*, *S. melanura*, *Taxigramma multipunctata*, and *Titanogrypa luculenta*). To further confirm these results, all bootstrap trees from all three RAxML runs were combined into a single sample and the RogueNaRok analysis was repeated on the combined treeset. The final set of accepted rogue taxa was exactly the same. Dropping these rogue taxa was clearly beneficial; RogueNaRok calculated that the rBIC (relative bipartition information criterion; see Aberer et al. 2013) score for the combined treeset would increase from 0.68 to 0.76 if the eight rogue taxa were dropped.

The final alignment matrix with rogue taxa removed was then used for Bayesian phylogenetic inference with MrBayes. To take full advantage of all CPUs on the host machine, MrBayes was compiled with MPI (Message Passing Interface) support and run with the MPICH 3.0.4 implementation of the MPI standard (http://www.mpich.org/) to enable parallel $(MC)^3$ (Metropolis-coupled Markov chain Monte Carlo; see Ronquist et al. (2009) for an introduction) processing (Altekar et al. 2004). Prior to running the $(MC)^3$ analysis, the alignment was analyzed again in PartitionFinder to identify an optimal partitioning scheme and model(s) of molecular evolution, this time with a set of candidate models that included all molecular evolution models supported by MrBayes, which is a much larger set than those supported by RAxML. The best partitioning scheme was the same as that found for the RAxML analyses except that the first codon position for EF1-α was placed in its own partition. The molecular evolution models chosen by PartitionFinder varied, with half of the partitions receiving either the GTR+I+G or GTR+I models and the remainder receiving parameter-constrained simplifications of the full GTR model. All model parameters except for topology and branch lengths were unlinked among the partitions (i.e., each partition had its own set of model parameters). MrBayes's default prior distributions were used for all model parameters. The full analysis consisted of three independent $(MC)^3$ runs, with each run consisting of three "heated" chains and one "cold" chain. The chains were run for 20,000,000 generations and the cold chains were sampled every 1,000 generations. The first 25% of the samples were discarded as burn-in. To verify convergence among the three independent $(MC)^3$ analyses, potential scale reduction factor statistics (PSRF; Gelman and Rubin 1992, Brooks and Gelman 1998) were examined for all numeric parameter samples and the average standard deviation of split frequencies was examined

for the tree samples. Effective sample size (ESS) estimates, as calculated by MrBayes, were also examined to verify that the sampling scheme was adequate for reasonable estimation precision.

### *Evolution of sarcophagid larval feeding strategies*

#### *Larval feeding strategies of extant species*

A thorough literature review was conducted to determine, if possible, the larval feeding habits of each species included in the final phylogenetic analysis. Unfortunately, the life history literature for the Sarcophagidae is widely scattered, and for most species, has never been comprehensively reviewed. Furthermore, many important, older records are found in relatively obscure journals that are not easily accessible and have not been fully digitized. Some important references, especially for Old World species, are not available in English. Considering these challenges, the life history knowledge assembled here cannot be considered exhaustive, but it is undoubtedly the most complete yet published for many of these species.

Many references were located by searching online indexes, such as Google Scholar (https://scholar.google.com/). Additional, usually older references and life history notes were located by consulting major taxonomic and systematic works (e.g., Aldrich 1916, Lopes 1950, Roback 1954, Pape 1994, Dahlem and Downes 1996), faunistic and ecological catalogues (e.g., Hall 1929, Hallock 1940a, 1940b, 1942, James 1947, Lopes 1973, Rees 1973, Pape 1987, Povolný and Verves 1997, Coupland and Barker 2004), parasitoid/host catalogues (van Emden 1950, Thompson 1951, Greathead 1963), and original species descriptions. Some additional references were found in the *Review of Applied Entomology*. References obtained from these sources were then examined for further references, and so on. As time permitted, works in French, German, Portuguese, or Spanish were translated and included in the review, but several papers originally published in Russian could not be included due to a lack of translation

resources. Natural history data were only accepted if the original, primary sources could be retrieved and examined.

Following the literature review, all species included in the final phylogeny were assigned one of four possible larval feeding character states: parasitoid, scavenger, facultative parasitoid/scavenger, or unknown. For this study, "parasitoid" means that, under natural conditions, a species is only known to develop as a parasitoid; "scavenger" means a species is only known to develop as a scavenger of carrion, animal excrement, or other organic material; and "facultative parasitoid/scavenger" means a species can develop as either a scavenger or a true parasitoid.

Any effort to accurately determine sarcophagid larval feeding habits by interpreting natural history records in the literature is beset by at least three major difficulties. First, feeding data for many species are only available for the adult stage, but adult food preferences do not necessarily indicate larval feeding habits. For example, necrophagous sarcophagids have often been studied by trapping adult flies on carrion baits, but there is now ample evidence that adult flies of many species will visit carrion as a food source regardless of whether they use carrion as a substrate for larviposition (e.g., Kirchberg 1954, Davis and Turner 1978, Souza and Linhares 1997, Bänziger and Pape 2004). Second, laboratory rearing records can be misleading because larval food substrates used in the laboratory might not match natural larviposition behaviors. To give but two examples, the larvae of both *Boettcheria cimbicis* (Townsend) and *Blaesoxipha kellyi* (Aldrich) can be reared in the laboratory on decaying beef or other meats (Knipling 1936, Downes 1955), but female *B. cimbicis* will not voluntarily larviposit on ground beef (Dahlem and Downes 1996) and *B. kellyi* is a well-known parasitoid of grasshoppers (e.g., Kelly 1914, Rees 1973) that has never been reported as a scavenger of vertebrate carrion. Moreover, female

flies confined in laboratory conditions will sometimes larviposit on substrates they would not normally accept in nature (Bänziger and Pape 2004), so rearings in artificial environments are of limited utility in establishing natural feeding behaviors. Third, records of species developing as "parasitoids" might reflect scavenging on dead insects or other animals rather than true parasitization of live hosts. This concern has already been raised by a multitude of previous authors (e.g., Aldrich 1916, Lopes 1950, Ferrar 1987, Dahlem and Downes 1996, Coupland and Barker 2004) and pertains to any records for which larviposition after death of the "host" cannot be ruled out. Such records could arise if the supposed host was already dead when collected in the field or if it was housed in conditions that did not exclude larviposition by scavenging female flies after its death. The literature is replete with rearing records of supposed sarcophagid parasitoids that include little or no methodological detail, and these must therefore be regarded with a great deal of caution.

In light of these difficulties, a conservative approach was taken to inferring larval feeding habits from the literature, using the following criteria: 1) A species was classified as a parasitoid only if, at a minimum, it had been reared from living host animals in conditions that minimized the chance of infestation after the death of the host or if other, equally compelling evidence was available. 2) A species was classified as a scavenger only if it had been reared from carrion, animal excrement, or other non-living organic material on which voluntary larviposition occurred in the field. 3) A species that met both criterion 1 and 2 was classified as a facultative parasitoid/scavenger. 4) A species that met neither criterion 1 nor 2 was classified as unknown.

The above criteria helped minimize the risk of misinterpreting ambiguous literature records, but an additional, more general problem, is that misidentifications and other mistakes will inevitably creep into any large, heterogeneous collection of natural history data.

Sarcophagids are notoriously difficult to identify, and early work on this group was often plagued by taxonomic and nomenclatural confusion. More recent decades have seen numerous changes in nomenclature as various taxonomic issues have finally come to light and old, poorly described type specimens have been re-examined. This is true even for some common, widespread species, such as the nearly cosmopolitan *Sarcophaga africa* (Wiedemann). This species has frequently been identified in the literature as *S. haemorrhoidalis* (Fallén), *S. georgina* Wiedemann, or *S. cruentata* Meigen, and it was not until the publication of Pape's catalogue of world species (1996) that the issue was fully resolved. Such confusion means that many early natural history records for sarcophagids must be viewed with caution because species identifications might be unreliable.

It is of course impossible (or at least impractical) to somehow detect and eliminate all records based on incorrect identifications. Instead, the reliability of species determinations was roughly assessed by whether the records predated or postdated the publication of Aldrich's landmark revision of the Sarcophaginae in 1916. This work provided the first firm foundation for the identification of the North American fauna, and species identifications for natural history records published prior to Aldrich's treatise must be regarded with considerable skepticism. In fact, Hall (1929) went so far as to state that "records based upon specimens determined before 1916 should be disregarded." A slightly less pessimistic view was taken here, but records predating Aldrich's 1916 revision were only included if the identifications were highly likely to be reliable (e.g., if specimens were known to have been identified by Aldrich or another recognized authority at the time). In addition, every effort was made to track nomenclatural changes and known synonymies back in time so that records for species previously associated with a different name (or names) could be properly matched with their contemporary names.

The nomenclature and taxonomy used for this study mostly followed Pape's world catalogue (1996). In the years since the publication of Pape's catalogue, newer revisions of the *Sarcophaga* subgenus *Neobellieria* (Giroux and Wheeler 2009, 2010) and the genus *Peckia* (Buenaventura and Pape 2013) have become available; the taxonomic conclusions of these newer works were followed in preference to Pape's catalogue. This study also followed Pape et. al (2004) and Pape and Dahlem (2010) in considering *Duckemyia* as a subgenus of *Lepidodexia*.

*Evolution of larval feeding strategies*

The evolution of sarcophagid larval feeding strategies was analyzed in a Bayesian framework using the software BayesTraits (Pagel et al. 2004). BayesTraits models the evolution of character states on a phylogeny as a continuous-time Markov process where each possible pair of state transitions can optionally have its own rate parameter. BayesTraits also uses a posterior sample of trees from a Bayesian phylogenetic analysis (e.g., from MrBayes) to account for uncertainty about the phylogenetic reconstruction in its estimate of the model of trait evolution.

Three subfamilies are currently widely recognized within Sarcophagidae: Miltogramminae, Paramacronychiinae, and Sarcophaginae, with the first two having sometimes been considered tribes of a single subfamily (e.g., Downes 1955, 1965, Pape 1987, 1996, Shewell 1987). As mentioned earlier, Sarcophaginae includes *Emblemasoma* along with the vast majority of sarcophagid species. Although the present phylogenetic analysis included representatives of all three subfamilies, taxa sampling for the subfamilies Miltogramminae and Paramacronychiinae was rather limited, with only 2 and 5 species included, respectively. In addition, of the species from these subfamilies that were included, definite life history data could not be found for either of the two species of Miltogramminae and for only two of the five species

of Paramacronychiinae. Considering these limitations, the analysis of life history evolution was confined to the subfamily Sarcophaginae.

Prior to running the analysis with BayesTraits, the burn-in trees (i.e., the first 25%) from each of the three independent $(MC)^3$ MrBayes phylogenetic analyses were deleted, and the remaining trees from the three analyses were combined into a single sample of 45,000 trees from the posterior distribution. Next, all trees were rooted according to the outgroup and then the outgroup taxa and species of Miltogramminae and Paramacronychiinae were pruned from the trees. Within Sarcophaginae, species that were represented by more than one set of sequence data were pruned so that each species was represented only once in each tree. An exception was made for *Sarcophaga africa*, which was represented by two sets of sequence data, one from the USA and one from China. Available natural history data suggested that there might be differences in larval feeding strategies between the New and Old Worlds representatives of this species (see references in Appendix C), so both sequence sets were retained to allow for this possibility. It also was not obvious how to best prune *Emblemasoma erro* and *E. faciale*. These two species appeared to form a complex, with the data suggesting that perhaps neither species was monophyletic as currently recognized (see results below). However, three distinct, strongly supported clades emerged, so the tree was pruned to capture these clades: Great Plains *E. erro*, southwestern U.S. *E. erro*, and southeastern U.S. *E. erro*/*E. Faciale*.

For a continuous-time Markov model of evolution with three character states (parasitoid, scavenger, and facultative parasitoid/scavenger), there are six possible state transitions and therefore six possible transition rate parameters. BayesTraits was run using the "full" model with all six rate parameters (that is, no rate parameters were constrained to equal zero or to equal other rate parameters). The prior probability for each rate parameter was specified with an

exponential distribution and the mean of each exponential prior was specified using a uniform hyperprior on the interval [0,12]. Prior specification was guided, in part, by an initial maximum likelihood analysis of the data (also completed using BayesTraits). The maximum value of the uniform hyperpriors exceeded the largest ML transition rate estimate, and the use of exponential priors emphasized that the transition rates were expected to be relatively low while still allowing for the possibility of high transition rates. Otherwise, the priors were essentially uninformative in the sense that the prior beliefs about the values of all six transition rates were exactly the same.

Ancestral larval feeding strategies were reconstructed for 12 key clades that had very high posterior probabilities in the Bayesian phylogenetic analysis (all probs. > 0.96; see results below): 1) the entire subfamily Sarcophaginae; 2) Sarcophaginae except for *Notochaeta*+*Sinopiella*; 3) *Sarcophaga*+sister clade to *Sarcophaga*; 4) the sister clade of *Sarcophaga*; 5) *Sarcophaga*; 6) the clade comprising *Ravinia*, *Oxysarcodexia*, *Blaesoxipha*, *Spirobolomyia*, and *Emblemasoma*; 7) *Ravinia*+*Oxysarcodexia*; 8) *Ravinia*; 9) the clade comprising *Blaesoxipha*, *Spirobolomyia*, and *Emblemasoma*; 10) *Blaesoxipha*+*Spirobolomyia*; 11) *Blaesoxipha*; and 12) *Emblemasoma*. The first four clades were chosen because they are important for understanding the evolution of feeding strategies in the subfamily Sarcophaginae as a whole. The remaining clades were chosen primarily because they are significant for understanding the evolution of *Emblemasoma* and acoustic eavesdropping.

Three independent MCMC analyses with BayesTraits were completed, where each analysis consisted of a chain run for 48,000,000 generations, sampled every 1,000 generations, and with the first 25% of the samples discarded as burn-in. Convergence of the three independent runs was checked by calculating PSRF statistics for each rate parameter. The

adequacy of the burn-in period was evaluated by calculating Geweke's diagnostic (Geweke 1992) for each rate parameter from each run. After confirming that the analyses all converged on the posterior distribution, the samples from all three analyses, minus the burn-in, were combined to create a single sample of size $n$ = 108,000 from the posterior distribution. To verify that the sampling scheme was sufficient for reasonably precise model estimation, the ESS was calculated for each rate parameter as described by Kass et. al (1998), using autocorrelation time estimates based on the initial positive sequence method of Geyer (1992).

## 6.3 Results

### *Phylogeny of Sarcophagidae and* Emblemasoma

The majority-rule consensus tree for the posterior tree samples from all three $(MC)^3$ runs combined (without the 25% burn-in, for a total sample size of $n$ = 45,000 trees) is shown in Figure 6.1. All convergence diagnostics strongly suggested that the three $(MC)^3$ runs converged and were sampling from the true posterior distribution: The PSRF values for all numerical model parameters, including branch lengths, deviated from 1.0 by less than 0.0004, and the final mean standard deviation of tree split frequencies was less than 0.0018.

In general, the inferred phylogeny was very well resolved. The mean posterior branch probability for the majority-rule consensus tree was 0.957, but several of the most poorly resolved branches were merely due to uncertainty about how multiple representatives of the same species were related. All species with multiple representative specimens were in the subfamily Sarcophaginae, and if these "duplicates" were pruned from the trees as described above in the '*Inference of life history evolution*" subsection of the Methods, the mean posterior branch probability of the majority-rule consensus tree increased to 0.966. At the taxonomic level of subgenus or higher, only four clades had posterior branch probabilities less than 0.93:

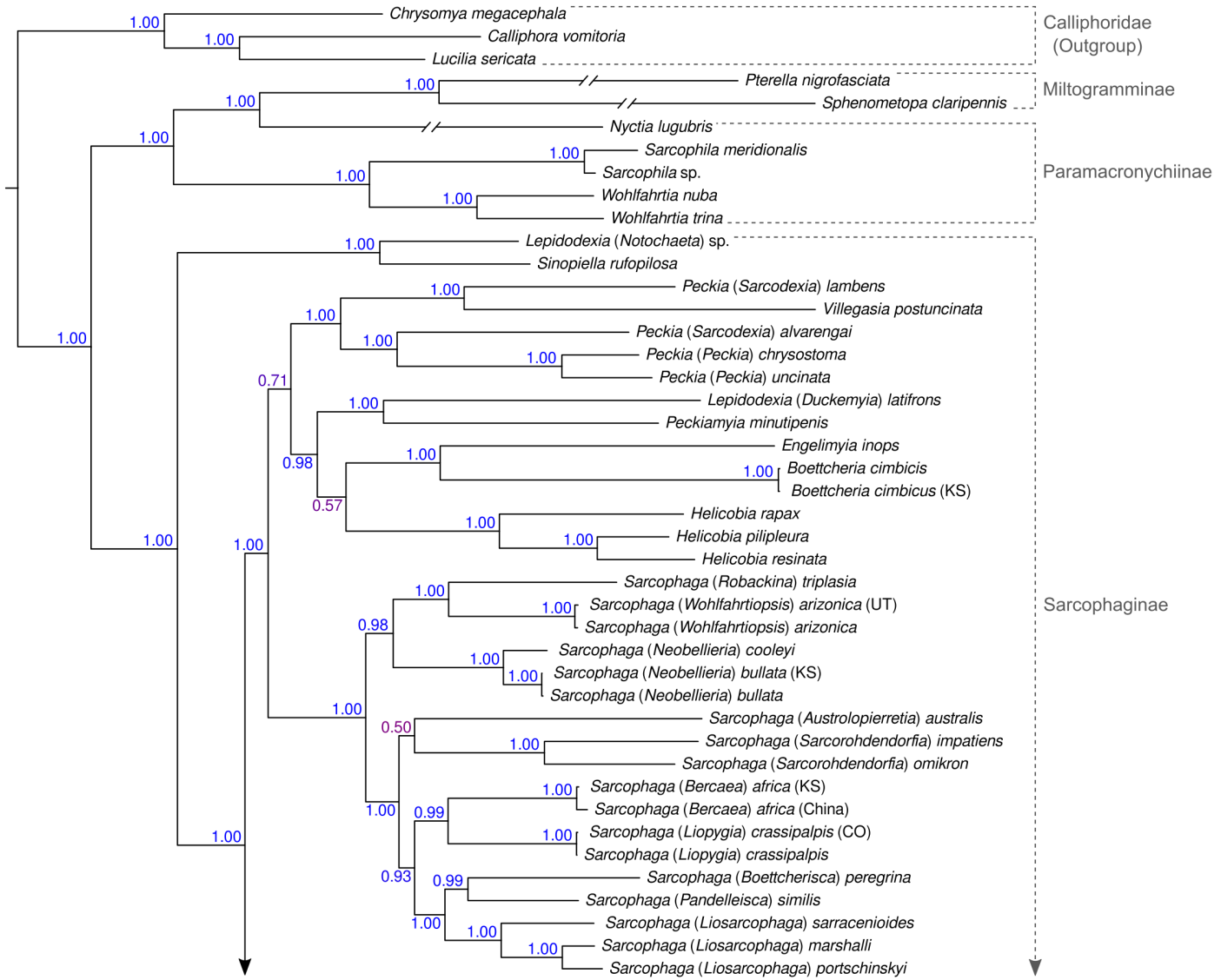


**Figure 6.1** See full caption on the next page.

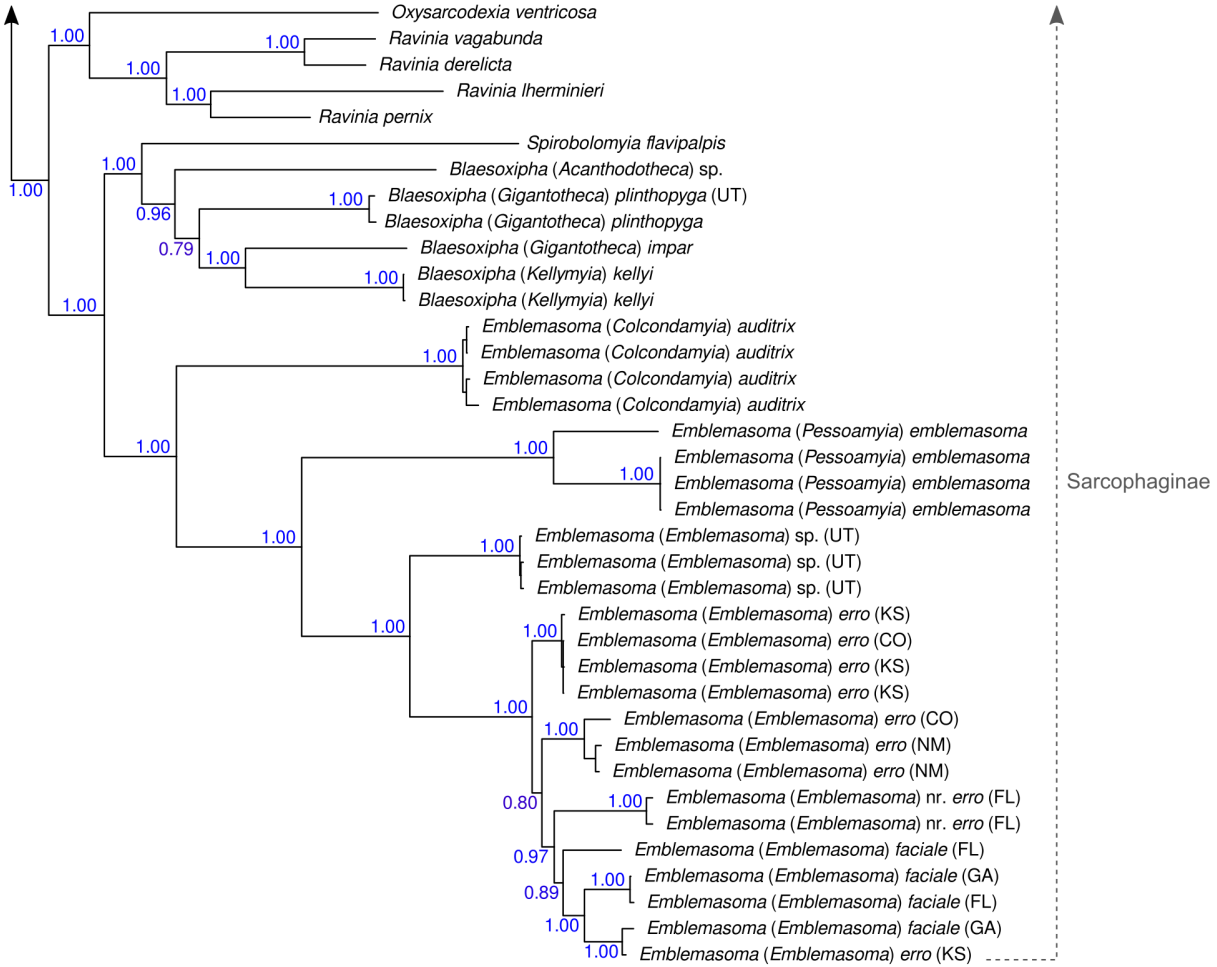


**Figure 6.1** Majority-rule consensus phylogeny of the posterior probability distribution of phylogenetic trees. Branch support values indicate the estimated posterior probability of each clade. Support values are colored from a gradient of red = 0 to blue = 1.0. Note that the terminal branches for the miltogrammine species have been shortened for display purposes.

The clade joining *Sarcophaga* (*Australopierretia*) as sister to *Sarcophaga* (*Sarcorohdendorfia*) [probability = 0.504], the clade joining *Helicobia* as sister to *Boettcheria+Engelimyia* [prob. = 0.568], the clade inferred as the sister to *Sarcophaga* [prob. = 0.711], and the clade encompassing *Blaesoxipha* (*Gigantotheca*) and *Blaesoxipha* (*Kellymyia*) [prob. = 0.791]. At the subfamily level, the monophyly of Sarcophaginae was strongly supported [prob. = 1.0]. In contrast, Paramacronychiinae was paraphyletic with respect to the remaining subfamily, Miltogramminae. The two species of Miltogramminae included in the analysis were placed as sister taxa.

Within the subfamily Sarcophaginae, after an initial basal split between (*Lepidodexia* (*Notochaeta*)+*Sinopiella*) and the rest of the subfamily, there were two large, very strongly supported clades (all probs. > 0.99) that represented two major sarcophagine lineages. One included the extremely speciose genus *Sarcophaga* and its relatives, while the other comprised the genera *Ravinia*, *Oxysarcodexia*, *Blaesoxipha*, *Spirobolomyia*, and *Emblemasoma*. These two major lineages appear to account for the majority of sarcophagine species-level diversity.

Among genus-level taxa, most currently recognized concepts were recovered as monophyletic, with a few exceptions. *Lepidodexia* is apparently polyphyletic, with the subgenera *Duckemyia* and *Notochaeta* widely separated from each other in the tree. *Peckia* was paraphyletic with respect to *Villegasia*, and the subgenus *Peckia* (*Sarcodexia*) was paraphyletic with respect to *Peckia* (*Peckia*). Finally, the subgenus *Blaesoxipha* (*Gigantotheca*) was paraphyletic with respect to *Blaesoxipha* (*Kellymyia*).

The analysis confirmed that *Emblemasoma*, in the broad sense proposed by Pape (1990), is a monophyletic group [prob. = 1.0], with the originally described genera *Pessoamyia+Emblemasoma* (*sensu stricto*) as sister to *Colcondamyia*. *Emblemasoma* is sister to

the clade *Blaesoxipha+Spirobolomyia*, and the clade encompassing these three genera is, in turn, sister to the clade *Oxysarcodexia+Ravinia*.

***Larval feeding strategies of extant species***

Larval feeding strategies could be determined for 30 of the 45 terminal taxa (67%) included in the phylogeny of the subfamily Sarcophaginae. Of those 30, 10 (33%) were parasitoids, 15 (50%) were scavengers, and 5 (17%) were facultative parasitoids/scavengers. Larval feeding strategies for all sarcophagines included in this study are summarized in Figure 6.2, and detailed assessments for each species, with all references, are provided in Appendix C.

For most species identified as having larvae that can develop as parasitoids (i.e., species that were identified as either parasitoids or facultative parasitoids/scavengers), the determination was based on positive rearing records from live hosts in controlled conditions. For three species, however, inference of a parasitoid lifestyle was based on other kinds of evidence, and each of these exceptions is briefly explained here.

*Blaesoxipha* (*Acanthodotheca*) sp.: This specimen (and others collected with it) could not be conclusively identified as any currently described species of *Blaesoxipha* (*Acanthodotheca*), but it is very similar to *B.* (*A.*) *prohibita* (Aldrich) and *B.* (*A.*) *savoryi* (Parker), and perhaps belongs to one of those species. This specimen was coded as a parasitoid for the following reasons. First, *Acanthodotheca* is a very well-defined group within *Blaesoxipha*, and all available evidence suggests these flies are almost exclusively parasitoids, most of which use beetles as hosts. Rearing data are available for 16 of the 71 described species of *Acanthodotheca* (23%), and in nearly every case, they suggest a parasitoid lifestyle (Pape 1994, Reeves et al. 2000). The only definite exception is a record of *B.* (*A.*) *dampfi* (Hall) that were reared from a "dead rodent" (Reeves et al. 2000), and Pape (1994) concluded that "most if

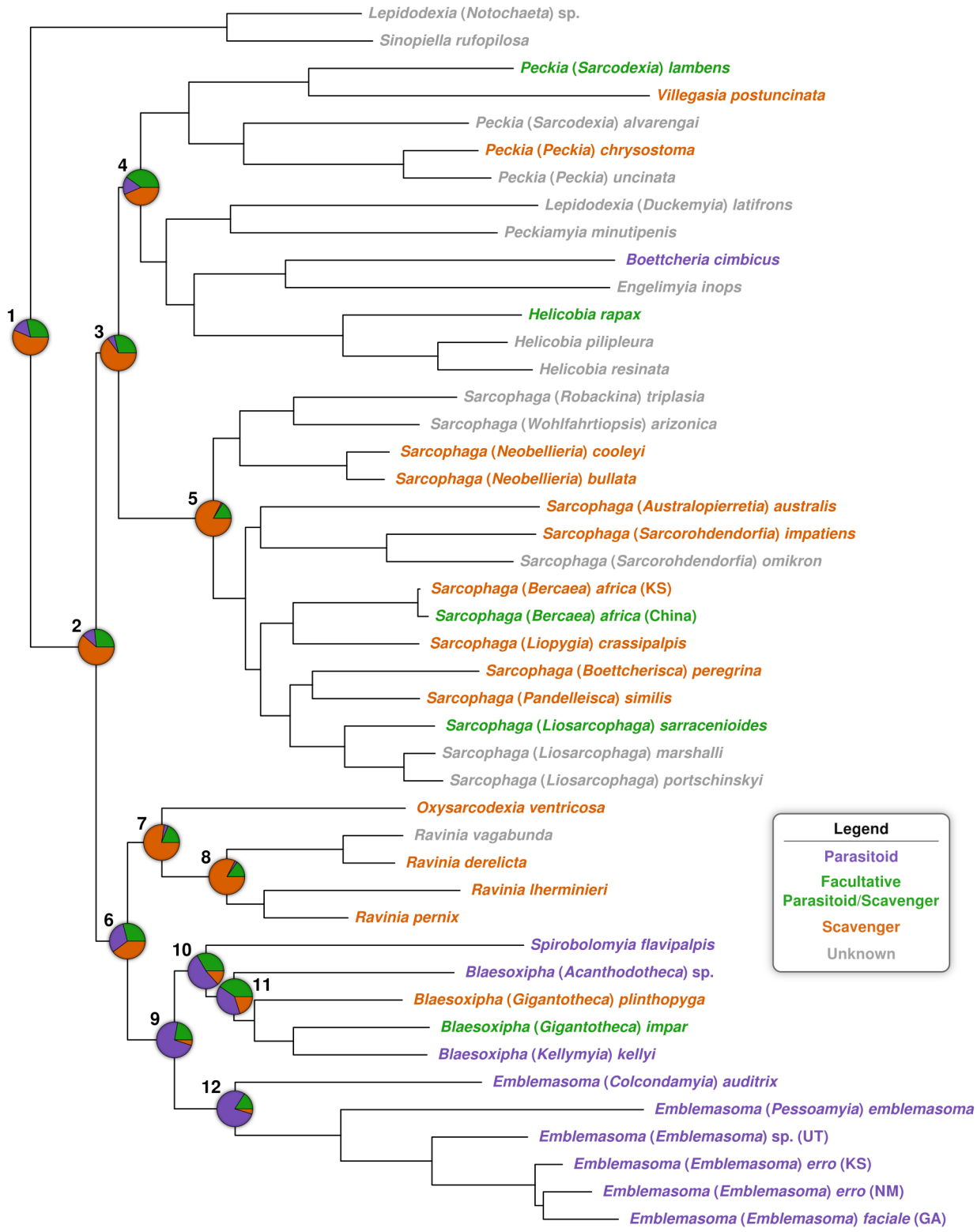


**Figure 6.2** Summary of ancestral state reconstructions for key clades of the sarcophagid subfamily Sarcophaginae. The pie charts depict the mean values of the posterior distributions of the reconstructed states from the BayesTraits analysis. Clade numbers correspond with the clade numbers in Table 6.3.

not all [*Acanthodotheca*] are beetle parasites". Second, rearing records for *B.* (*A.*) *prohibita* and *B.* (*A.*) *savoryi*, the two species closest to the specimen in this study, suggest that both species are parasitoids of beetles, and *B.* (*A.*) *prohibita* has even been observed larvipositing on live beetles (Hall 1929, Pape 1994). Consequently, it seemed very likely that the specimen in question was also a parasitoid, and classifying it as such also made sense in terms of the known biology of the entire subgenus *Acanthodotheca*.

*Emblemasoma faciale* Aldrich: Although this species has not yet been reared, it was considered a parasitoid of cicadas for two reasons. First, it is morphologically nearly indistinguishable from *E. erro*, which is a known parasitoid of cicadas (Stucky 2015), and *E. faciale* might, in fact, merely be a synonym of *E. erro* (Downes 1965; also see the results below). Second, female *E. faciale* in the field perform positive phonotaxis in response to cicada calling songs in a manner that appears to be virtually identical to the host-finding behavior of *E erro* (Appendix C). All specimens of *E. faciale* captured for this study were attracted in this manner, so it seems almost certain that *E. faciale* is, like *E. erro*, a parasitoid of cicadas.

*Spirobolomyia flavipalpis* (Aldrich): There are no rearing records available for this species with details about the rearing methods or the condition of the supposed host animals when captured. However, there is convincing circumstantial evidence that this species is a parasitoid of millipedes. First, all rearing records for the genus *Spirobolomyia*, including records for *S. flavipalpis*, suggest that these flies parasitize millipedes (Aldrich 1916, Pape 1990). Second, the geographic range of *Spirobolomyia* in the Nearctic is approximately the same as the range of the spirobolid millipede genus *Narceus*, which includes the species from which *Spirobolomyia* have been reared (Pape 1990). Finally, *Spirobolomyia* are known to be attracted by the defensive secretions of millipedes, which suggests a possible host-finding mechanism

(Eisner et al. 1998). Although Eisner et al. did not report the species of *Spirobolomyia* encountered in their study, the location of their field site in central Florida, along with the known distribution of *Spirobolomyia* species (Pape 1996), indicates that the species was very likely to have been *S. flavipalpis*.

Finally, it should be mentioned that at least eight of the species identified here as scavengers or facultative parasitoids/scavengers can also, under exceptional circumstances, infect vertebrates (including humans) as apparent facultative parasites. These species are *Blaesoxipha* (*Gigantotheca*) *plinthopyga* (Wiedemann), *Peckia* (*Sarcodexia*) *lambens* (Wiedemann), *Sarcophaga* (*Bercaea*) *africa*, *S.* (*Boettcherisca*) *peregrina* (Robineau-Desvoidy), *S.* (*Liopygia*) *crassipalpis* Macquart, *S.* (*Neobellieria*) *bullata* Parker, *S.* (*N.*) *cooleyi* Parker, and *S.* (*Pandelleisca*) *similis* Meade (Stewart 1934, James 1947, Shiota et al. 1990, Hatsushika et al. 2002, Fernandes et al. 2009). However, there is little evidence that any of these species regularly, if ever, develop as primary parasites of healthy tissue. Instead, they are probably all "accidental" parasites that are initially attracted to larviposit on feces-contaminated body surfaces or on necrotic, festering wounds, and in severe cases, may then secondarily damage healthy tissues (Shewell 1987, Bänziger and Pape 2004). Alternatively, some species might initially larviposit on human foods and then, if the food is ingested, the fly larvae can incidentally invade the gastrointestinal tract (Haseman 1917, Shiota et al. 1990). Either way, there did not seem to be compelling evidence for classifying these species as anything other than scavengers.

***Evolution of sarcophagine larval feeding strategies***

The results of the reconstructions of ancestral larval feeding strategies in the subfamily Sarcophaginae are depicted in Figure 6.2, and the posterior distributions of the reconstructions

are summarized in Table 6.3. Summaries of the posterior distributions for the model parameter estimates are given in Table 6.4. The PSRF values for all model rate parameters deviated by less than 0.0002 from 1.0, which strongly suggested that all three BayesTraits MCMC runs converged and were sampling from the true posterior distribution. The Geweke diagnostics also did not indicate any problems with convergence or an inadequate burn-in period.

As might be expected given the size of the dataset and the number of species for which larval feeding strategies were coded as "unknown", there was considerable uncertainty in the estimates of the model state transition rates and in the estimated probabilities of ancestral states at many of the selected nodes. Nevertheless, the likely ancestral larval feeding strategies were still relatively clear in many cases.

**Table 6.3** Ancestral state reconstructions from the BayesTraits results. The clade numbers correspond with the clade numbers in Figure 6.1. "P" = parasitoid, "F" = facultative parasitoid/scavenger, "S" = scavenger. Posterior ancestral state probabilities are conditional on the target clade actually existing; unconditional probabilities can be approximated by multiplying the ancestral state probability and the posterior probability of the target clade given in Figure 6.1 (Pagel et al. 2004).

| Clade | Clade number | Feeding strategy | Posterior mean | Posterior median | 95% HPD interval |
|---|---|---|---|---|---|
| Sarcophaginae | 1 | F | 0.285 | 0.303 | 0.009 - 0.498 |
| | | P | 0.151 | 0.099 | 0 - 0.452 |
| | | S | 0.565 | 0.538 | 0.221 - 0.975 |
| Sarcophaginae, exept for (*Notochaeta*+*Sinopiella*) | 2 | F | 0.267 | 0.267 | 0 - 0.568 |
| | | P | 0.119 | 0.047 | 0 - 0.476 |
| | | S | 0.614 | 0.599 | 0.195 - 1 |
| *Sarcophaga*+sister | 3 | F | 0.287 | 0.300 | 0 - 0.527 |
| | | P | 0.065 | 0.038 | 0 - 0.219 |
| | | S | 0.648 | 0.628 | 0.377 - 1 |
| sister to *Sarcophaga* | 4 | F | 0.401 | 0.404 | 0.112 - 0.662 |
| | | P | 0.163 | 0.148 | 0 - 0.36 |
| | | S | 0.436 | 0.415 | 0.142 - 0.818 |

| Clade | Clade number | Feeding strategy | Posterior mean | Posterior median | 95% HPD interval |
|---|---|---|---|---|---|
| *Sarcophaga* | 5 | F | 0.162 | 0.094 | 0 - 0.483 |
| | | P | 0.014 | 0.005 | 0 - 0.056 |
| | | S | 0.824 | 0.889 | 0.498 - 1 |
| (*Ravinia*+*Oxysarcodexia*)+((*Blaesoxipha*+*Spirobolomyia*)+*Emblemasoma*) | 6 | F | 0.289 | 0.290 | 0 - 0.572 |
| | | P | 0.313 | 0.280 | 0 - 0.75 |
| | | S | 0.397 | 0.373 | 0.024 - 0.817 |
| *Ravinia*+*Oxysarcodexia* | 7 | F | 0.190 | 0.161 | 0 - 0.453 |
| | | P | 0.036 | 0.018 | 0 - 0.131 |
| | | S | 0.774 | 0.799 | 0.498 - 1 |
| *Ravinia* | 8 | F | 0.153 | 0.113 | 0 - 0.42 |
| | | P | 0.022 | 0.009 | 0 - 0.088 |
| | | S | 0.825 | 0.862 | 0.55 - 1 |
| (*Blaesoxipha*+*Spirobolomyia*)+*Emblemasoma* | 9 | F | 0.221 | 0.161 | 0 - 0.602 |
| | | P | 0.729 | 0.776 | 0.32 - 1 |
| | | S | 0.050 | 0.022 | 0 - 0.198 |
| *Blaesoxipha*+*Spirobolomyia* | 10 | F | 0.333 | 0.334 | 0 - 0.693 |
| | | P | 0.534 | 0.512 | 0.131 - 1 |
| | | S | 0.133 | 0.104 | 0 - 0.35 |
| *Blaesoxipha* | 11 | F | 0.404 | 0.409 | 0 - 0.726 |
| | | P | 0.395 | 0.377 | 0 - 0.815 |
| | | S | 0.201 | 0.181 | 0 - 0.442 |
| *Emblemasoma* | 12 | F | 0.160 | 0.123 | 0 - 0.42 |
| | | P | 0.793 | 0.827 | 0.505 - 1 |
| | | S | 0.047 | 0.030 | 0 - 0.15 |

**Table 6.4** Larval feeding strategy transition rate estimates from the BayesTraits analysis. "P" = parasitoid, "F" = facultative parasitoid/scavenger, "S" = scavenger.

| Parameter | Mean | Median | 95% HPD interval |
|---|---|---|---|
| q(F→P) | 8.295 | 6.432 | 0 - 22.374 |
| q(F→S) | 12.250 | 9.549 | 0 - 32.697 |
| q(P→F) | 3.040 | 2.281 | 0 - 8.488 |
| q(P→S) | 2.470 | 1.783 | 0 - 7.094 |
| q(S→F) | 5.588 | 4.751 | 0.502 - 12.745 |
| q(S→P) | 1.859 | 1.361 | 0 - 5.257 |

Two of the deepest nodes in the tree – the whole of Sarcophaginae, and Sarcophaginae minus *Notochaeta*+*Sinopiella* – had similar ancestral state reconstructions. The ancestors at these nodes were inferred as most likely not strictly parasitoids and could have reasonably been either obligate scavengers or facultative parasitoids/scavengers, although scavenger was considered most probable for both nodes (the posterior probabilities (PPs) that the ancestors of these two clades were scavengers were 0.56 and 0.61, respectively, as calculated by taking the means of the posterior distributions of the probability that the ancestral state was scavenger for each clade).

The common ancestor of the first major sarcophagine lineage, the clade comprising *Sarcophaga* and its relatives, probably had larval feeding habits similar to the whole of Sarcophaginae: it was most likely a scavenger (PP = 0.65), but very possibly could have been a facultative parasitoid/scavenger (PP = 0.29). The common ancestor of *Sarcophaga*, however, was most likely strictly a scavenger (PP = 0.82).

The other major lineage of Sarcophaginae, the clade encompassing *Ravinia*, *Oxysarcodexia*, *Blaesoxipha*, *Spirobolomyia*, and *Emblemasoma*, had an almost completely ambiguous ancestral state reconstruction, with none of the three possible states clearly favored (or not) over the others. After this ambiguously reconstructed common ancestor, however, there are two lineages with essentially divergent larval feeding strategies. The ancestors in the first lineage, represented by the clades *Oxysarcodexia*+*Ravinia* and *Ravinia*, were most likely obligate scavengers (PP = 0.77), with some probability that they could have been facultative parasitoids/scavengers (PP = 0.19). In contrast, the common ancestor of the second lineage, represented by the clade comprising *Blaesoxipha*, *Spirobolomyia*, and *Emblemasoma*, was most likely an obligate parasitoid (PP = 0.73) or could possibly have been a facultative

parasitoid/scavenger (PP = 0.22), but was most likely not an obligate scavenger (PP = 0.05). The ancestors of *Blaesoxipha*+*Spirobolomyia* and *Blaesoxipha* were probably either parasitoids (PPs = 0.53 and 0.40, respectively) or facultative parasitoids/scavengers (PPs = 0.33 and 0.40) and not obligate scavengers (PPs = 0.13 and 0.20), but for these two clades, the ancestral state reconstructions were relatively ambiguous. Finally, the common ancestor of *Emblemasoma* was most likely an obligate parasitoid (PP = 0.79), but could have possibly been a facultative parasitoid/scavenger (PP = 0.16). It was most likely not an obligate scavenger (PP = 0.05).

## 6.4 Discussion

### *Phylogeny of Sarcophagidae and* Emblemasoma

Although the primary focus of this study was on the evolution of *Emblemasoma*, some of the findings from the broader phylogenetic analysis of Sarcophagidae are also noteworthy, especially in the context of other, recent morphological and molecular phylogenetic studies of this family. Here, I briefly discuss my results in comparison with the conclusions of some of these other studies, as well as the possible implications of these results for the taxonomy of the group. Note that this is not intended to be an exhaustive synthesis of previous studies; rather, I merely emphasize a few key points. I also discuss the phylogeny of *Emblemasoma* and its relationship to the rest of Sarcophagidae.

#### *Sarcophagid subfamilies*

Perhaps the most striking result of the present study, from a taxonomic standpoint, is the strong evidence that the subfamily Paramacronychiinae is paraphyletic. The two included species of the subfamily Miltogramminae (*Pterella nigrofasciata* and *Sphenometopa claripennis*) are nested within Paramacronychiinae as sister to *Nyctia*, and the posterior probability of this

relationship is nearly 1.0 (prob. = 0.997). Together, the species of Paramacronychiinae and Miltogramminae form a strongly supported, monophyletic clade (prob. = 1.0).

The recognition of three sarcophagid subfamilies was initially based on morphological evidence (Downes 1955, Pape 1987, 1996). So far, few molecular studies have addressed the validity of these groups, and the results of these studies have been largely inconclusive. Kutty et al. (2010) found some support for the monophyly of all three subfamilies, but the support values for a monophyletic Paramacronychiinae were quite low, lending considerable doubt to any conclusions about subfamily classification. In contrast, the results of Piwczyński et al. (2014) corroborated the present study by finding that Paramacronychiinae was probably paraphyletic, but the support values for this part of the tree from their study were also far too low to be conclusive. In addition to these two molecular studies, the recent parsimony-based morphological analysis of Giroux et al. (2010) found Paramacronychiinae to be monophyletic, but their study did not include *Nyctia* or either of the miltogrammine genera included here (*Pterella* and *Sphenometopa*), and so their results could have been a consequence of taxa sampling.

My results strongly suggest that the current concepts of Miltogramminae and Paramacronychiinae are not tenable from a phylogenetic standpoint. The simplest way to resolve this issue taxonomically would be to unite the species from both subfamilies into a single, broad concept of Miltogramminae. Indeed, there is historical precedent for such a broadly defined Miltogramminae as the only other sarcophagid subfamily besides Sarcophaginae (Downes 1955, 1965, Shewell 1987, McAlpine 1989). An alternative solution would be to try to retain both Miltogramminae and Paramacronychiinae as subfamilies, but redefine them to properly reflect phylogeny. A revision of this nature, however, would need to wait until a more comprehensive

analysis can clarify the true relationships among the numerous species currently divided between these two subfamilies.

*Monophyly of the genera* Lepidodexia *and* Peckia

The present phylogenetic analysis also revealed that the genus *Lepidodexia*, as currently recognized, is not monophyletic. Pape (1996) reclassified a large number of genera as subgenera within *Lepidodexia*, and Pape et al. (2004) and Pape and Dahlem (2010) added *Duckemyia* as another subgenus of *Lepidodexia*. My study included representatives of only two subgenera, *Notochaeta* and *Duckemyia*. These two subgenera were widely separated on the tree, with *Lepidodexia* (*Duckemyia*) placed as sister to *Peckiamyia* and *Lepidodexia* (*Notochaeta*) placed as sister to *Sinopiella*. Both of these relationships were very strongly supported (probs. > 0.99). Kutty et al. (2010) also found that *Duckemyia* and *Notochaeta* were probably not closely related. Thus, if *Lepidodexia* is defined to include both *Duckemyia* and *Notochaeta*, it is clearly polyphyletic. Both Pape (1996) and Pape and Dahlem (2010) had already indicated that the concept of *Lepidodexia* was likely to change as more information became available, and this study appears to support their predictions.

*Peckia* is another genus that was not inferred as a monophylum. *Peckia* was recently comprehensively revised by Buenaventura and Pape (2013). Their expanded concept of *Peckia* does not include the genus *Villegasia*, but the present analysis very strongly suggests that *Peckia* is paraphyletic with respect to *Villegasia*, with *Villegasia postuncinata* as the sister taxon of *Peckia* (*Sarcodexia*) *lambens* (prob. = 1.0). Furthermore, Buenaventura's and Pape's revised concept of the *Peckia* subgenus *Sarcodexia* is evidently also paraphyletic, with *Peckia* (*Peckia*) nested within *Peckia* (*Sarcodexia*) (all branch probs. > 0.99). Two prior molecular studies (Kutty

et al. 2010, Piwczyński et al. 2014) had already hinted at both of these problems, but many of the branch support values within the *Peckia* clade from those studies were quite low.

*Sister taxon of* Sarcophaga *and the placement of* Helicobia

Recent molecular studies have generally determined that *Peckia* (sensu Buenaventura and Pape 2013) (with *Villegasia*) is the sister group of the large genus *Sarcophaga* (*sensu lato*), although support for this relationship has often been rather low (Kutty et al. 2010, Stamper et al. 2013, Piwczyński et al. 2014). The results here present a different conclusion, with *Peckia* and *Villegasia* part of a larger clade that also includes *Boettcheria*, *Engelimyia*, *Helicobia*, *Lepidodexia* (*Duckemyia*), and *Peckiamyia*, and with this larger clade placed as the sister of *Sarcophaga*. However, the monophyly of this possible sister clade had only modest support (prob. = 0.71), so further work is needed to better establish how *Sarcophaga* is related to the rest of the sarcophagine genera. Nevertheless, *Sarcophaga* and its proposed sister clade together form a group that is almost certainly monophyletic (prob. = 1.0), so these genera must be rather closely related even if the correct sister taxa relationships among them have not yet been conclusively resolved.

As mentioned in the previous paragraph, *Helicobia* appears to be part of the sister clade to *Sarcophaga*, but the precise phylogenetic relationships of this genus remain elusive. Previous molecular studies have all found that *Helicobia* is clearly monophyletic, but none were able to determine the sister group of *Helicobia* with any certainty. My results also show that *Helicobia* is undoubtedly monophyletic (prob. = 1.0), and it is part of a strongly-supported clade that also includes *Boettcheria*, *Engelimyia*, *Lepidodexia* (*Duckemyia*), and *Peckiamyia* (prob. = 0.98), but the placement of *Helicobia* within this clade was not clear. The parsimony-based morphological study of Giroux et al. (2010) suggested that *Helicobia* belonged within *Sarcophaga* and should

be considered a subgenus of the latter, but that hypothesis is not supported by my results: *Sarcophaga* without *Helicobia* is clearly monophyletic (prob. = 1.0), and *Helicobia* appears to be more closely related to the other genera mentioned than it is to *Sarcophaga*.

*Relationship of the genera* Oxysarcodexia *and* Ravinia

Morphological data have long supported a close relationship between the genera *Oxysarcodexia* and *Ravinia* (Roback 1954, Downes 1955, Lopes 1982, Pape 1994, Giroux et al. 2010), so it is somewhat surprising that two recent molecular studies found them to be widely separated on the sarcophagid phylogeny (Kutty et al. 2010, Piwczyński et al. 2014). However, it must be stressed that branch supports for the placement of *Oxysarcodexia* and *Ravinia* in both of those molecular studies were very low. My results concur with morphological studies and the molecular analysis of Stamper et al. (2013) by inferring a sister group relationship between *Oxysarcodexia* and *Ravinia*, and in the present study, the posterior probability of this relationship was nearly 1.0, providing strong evidence that these two genera are, indeed, very closely related. Although taxa sampling within *Ravinia* was much more limited in the present study, my results also agree with Wong et al. (2015) in finding that the species originally placed in *Chaetoravinia* and *Ravinia* (*sensu stricto*) form two monophyletic groups within *Ravinia*.

*Monophyly of the genus* Blaesoxipha *and subgenus* Blaesoxipha *(*Gigantotheca*)*

Another point of contention in recent studies has been the monophyly of *Blaesoxipha*. Of molecular studies, both Kutty et al. (2010) and Piwczyński et al. (2014) found *Blaesoxipha* to be polyphyletic, while Stamper et al. (2013) recovered a monophyletic *Blaesoxipha*. However, the studies of both Kutty et al. (2010) and Piwczyński et al. (2014) included only two species of *Blaesoxipha*, *B.* (*Gigantotheca*) *plinthopyga* and *B*. (*Blaesoxipha*) *setosa*, and branch support values were very low for the placement of either species. Regarding morphological studies, Pape

(1994) found *Blaesoxipha* to be monophyletic, while Giroux et al. (2010) determined that *Blaesoxipha* was paraphyletic with respect to *Spirobolomyia*. The present study corroborated *Blaesoxipha* as monophyletic (prob. = 0.96), with *Spirobolomyia* as its sister group (prob. > 0.99). Both molecular studies that found *Blaesoxipha* to be polyphyletic included the subgenus *Blaesoxipha* (*Blaesoxipha*), which contains almost exclusively Old World species, while the molecular studies inferring a monophyletic *Blaesoxipha* included only New World species. An intriguing possibility is that "*Blaesoxipha*", exclusive of the primarily Old World *Blaesoxipha* (*sensu stricto*), is a monophyletic group. An analysis with much more comprehensive taxa sampling within *Blaesoxipha* would be needed to test this. It might be especially important to include the subgenus *Blaesoxipha* (*Servaisia*), which is the only other subgenus with a substantial number of Old World species, and for which no molecular data are yet available. Regardless, Pape's (1994) parsimony-based morphological study included all 10 currently recognized subgenera and determined that *Blaesoxipha* was probably monophyletic. The present study is also the first molecular study to include the *Blaesoxipha* subgenus *Kellymyia*, and the results indicate that *Kellymyia* is contained within *Blaesoxipha* (*Gigantotheca*), rendering the latter paraphyletic. The earlier work of Pape (1994) had already suggested this possibility.

*Phylogeny of* Emblemasoma *and its relationship to other sarcophagids*

This study is the first to use molecular data to infer the relationships among the three genera currently synonymized with *Emblemasoma* (*Colcondamyia*, *Emblemasoma* [*sensu stricto*], and *Pessoamyia*) as well as the placement of *Emblemasoma* in the broader sarcophagid phylogeny. The results confirm that the broad concept of *Emblemasoma* proposed by Pape (1990), which is equivalent to the tribe Emblemasomatini proposed by Lopes (1974b, 1982), defines a monophyletic group. As might be expected from their morphological similarities,

*Emblemasoma* (*sensu stricto*) and *Pessoamyia* are sister taxa, and together they form the sister clade of *Colcondamyia* (all probs. = 1.0). Because *Colcondamyia* and *Pessoamyia* were each represented by only one species, the monophyly of these former genera could not be tested. At least with regard to the species included in this analysis, *Emblemasoma* (*sensu stricto*) was monophyletic (prob. = 1.0).

Multiple specimens of *E. erro* and *E. faciale* from a variety of localities were included in the analysis, and the results did not confirm the monophyly of these two species concepts. Instead, these specimens were all placed in a single, strongly supported clade (prob. = 1.0), and within this clade, three well-supported subclades were evident: *E. erro* from the Great Plains, *E. erro* from the southwestern U.S., and *E. erro*/*E. faciale* from the southeastern U.S. (all probs. > 0.97). One specimen of *E. erro* from Kansas was anomalous and grouped with the southeastern U.S. clade rather than the Great Plains clade. These results suggest that either *E. erro* and *E. faciale* are really a single, widespread species, as had been suggested earlier by Downes (1965), or they represent a complex of previously unrecognized cryptic species. Given the morphological and behavioral similarities throughout the complex (B. Stucky, unpublished data), along with the grouping of a Kansas *E. erro* with *E. faciale*, I suspect the former explanation is more likely, but more thorough biogeographic and biological data will be needed to distinguish between these hypotheses.

*Emblemasoma* (*sensu lato*) was very strongly supported as the sister group to the clade consisting of *Blaesoxipha*+*Spirobolomyia* (prob. = 1.0). I am unaware of any previously published, explicit phylogenetic hypotheses about the relationship of *Emblemasoma* to the rest of Sarcophagidae, but Pape (1990) noted that *Colcondamyia* shares several morphological features with *Blaesoxipha* and suggested that the genera might be closely related. In fact, Pape

previously classified *Colcondamyia* as part of *Blaesoxipha* (Pape 1987) before revising this change and synonymizing *Colcondamyia* with *Emblemasoma* (Pape 1990). The present analysis indicates that, as suggested by Pape, *Emblemasoma*, including *Colcondamyia*, is closely related to, yet distinct from, *Blaesoxipha*. Furthermore, there are remarkable similarities in the host infection behaviors of some species of *Blaesoxipha* and *Emblemasoma* (Stucky 2015), and these shared behaviors might also be indicative of shared ancestry.

***Evolution of larval feeding strategies in Sarcophaginae***

Despite uncertainty at some nodes, the ancestral state reconstructions still sketch at least a partial picture of the evolution of sarcophagine larval feeding strategies. The common ancestor of all sarcophagines was most likely a scavenger or possibly a facultative parasitoid/scavenger, and this basic biological ground plan was retained by at least some successful descendant lineages, such as the branches leading to the highly speciose *Sarcophaga*. Obligate parasitoidism appears to be a derived feeding strategy in the Sarcophaginae, and at least one major lineage of Sarcophaginae, here represented by the species-rich clade comprising *Blaesoxipha*, *Spirobolomyia*, and *Emblemasoma*, has more or less specialized on the parasitoid lifestyle. The closest relatives of this parasitoid clade, the species of *Oxysarcodexia*+*Ravinia*, appear to be obligate scavengers with little or no parasitoidism at all.

The rate parameter estimates for transitions between larval feeding strategies also reveal general patterns of evolutionary change in the Sarcophaginae. The facultative parasitoid/scavenger lifestyle appears to be a common pathway to greater specialization as either scavengers or parasitoids, because transitions from facultative parasitoid/scavenger to obligate scavenger or obligate parasitoid are relatively frequent. Transitions from strict scavenging to facultative parasitoid/scavenger are also not uncommon. On the other hand, transitions from

obligate parasitoid to the other two strategies are comparatively uncommon, and transitions from obligate scavenger to obligate parasitoid are also rare.

It should also be noted that even though the earliest sarcophagines were inferred to have most likely been scavengers, there is also a reasonably strong probability that they were facultative parasitoids/scavengers. Thus, even though Feener and Brown (1997) stated that "it is clear the parasitoid life-style evolved many times" in Sarcophagidae, the results of this study suggest that the parasitoid lifestyle could have been a characteristic of the very earliest sarcophagines, the descendants of which now account for the vast majority of sarcophagid diversity.

This study provides the first explicitly model-based statistical analysis of the evolution of sarcophagid feeding strategies, and the broad conclusions are generally consistent with evolutionary hypotheses that have previously been proposed by specialists of the group. Numerous authors have concluded that the earliest sarcophagids were most likely scavenging coprophages or necrophages, or possibly facultative parasitoids/scavengers (Roback 1954 [refers to Sarcophaginae only], Pape 1987, McAlpine 1989, Povolný and Verves 1997), and although my study did not encompass the entire family, the results are certainly concordant with these views. Several authors have also argued that sarcophagid parasitoids most likely evolved from necrophage ancestors and that the strategy of facultative parasitoid/scavenger might represent a transitional state in the evolution of obligate parasitoids (Aldrich 1915, 1916, Eggleton and Belshaw 1992, Povolný and Verves 1997). Again, my results support these earlier ideas.

Although it is fairly clear that facultative parasitoidism/scavenging can provide a stepping stone to greater specialization, extant species also provide ample evidence that the facultative strategy has been, in and of itself, very successful. Indeed, some common,

widespread sarcophagids utilize this strategy, such as *Helicobia rapax*, *Peckia* (*Sarcodexia*) *lambens* and *Sarcophaga* (*Liosarcophaga*) *sarracenioides* (e.g., Aldrich 1915, 1916, 1927, Hall 1929, Hallock 1940a, 1942, Coupland and Barker 2004). Even for species that are primarily parasitoids, it is not difficult to imagine that retaining the ability to facultatively develop as a scavenger could be a distinct advantage when host animals are uncommon or absent. Unfortunately, facultative parasitoidism appears to be a rather poorly understood phenomenon in general. One early study of at least some relevance found that the sarcophagid *Wohlfahrtia vigil* (Walker), which is generally considered to be an obligate vertebrate parasite, usually completely ignores carrion as a possible larviposition substrate unless the female fly is near the end of her life and is unable to infect living hosts (Walker 1931). It seems plausible that similar trade-offs might be important for at least some species of facultative parasitoids/scavengers, but experimental work is needed to test this. In any case, although facultative parasitoidism/scavenging is certainly not unique to Sarcophagidae (e.g., Eggleton and Belshaw 1992, Coupland and Barker 2004), it is nevertheless a conspicuous and apparently widespread sarcophagid feeding strategy, at least among species of the subfamily Sarcophaginae.

In addition to the uncertainty in the statistical results and potential misidentifications in literature records, two problems which have already been mentioned, there are at least five other potential limitations of this analysis which must also be considered. First, missing life history data could certainly have impacted the results. Perhaps the most glaring potential problem with missing data is the complete absence of information about larval feeding strategies for the basal group *Lepidodexia* (*Notochaeta*)+*Sinopiella* near the root of Sarcophaginae. At least some species of *Notochaeta* are parasitoids or parasites (Lopes 1969a, 1973), and it is quite possible that accounting for the larval feeding strategies of this group could change the inference about

ancestral states at the root of Sarcophaginae. It seems unlikely, though, that ancestral state reconstructions deeper in the phylogeny would change significantly.

Second, inadequate taxa sampling might have also been a problem. It is possible that the conclusions are biased due to the absence of biologically important genera or higher clades. In addition, if the species that were sampled for a given clade are not representative of the "average" biology of the clade, then the results might also be biased. This could have been a problem, for example, with the very large genus *Blaesoxipha*. *Blaesoxipha* includes many species of obligate parasitoids (Pape 1994), and it is probable that the species included in this analysis underrepresent the prevalence of the parasitoid lifestyle in this clade. It seems likely, then, that a more thorough sampling of *Blaesoxipha* species would result in a much lower probability that the common ancestor of *Blaesoxipha* could have been a scavenger.

Third, the included species do not cover the full breadth of larval feeding strategies of the Sarcophaginae. For instance, at least some species of sarcophagines are evidently obligate vertebrate parasites, such as *Cistudinomyia cistudinis* (Aldrich), which parasitizes turtles (Aldrich 1916, Peters 1948, Dodge 1955), and *Lepidodexia* (*Notochaeta*) *blakeae* (Dodge), which parasitizes lizards (Blake 1955, Dodge 1955, Irschick et al. 2006). Still, our current knowledge of Sarcophaginae suggests that the three feeding strategies included in the present study provide a reasonable classification for the vast majority of the species in this subfamily, so it seems unlikely that ancestral reconstructions for most nodes would change very drastically with the inclusion of additional feeding strategies.

Fourth, it is all but certain that literature records to date do not fully represent the breadth of larval feeding habits for the species included in this study. Natural history data for many species are limited to only one or a very few records, so it is likely, for instance, that further work

will reveal that some species currently known only as scavengers can also develop as facultative parasitoids, or that some species currently considered obligate parasitoids also develop as scavengers in exceptional circumstances. Incomplete life history information is likely to result in a bias towards recognizing species as scavengers but not parasitoids, because studies of necrophagous flies are relatively common and it is generally much more difficult to unambiguously demonstrate that a particular species can develop as a true parasitoid than it is to demonstrate a species develops as a scavenger. Consequently, one might predict that if we had complete knowledge of the habits of all included species, the overall proportion of species known to develop as parasitoids would increase, while the proportions of obligate scavengers and obligate parasitoids would decrease. It is uncertain what effect this might have on the ancestral state reconstructions, but it at least seems likely that the probability of some form of parasitoidism would increase for some nodes.

Fifth, given the well-known difficulties of sarcophagid morphological taxonomy, it is entirely possible, perhaps even likely, that our current understanding of larval feeding strategies is misguided by the presence of cryptic species diversity. For example, the geographically widespread species *Peckia* (*Sarcodexia*) *lambens* appears to "do everything": It is reliably documented as a scavenger of mammalian excrement, dead arthropods, and vertebrate carrion; as a parasitoid of living insects and frogs; and as a facultative agent of myiasis in vertebrates (e.g., Drake 1920, Aldrich 1927, Graenicher 1931, James 1947, Lopes 1973, D'Almeida 1994, Hagman et al. 2005, Fernandes et al. 2009). It is possible that what is currently recognized as *P.* (*S.*) *lambens* is actually a complex of cryptic species, each specialized on a particular feeding strategy. Conflicting natural history data have already suggested that some species, such as *S.* (*Bercaea*) *africa*, might use different feeding strategies in different parts of their range (this

study, Bänziger and Pape 2004). Moreover, it is certainly not unprecedented for "species" of insects once thought to be generalists of some sort to be recognized as a complex of cryptic specialists upon closer examination (e.g., Kankare et al. 2005, Smith et al. 2007, 2008, Burns et al. 2008). If Sarcophagidae includes many such cryptic species complexes, then it could turn out that facultative parasitoids/scavengers are less common then currently thought.

The potential problems outlined above all indicate that much work remains to improve our knowledge of the ecological diversity and evolutionary history of Sarcophagidae. More molecular data is certainly needed to construct a more complete flesh fly phylogeny, but perhaps the most serious current limitation is the total lack of natural history data for so many sarcophagid species. Natural history studies are often logistically challenging, time intensive, and not always easy to fund, so it is perhaps not surprising that so little information is available for so many species. Nevertheless, more thorough taxa sampling and more complete natural history knowledge are the obvious remedies for most of the problems discussed above, and careful behavioral and molecular studies could also reveal the presence of cryptic species complexes. Despite the potential shortcomings, however, the present study covers the major sarcophagine larval feeding strategies and includes representatives from many of the most speciose taxonomic groups, so it seems likely that the results should at least provide a reasonable sketch of broad patterns in the evolution of larval feeding strategies in this subfamily.

***Evolution of hearing and acoustic eavesdropping in Sarcophagidae***

I turn finally to the primary focus of this study and discuss the implications of my results for our understanding of the evolution of hearing and acoustic eavesdropping in Sarcophagidae. To begin with, the conclusions of the phylogenetic reconstruction and the analysis of the evolution of larval feeding strategies are fully consistent with the host-finding hypothesis as an

explanation of the origins of sarcophagid hearing. First, *Emblemasoma* represents an exceedingly well supported monophyletic clade of sarcophagids (PP = 1.0) that corresponds with the evolution of tympanal hearing in Sarcophagidae. The four species of *Emblemasoma* so far subjected to behavioral study – *E. auditrix*, *E. emblemasoma*, *E. erro*, and *E. faciale* – all have well developed hearing that is used to orient toward cicada calling songs (Soper et al. 1976, Stucky 2015, Appendix C). In these flies, the tympanal organs are located on a greatly expanded prosternal region (Lakes-Harlan et al. 1999, Farris et al. 2008), and all known species of *Emblemasoma*, but no other known species of Sarcophagidae, possess this inflated prosternal area (Lopes 1988). Clearly, tympanal organs for long-range hearing are an autapomorphy of *Emblemasoma* that originated with the common ancestor of the clade. Second, the ancestral state reconstructions strongly suggest that the parasitoid lifestyle was already in place well before this common ancestor evolved. The parent clade of *Emblemasoma*, which comprises *Blaesoxipha*, *Spirobolomyia*, and *Emblemasoma*, was ancestrally most likely an obligate parasitoid (mean posterior probability [MPP] = 0.729) or possibly a facultative parasitoid/scavenger (MPP = 0.221), but was very unlikely to have been a scavenger (MPP = 0.05). Putting these results together, then, it is clear that in the lineage leading to *Emblemasoma*, parasitoidism evolved before tympanal hearing. This is exactly as the host-finding hypothesis would predict, and it means that the ecological context needed to select for efficient host-finding behaviors, such as acoustic eavesdropping, could have been firmly established by the time tympanal hearing began to evolve.

Furthermore, it is unlikely that hearing was ancestrally associated with any other function for *Emblemasoma*, because alternative explanations for the evolution of hearing in this genus simply do not fit the available evidence. I discuss two possibilities: First, the hypothesis that

hearing evolved to detect bats, and second, the hypothesis that hearing evolved for intraspecific communication.

If sarcophagid hearing evolved as a mechanism to avoid bat predation, then the appearance of hearing should correlate with nocturnal behavior, but this is clearly not the case. For one thing, neither *Emblemasoma* nor their cicada hosts are nocturnal. Although cicadas will sometimes call well past dusk in certain circumstances (e.g., very warm, humid weather with bright moonlight, or in the presence of artificial lighting) (Beamer 1928, Sanborn et al. 2005, B. Stucky, personal obs.), such behavior is uncommon and cicadas are, as a whole, only active during daylight (Myers 1929, Moore 1966). Indeed, many species limit the majority of their calling activity to the brightest, sunniest portion of the day (e.g., Myers 1929, Sugden 1940, Alexander and Moore 1958, Hastings and Toolson 1991, Sanborn and Phillips 1992, Lakes-Harlan et al. 2000, Sanborn et al. 2002, Cole 2008, Stucky 2013). Correspondingly, extant *Emblemasoma* are, as far as we know, also active during the day. In fact, for *E. auditrix*, *E. emblemasoma*, and *E. erro*, the only species for which host infection behavior has been studied, visual orientation plays an important role in the larviposition sequence (Schniederkötter and Lakes-Harlan 2004, Stucky 2015, B. Stucky, unpublished data). Therefore, unlike tachinid acoustic parasitoids, which can larviposit in darkness using only acoustic information (Cade 1979, Fowler 1987, Allen et al. 1999), the known infection behaviors of *Emblemasoma* would most likely be impossible without at least some sunlight. Given what is currently known about extant species of *Emblemasoma*, then, it seems very unlikely that the ancestor of this group was a nocturnally active parasitoid that first evolved hearing to escape bat predation, then later switched to diurnally active hosts and vision-dependent host infection behaviors.

Moreover, not only do extant *Emblemasoma* not support the bat predation hypothesis, but there are evidently no truly nocturnal species known anywhere in the whole of Sarcophagidae. This possibility has been most thoroughly investigated for sarcophagid scavengers of vertebrate carrion. Multiple studies have found that sarcophagid scavengers are essentially active only during the day and rarely or never larviposit at night (Tessmer et al. 1995, Baldridge et al. 2006, Singh and Bharti 2008, Stamper et al. 2009). Similarly, a bait-trapping study of adult flies in Malaysia found that sarcophagids were almost exclusively active during the day (Nazni et al. 2007). There have been a few reports of sarcophagids attracted to artificial lights at night (Shannon 1914, Walton 1915, Audcent 1951, Sparks et al. 1986), but these scattered records are hardly indicative of habitual nocturnal activity. After all, it is well known that many insects, if disturbed, will fly to artificial lights at night even if they are normally diurnally active. Otherwise, there is little, if any, other evidence for nocturnal activity in sarcophagids. Lopes (1981) speculated that *Lepidodexia* (*Notochaeta*) *bufonivora* (Lopes and Vogelsang), a parasitoid of frogs, might be nocturnal, but presented no evidence to support this hypothesis. Similarly, Shi et al. (2015) suggested that *Sarcophaga* (*Liosarcophaga*) *dux* Thompson, a facultative parasitoid of scorpions, might larviposit at night, but they did not directly observe nocturnal behavior. Pape (1987) stated that "adults [of *Blaesoxipha*] often have a complex larviposition behaviour, and some species are nocturnal or crepuscular", but he did not offer any further details and made no mention of it in his later comprehensive revision of *Blaesoxipha* (Pape 1994). Indeed, Pape et al. (2002) noted that "adult Sarcophagidae are known to be strictly diurnal". The apparent absence of any nocturnal species among extant sarcophagids makes the possibility of a nocturnal ancestor to *Emblemasoma* even more remote. Overall, the bat predation hypothesis does not seem to be a viable explanation for hearing in *Emblemasoma*.

Another possibility is that hearing initially evolved to facilitate intraspecific acoustic sexual communication, but this does not fit the available evidence, either. No sarcophagids outside of *Emblemasoma* are known to have long-range hearing, and no species of sarcophagids, including those of *Emblemasoma*, are known to engage in long distance acoustic communication. Lakes-Harlan et al. (2014) investigated this possibility in some detail for *E. auditrix*, but found no evidence that these flies used their hearing for any sort of intraspecific acoustic communication. Of course, hearing could still be useful for mate finding even if the flies do not produce acoustic signals themselves by allowing males and females to meet at a mutually attractive sound source. As shown in Chapter 3, at least one species of *Emblemasoma*, *E. erro*, uses the mating calls of its cicada hosts for exactly this purpose. Although it is possible that this application played a role in the evolution of hearing, it seems unlikely. Individuals of the *E. erro*/*E. faciale* complex are, at this point, the only *Emblemasoma* known for which males exhibit positive phonotaxis to host calling songs. Males of the other species that have been investigated, *E. auditrix* and *E. emblemasoma*, are apparently not attracted by the signals of their hosts (Lakes-Harlan et al. 2014, B. Stucky, unpublished data). Thus, even though the evidence is limited, it suggests that use of the host calling song for mate finding is a derived feature of the *Emblemasoma* (*sensu stricto*) lineage and not ancestral to the entire *Emblemasoma* (*sensu lato*) clade. This means that, if the mate-finding hypothesis were correct, it would require that the ancestors of *Emblemasoma* initially acquired hearing to detect the sexual signals of other insects for the purpose of finding mates, later (or concurrently) expanded their use of hearing to include host finding, then subsequently lost the original mate finding functionality multiple times. The alternative, under the host-finding hypothesis, is that the ancestors of *Emblemasoma* initially acquired hearing to assist in host finding, then later expanded their use of hearing in at least one

lineage to include mate finding. The latter is certainly the most parsimonious explanation and, given the ubiquity of hearing as a host-finding mechanism in *Emblemasoma*, seems a much better fit to the available data. Overall, the host-finding hypothesis is the most compelling explanation of the origins of hearing in Sarcophagidae.

## 6.5 Conclusions

The results of this study provide the best evidence yet for the host-finding hypothesis as an explanation for hearing, and eavesdropping, in some parasitoid insects. In the introduction, I outlined three criteria that a lineage of parasitoids should satisfy to provide strong evidence for the host-finding hypothesis, and *Emblemasoma* meets all three: First, the parasitoid lifestyle was probably already established before hearing began to evolve; second, tympanal hearing is clearly an autapomorphy of *Emblemasoma*; and third, there is very little evidence that hearing was ancestrally used for any purpose besides finding hosts. Researchers have long suspected that host finding was responsible for the evolution of hearing in acoustic parasitoids (Robert et al. 1992, 1996, Lakes-Harlan et al. 1999), and for *Emblemasoma*, at least, it appears they were correct. There simply is no other explanation that better fits the available evidence.

More broadly, this study demonstrates the utility of sarcophagids for testing hypotheses about the evolution of insect life histories and feeding strategies, the potential of which, I believe, has barely been tapped. Sarcophagidae is among the most ecologically diverse families of Diptera (or any order of insects, for that matter) and encompasses a staggering range of natural histories, including necrophages, coprophages, predators, cleptoparasites, obligate vertebrate parasites, facultative vertebrate parasites, obligate parasitoids, and facultative parasitoids (Ferrar 1987, Shewell 1987, Pape 1996, Povolný and Verves 1997). It is not uncommon to find several of these feeding strategies even within a single genus (e.g., Figure

6.2). Moreover, the results of my study suggest that at least some of these feeding strategies have probably evolved multiple times *within* Sarcophagidae. Given such outstanding, multifaceted diversity within a family of about 2,500 species, sarcophagids could be ideal for exploring fundamental questions about the evolutionary ecology of parasitoids and other trophic strategies that go well beyond this study's focus on eavesdropping. To give but a few examples: How common are evolutionary transitions between feeding strategies? Are some feeding niches more phylogenetically conserved than others, and if so, why? In what ecological contexts do parasitoids evolve? Answering any of these questions will, however, require more fine-grained analysis of both the phylogeny and life history evolution of sarcophagids, which ultimately means that more complete taxa sampling, in terms of both DNA sequences and life history data, is needed first. The present study shows that even within the rather severe constraints of current phylogenetic and life history knowledge, sarcophagids can shed new light on old questions. It would be exciting to see what we could learn if our understanding of the extant diversity and natural histories of these flies were more complete.

## References


Abdel-Kareim AI, Kozár F (1988) Extraction and bioassay of the sex pheromone of the red pear scale, *Epidiaspis leperii*. *Entomologia Experimentalis et Applicata* 46: 79–84. doi: 10.1111/j.1570-7458.1988.tb02270.x

Aberer AJ, Krompass D, Stamatakis A (2013) Pruning rogue taxa improves phylogenetic accuracy: an efficient algorithm and webservice. *Systematic Biology* 62: 162–166. doi: 10.1093/sysbio/sys078

Adamo SA, Robert D, Hoy RR (1995) Effects of a tachinid parasitoid, *Ormia ochracea*, on the behaviour and reproduction of its male and female field cricket hosts (*Gryllus* spp). *Journal of Insect Physiology* 41: 269–277. doi: 10.1016/0022-1910(94)00095-X

Adamo SA, Robert D, Perez J, Hoy RR (1995) The response of an insect parasitoid, *Ormia ochracea* (Tachinidae), to the uncertainty of larval success during infestation. *Behavioral Ecology and Sociobiology* 36: 111–118. doi: 10.1007/BF00170716

Adham FK, Zohdy NZ, Yeramian MA (1980) Studies on the mating behaviour of the flesh fly *Parasarcophaga argyrostoma* (Robineau-Desvoidy) (Diptera: Sarcophagidae). *Journal of the Egyptian Society of Parasitology* 10: 349–357.

Agresti A, Coull BA (1998) Approximate is better than "exact" for interval estimation of binomial proportions. *The American Statistician* 52: 119. doi: 10.2307/2685469

Aidley DJ (1969) Sound production in a Brazilian cicada. *Journal of Experimental Biology* 51: 325–337.

Akaike H (1973) Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Caski F (Eds), *Proceeding of the Second International Symposium on Information Theory*. Akademiai Kiado, Budapest, 267–281.

Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723. doi: 10.1109/TAC.1974.1100705

Alcock J (2000) The natural history of a miltogrammine fly, *Miltogramma rectangularis* (Diptera: Sarcophagidae). *Journal of the Kansas Entomological Society* 73: 208–219.

Alcock J, Schaefer JE (1983) Hilltop territoriality in a Sonoran desert bot fly (Diptera: Cuterebridae). *Animal Behaviour* 31: 518. doi: 10.1016/S0003-3472(83)80074-8

Aldrich JM (1915) The economic relations of the Sarcophagidae. *Journal of Economic Entomology* 8: 242–247. doi: 10.1093/jee/8.2.242

Aldrich JM (1916) *Sarcophaga and Allies in North America*. Murphey-Bivins Co. Press, Lafayette, IN, 301 pp.

Aldrich JM (1927) The dipterous parasites of the migratory locust of tropical America, *Schistocerca paranensis* Burmeister. *Journal of Economic Entomology* 20: 588–593. doi: 10.1093/jee/20.4.588

Aldrich JM (1930) Notes on the types of American two-winged flies of the genus *Sarcophaga* and a few related forms, described by the early authors. *Proceedings of the United States National Museum* 78: 1–39.

Aldrich JR (1985) Pheromone of a true bug (Hemiptera-Heteroptera): attractant for the predator, *Podisus maculiventris*, and kairomonal effects. In: Acree TE, Soderlund DM (Eds), *Semiochemistry: Flavors and Pheromones*. W. de Gruyter, Berlin ; New York, 95–119.

Aldrich JR, Hoffmann MP, Kochansky JP, Lusby WR, Eger JE, Payne JA (1991) Identification and attractiveness of a major pheromone component for Nearctic *Euschistus* spp. stink bugs (Heteroptera: Pentatomidae). *Environmental Entomology* 20: 477–483. doi: 10.1093/ee/20.2.477

Aldrich JR, Khrimian A, Zhang A, Shearer PW (2006) Bug pheromones (Hemiptera: Heteroptera) and tachinid fly host-finding. *Denisia* 19: 1015–1031.

Aldrich JR, Kochansky JP, Abrams CB (1984) Attractant for a beneficial insect and its parasitoids: pheromone of the predatory spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae). *Environmental Entomology* 13: 1031–1036. doi: 10.1093/ee/13.4.1031

Aldrich JR, Lusby WR, Marron BE, Nicolaou KC, Hoffmann MP, Wilson LT (1989) Pheromone blends of green stink bugs and possible parasitoid selection. *Naturwissenschaften* 76: 173–175. doi: 10.1007/BF00366402

Aldrich JR, Oliver JE, Lusby WR, Kochansky JP, Lockwood JA (1987) Pheromone strains of the cosmopolitan pest, *Nezara viridula* (Heteroptera: Pentatomidae). *Journal of Experimental Zoology* 244: 171–175. doi: 10.1002/jez.1402440121

Aldrich JR, Oliver JE, Taghizadeh T, Ferreira JTB, Liewehr D (1999) Pheromones and colonization: reassessment of the milkweed bug migration model (Heteroptera: Lygaeidae: Lygaeinae). *Chemoecology* 9: 63–71. doi: 10.1007/s000490050035

Alexander RD (1960) Sound communication in Orthoptera and Cicadidae. In: Lanyon WE, Tavolga WN (Eds), *Animal Sounds and Communication*. American Institute of Biological Sciences, Washington, D.C., 38–92.

Alexander RD (1967) Acoustical communication in arthropods. *Annual Review of Entomology* 12: 495–526.

Alexander RD, Marshall DC, Cooley JR (1997) Evolutionary perspectives on insect mating. In: Choe JC, Crespi BJ (Eds), *The Evolution of Mating Systems in Insects and Arachnids*. Cambridge University Press, Cambridge, 4–31.

Alexander RD, Moore TE (1958) Studies on the acoustical behavior of seventeen-year cicadas. *Ohio Journal of Science* 58: 107–127.

Allen GR (1995) The biology of the phonotactic parasitoid, *Homotrixa* sp. (Diptera: Tachinidae), and its impact on the survival of male *Sciarasaga quadrata* (Orthoptera: Tettigoniidae) in the field. *Ecological Entomology* 20: 103–110. doi: 10.1111/j.1365-2311.1995.tb00435.x

Allen GR (1998) Diel calling activity and field survival of the bushcricket, *Sciarasaga quadrata* (Orthoptera: Tettigoniidae): a role for sound-locating parasitic flies? *Ethology* 104: 645–660. doi: 10.1111/j.1439-0310.1998.tb00099.x

Allen GR, Hunt J (2001) Larval competition, adult fitness, and reproductive strategies in the acoustically orienting ormiine *Homotrixa alleni* (Diptera: Tachinidae). *Journal of Insect Behavior* 14: 283–297. doi: 10.1023/A:1011136310683

Allen GR, Kamien D, Berry O, Byrne P, Hunt J (1999) Larviposition, host cues, and planidial behavior in the sound-locating parasitoid fly *Homotrixa alleni* (Diptera: Tachinidae). *Journal of Insect Behavior* 12: 67–79. doi: 10.1023/A:1020929231430

D'Almeida JM (1993) Capture of caliptrate flies with different breeding substrates on beaches in Rio de Janeiro, RJ, Brazil. *Memórias do Instituto Oswaldo Cruz* 88: 215–220. doi: 10.1590/S0074-02761993000200007

D'Almeida JM (1994) Ovipositional substrates used by calyptrate Diptera in Tijuca forest, Rio de Janeiro. *Memórias do Instituto Oswaldo Cruz* 89: 261–264. doi: 10.1590/S0074-02761994000200027

van Alphen JJM, Visser ME (1990) Superparasitism as an adaptive strategy for insect parasitoids. *Annual Review of Entomology* 35: 59–79. doi: 10.1146/annurev.en.35.010190.000423

Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F (2004) Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. *Bioinformatics* 20: 407–415. doi: 10.1093/bioinformatics/btg427

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410. doi: 10.1016/S0022-2836(05)80360-2

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389–3402. doi: 10.1093/nar/25.17.3389

Anderson JF, Kaya HK (1974) Parasitism of the elm spanworm by *Telenomus alsophilae* and *Actia ontario* in Connecticut. In: Beard RL (Ed), *25th Anniversary Memoirs, Connecticut Entomological Society*. Connecticut Entomological Society, New Haven, 267–276.

Anderson JF, Kaya HK (1976) Parasitoids and diseases of the elm spanworm. *Journal of the New York Entomological Society* 84: 169–177.

Anton E, Niederegger S, Beutel RG (2011) Beetles and flies collected on pig carrion in an experimental setting in Thuringia and their forensic implications. *Medical and Veterinary Entomology* 25: 353–364. doi: 10.1111/j.1365-2915.2011.00975.x

Arakaki N, Wakamura S, Yasuda T (1996) Phoretic egg parasitoid, *Telenomus euproctidis* (Hymenoptera: Scelionidae), uses sex pheromone of tussock moth *Euproctis taiwana* (Lepidoptera: Lymantriidae) as a kairomone. *Journal of Chemical Ecology* 22: 1079–1085. doi: 10.1007/BF02027946

Arnaldos MI, Romera E, Presa JJ, Luna A, García MD (2004) Studies on seasonal arthropod succession on carrion in the southeastern Iberian Peninsula. *International Journal of Legal Medicine* 118. doi: 10.1007/s00414-004-0446-3

Arthur AP, Stainer JER, Turnbull AL (1964) The interaction between *Orgilus obscurator* (Nees) (Hymenoptera: Braconidae) and *Temelucha interruptor* (Grav.) (Hymenoptera: Ichneumonidae), parasites of the pine shoot moth, *Rhyacionia buoliana* (Schiff.) (Lepidoptera: Olethreutidae). *The Canadian Entomologist* 96: 1030–1034. doi: 10.4039/Ent961030-7

Askari A, Alishah A (1979) Courtship behavior and evidence for a sex pheromone in *Diaeretiella rapae* (Hymenoptera: Braconidae), the cabbage aphid primary parasitoid. *Annals of the Entomological Society of America* 72: 749–750. doi: 10.1093/aesa/72.6.749

Audacity Team (2014) *Audacity: Free Audio Editor and Recorder*. Available from: http://audacity.sourceforge.net/.

Audcent HLF (1951) Midnight flies. *Entomologist's Monthly Magazine* 87: 133.

El-Azazy OME, El-Metenawy TM (2004) Cutaneous myiasis in Saudi Arabia. *Veterinary Record* 154: 305–306. doi: 10.1136/vr.154.10.305

Baer W (1921) Die Tachinen als Schmarotzer der schädlichen Insekten. lhre Lebensweise, wirtschaftliche Bedeutung und systematische Kennzeichnung. (Schluß.). *Zeitschrift für Angewandte Entomologie* 7: 349–423.

Bailey WJ (1991) *Acoustic Behaviour of Insects: An Evolutionary Perspective*. 1st ed. Chapman and Hall, London; New York, 225 pp.

Bailey WJ, Haythornthwaite S (1998) Risks of calling by the field cricket *Teleogryllus oceanicus*; potential predation by Australian long-eared bats. *Journal of Zoology* 244: 505–513. doi: 10.1111/j.1469-7998.1998.tb00056.x

Bai S-X, Wang Z-Y, He K-L, Wen L-P, Zhou D-R (2004) Olfactory responses of *Trichogramma ostriniae* Pang et Chen to kairomones from eggs and different stages of female adults of *Ostrinia furnicalis* (Guenée). *Acta Entomologica Sinica* 47: 48–54.

Baldridge RS, Wallace SG, Kirkpatrick R (2006) Investigation of nocturnal oviposition by necrophilous flies in central Texas. *Journal of Forensic Sciences* 51: 125–126. doi: 10.1111/j.1556-4029.2005.00022.x

Bänziger H, Pape T (2004) Flowers, faeces and cadavers: natural feeding and laying habits of flesh flies in Thailand (Diptera: Sarcophagidae, *Sarcophaga* spp.). *Journal of Natural History* 38: 1677–1694. doi: 10.1080/0022293031000156303

Baranoff N (1934) Mitteilungen über gezüchtete orientalische Larvaevoriden. *Entomologisches Nachrichtenblatt, Organ für Entomologie und Entomologische Hilfsmittel* 8: 41–49.

Barbosa RR, Mello-Patiu CA de, Mello RP de, Queiroz MM de C (2009) New records of calyptrate dipterans (Fanniidae, Muscidae and Sarcophagidae) associated with the decomposition of domestic pigs in Brazil. *Memórias do Instituto Oswaldo Cruz* 104: 923–926. doi: 10.1590/S0074-02762009000600018

Bates D, Mächler M, Bolker B, Walker S (2015) *lme4: linear mixed-effects models using Eigen and S4*. Available from: http://CRAN.R-project.org/package=lme4.

Battisti A (1989) Field studies on the behaviour of two egg parasitoids of the pine processionary moth *Thaumetopoea pityocampa*. *Entomophaga* 34: 29–38. doi: 10.1007/BF02372584

Bayoumy MH, Kydan MB, Kozár F (2011) Are synthetic pheromone captures predictive of parasitoid densities as a kairomonal attracted tool? *Journal of Entomological and Acarological Research* 43: 23. doi: 10.4081/jear.2011.23

Beamer RH (1928) Studies on the biology of Kansas Cicadidae. *The University of Kansas Science Bulletin* 18: 155–263.

Beaver RA (1986) Biological studies of muscoid flies (Diptera) breeding in mollusc carrion in Southeast Asia. *Japanese Journal of Sanitary Zoology* 37: 205–211.

Beckers OM, Wagner WE (2011) Male field crickets infested by parasitoid flies express phenotypes that may benefit the parasitoids. *Animal Behaviour* 82: 1151–1157. doi: 10.1016/j.anbehav.2011.08.013

Bedard WD (1965) The biology of *Tomicobia tibialis* (Hymenoptera: Pteromalidae) parasitizing <i>Ips confusus/i> (Coleoptera: Scolytidae) in California. *Contributions from the Boyce Thompson Institute for Plant Research* 23: 77–81.

Bell PD (1979) Acoustic attraction of herons by crickets. *Journal of the New York Entomological Society* 87: 126–127.

Belwood JJ, Morris GK (1987) Bat predation and its influence on calling behavior in neotropical katydids. *Science* 238: 64–67. doi: 10.1126/science.238.4823.64

Benelli G, Carpita A, Simoncini S, Raspi A, Canale A (2014) For sex and more: attraction of the tephritid parasitoid *Psyttalia concolor* (Hymenoptera: Braconidae) to male sex

pheromone of the olive fruit fly, *Bactrocera oleae*. *Journal of Pest Science* 87: 449–457. doi: 10.1007/s10340-014-0595-1

Bennet-Clark HC, Young D (1998) Sound radiation by the bladder cicada *Cystosoma saundersii*. *The Journal of Experimental Biology* 201 (Pt 12): 701–715.

Bennet-Clark H, Young D (1994) The scaling of song frequency in cicadas. *Journal of Experimental Biology* 191: 291–294.

Bequaert J (1925) The arthropod enemies of mollusks, with description of a new dipterous parasite from Brazil. *The Journal of Parasitology* 11: 201. doi: 10.2307/3270829

Bergsten J (2005) A review of long-branch attraction. *Cladistics* 21: 163–193. doi: 10.1111/j.1096-0031.2005.00059.x

Bermúdez C. SE, Buenaventura R. E, Couri M, Miranda RJ, Herrera JM (2010) Mixed myiasis by *Philornis glaucinis* (Diptera: Muscidae), *Sarcodexia lambens* (Diptera: Sarcophagidae) and *Lucilia eximia* (Diptera: Calliphoridae) in *Ramphocelus dimidiatus* (Aves: Thraupidae) chicks in Panama. *Boletín de la Sociedad Entomológica Aragoneasa* 47: 445–446.

Berner L (1960) Les myiases des hélicides. *Bulletin de la Société d'Histoire Naturelle du Doubs* 62: 9–12.

Berner L (1973) Sur le parasitisme des hélicidés par des mouches du genre *Sarcophaga*. *Bulletin du Muséum d'Histoire Naturelle de Marseille* 33: 87–91, 93–94.

Berrigan D, Locke SJ (1991) Body size and male reproductive performance in the flesh fly, *Neobellieria bullata*. *Journal of Insect Physiology* 37: 575–581. doi: 10.1016/0022-1910(91)90034-W

Blake DH (1955) Notes on the rearing of *Anolisimyia blakeae*, a sarcophagid fly from the American chameleon, *Anolis carolinensus* Voight. *Proceedings of the Entomological Society of Washington* 57: 187–188.

Blume RR (1985) A checklist, distributional record, and annotated bibliography of the insects associated with bovine droppings on pastures in America north of Mexico. *The Southwestern Entomologist, Supplements* 9: 1–55.

Blume RR (1986) Parasites of Diptera associated with bovine droppings on a pasture in east central Texas. *The Southwestern Entomologist* 11: 215–222.

Boo KS, Yang JP (2000) Kairomones used by *Trichogramma chilonis* to find *Helicoverpa assulta* eggs. *Journal of Chemical Ecology* 26: 359–375. doi: 10.1023/A:1005453220792

Borges M, Schmidt FGV, Sujii ER, Medeiros MA, Mori K, Zarbin PHG, Ferreira JTB (1998) Field responses of stink bugs to the natural and synthetic pheromone of the Neotropical

brown stink bug, *Euschistus heros* (Heteroptera: Pentatomidae). *Physiological Entomology* 23: 202–207. doi: 10.1046/j.1365-3032.1998.233086.x

Breakey EP (1929) Notes on the natural enemies of the iris borer, *Macronoctua onusta* Grote (Lepidoptera). *Annals of the Entomological Society of America* 22: 459–464. doi: 10.1093/aesa/22.3.459

Breakey EP (1931) Additional notes on the natural enemies of the iris borer, *Macronoctua onusta* Grote (Lepidoptera). *Annals of the Entomological Society of America* 24: 40–44. doi: 10.1093/aesa/24.1.40

Brodeur J, Boivin G (2004) Functional ecology of immature parasitoids. *Annual Review of Entomology* 49: 27–49. doi: 10.1146/annurev.ento.49.061703.153618

Brooks SP, Gelman A (1998) General methods for monitoring convergence of iterative simulations. *Journal of Computational and Graphical Statistics* 7: 434–455. doi: 10.1080/10618600.1998.10474787

Broughton WB (1963) Method in bio-acoustic terminology. In: Busnel R-G (Ed), *Acoustic Behaviour of Animals*. Elsevier, Amsterdam, 3–24.

Brown BV, Feener DH Jr (1991) Behavior and host location cues of *Apocephalus paraponerae* (Diptera: Phoridae), a parasitoid of the giant tropical ant, *Paraponera clavata* (Hymenoptera: Formicidae). *Biotropica* 23: 182. doi: 10.2307/2388304

Brues CT (1950) Large raptorial birds as enemies of cicadas. *Psyche: A Journal of Entomology* 57: 74–75. doi: 10.1155/1950/49542

Buchler ER, Childs SB (1981) Orientation to distant sounds by foraging big brown bats (*Eptesicus fuscus*). *Animal Behaviour* 29: 428–432. doi: 10.1016/S0003-3472(81)80102-9

Buckell ER (1945) The grasshopper outbreak of 1944 in British Columbia. *The Canadian Entomologist* 77: 115–116. doi: 10.4039/Ent77115-6

Buckell ER, Spencer GJ (1945) A preliminary list of the flesh flies of British Columbia (Diptera: Sarcophagidae). *Proceedings of the Entomological Society of British Columbia* 42: 6.

Buenaventura E, Pape T (2013) Revision of the New World genus *Peckia* Robineau-Desvoidy (Diptera: Sarcophagidae). *Zootaxa* 3622: 1. doi: 10.11646/zootaxa.3622.1.1

Buenaventura R. E, Camacho C. G, García G. A, Wolff E. M (2009) Sarcophagidae (Diptera) de importancia forense en Colombia: claves taxonómicas, notas sobre su biología y distribución. *Revista Colombiana de Entomología* 35: 189–196.

Bulmer MG (1979) *Principles of Statistics*. Dover Publications, New York, 252 pp.

Burk T (1982a) Evolutionary significance of predation on sexually signalling males. *Florida Entomologist* 65: 90–104.

Burk T (1982b) Sticky panel catches of the tachinid *Euphasiopteryx ochracea*. *Florida Entomologist* 65: 291–292.

Burk T (1988) Acoustic signals, arms races and the costs of honest signalling. *Florida Entomologist* 71: 400–409.

Burns JM, Janzen DH, Hajibabaei M, Hallwachs W, Hebert PDN (2008) DNA barcodes and cryptic species of skipper butterflies in the genus Perichares in Area de Conservacion Guanacaste, Costa Rica. *Proceedings of the National Academy of Sciences* 105: 6350–6355. doi: 10.1073/pnas.0712181105

Buschman LL, Whitcomb WH (1980) Parasites of *Nezara viridula* (Hemiptera: Pentatomidae) and other Hemiptera in Florida. *Florida Entomologist* 63: 154–162.

Byrd JH, Castner JL eds. (2009) *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. 2nd ed. Taylor and Francis, Boca Raton : New York, 681 pp.

Cade WH (1975) Acoustically orienting parasitoids: fly phonotaxis to cricket song. *Science* 190: 1312–1313. doi: 10.1126/science.190.4221.1312

Cade WH (1979) The evolution of alternative male reproductive strategies in field crickets. In: Blum MS, Blum NA (Eds), *Sexual Selection and Reproductive Competition in Insects*. Academic Press, New York, 343–379.

Cade WH (1984) Effects of fly parasitoids on nightly calling duration in field crickets. *Canadian Journal of Zoology* 62: 226–228. doi: 10.1139/z84-037

Callan EM (1946) A note on *Sarcophaga lambens* (Wied.), a parasite of the South American bollworm, *Sacadodes pyralis* Dyar. *Revista de Entomologia* 17: 474–475.

Cameron AC, Windmeijer FAG (1996) R-squared measures for count data regression models with applications to health-care utilization. *Journal of Business & Economic Statistics* 14: 209. doi: 10.2307/1392433

Capinera JL, Scott RD, Walker TJ (2004) *Field guide to grasshoppers, katydids, and crickets of the United States*. Comstock Pub. Associates, Ithaca, 249 pp.

Carpenter J, Bithell J (2000) Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. *Statistics in Medicine* 19: 1141–1164. doi: 10.1002/(SICI)1097-0258(20000515)19:9<1141::AID-SIM479>3.0.CO;2-F

Carroll H, Beckstead W, O'Connor T, Ebbert M, Clement M, Snell Q, McClellan D (2007) DNA reference alignment benchmarks based on tertiary structure of encoded proteins. *Bioinformatics* 23: 2648–2649. doi: 10.1093/bioinformatics/btm389

Carroll RJ, Ruppert D (1996) The use and misuse of orthogonal regression in linear errors-in-variables models. *The American Statistician* 50: 1–6. doi: 10.2307/2685035

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552.

Cervenka VJ, Moon RD (1991) Arthropods associated with fresh cattle dung pats in Minnesota. *Journal of the Kansas Entomological Society* 64: 131–145.

Ceryngier P, Roy HE, Poland RL (2012) Natural enemies of ladybird beetles. In: Hodek I, van Emden HF, Honěk A (Eds), *Ecology and Behaviour of the Ladybird Beetles (Coccinellidae)*. John Wiley & Sons, Ltd, Chichester, UK, 375–443.

Chapman JA (1954) Studies on summit-frequenting insects in western Montana. *Ecology* 35: 41. doi: 10.2307/1931402

Chigusa Y, Tanaka K, Yokoi H, Matsuda H, Sasaki Y, Ikadatsu Y, Baba K (1994) Two cases of otomyiasis caused by *Sarcophaga peregrina* and *S. similis* (Diptera: Sarcophagidae). *Japanese Journal of Sanitary Zoology* 45: 153–157.

Cleare LD (1926) Appendix V, Biological Division. In: *Reports of the Department of Science and Agriculture (British Guiana), 1924*. British Guiana Department of Science and Agriculture, Georgetown, British Guiana, 65–68.

Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. *BioScience* 55: 323. doi: 10.1641/0006-3568(2005)055[0323:TBEOIV]2.0.CO;2

Colazza S, Rosi MC, Clemente A (1997) Response of egg parasitoid *Telenomus busseolae* to sex pheromone of *Sesamia nonagrioides*. *Journal of Chemical Ecology* 23: 2437–2444. doi: 10.1023/B:JOEC.0000006657.11973.27

Colazza S, Salerno G, Wajnberg E (1999) Volatile and contact chemicals released by *Nezara viridula* (Heteroptera:Pentatomidae) have a kairomonal effect on the egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae). *Biological Control* 16: 310–317. doi: 10.1006/bcon.1999.0763

Cole JA (2008) A new cryptic species of cicada resembling *Tibicen dorsatus* revealed by calling song (Hemiptera: Auchenorrhyncha: Cicadidae). *Annals of the Entomological Society of America* 101: 815–823. doi: 10.1603/0013-8746(2008)101[815:ANCSOC]2.0.CO;2

Conti E, Colazza S (2012) Chemical ecology of egg parasitoids associated with true bugs. *Psyche: A Journal of Entomology* 2012: 1–11. doi: 10.1155/2012/651015

Coppel HC (1960) Key to adults of dipterous parasites of spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *Annals of the Entomological Society of America* 53: 94–97. doi: 10.1093/aesa/53.1.94

Coquillett DW (1892) Report on the locust invasion of California in 1891. *Bulletin of the Division of Entomology of the U.S. Department of Agriculture* 27: 34–57.

Corbet SA (1971) Mandibular gland secretion of larvae of the flour moth, *Anagasta kuehniella*, contains an epideictic pheromone and elicits oviposition movements in a hymenopteran parasite. *Nature* 232: 481–484. doi: 10.1038/232481b0

Corbett GH (1937) Division of Entomology annual report for the year 1936. *General Series, Department of Agriculture of the Straits Settlements and Federated Malay States* 26: 29–48.

Coupland JB, Barker GM (2004) Diptera as predators and parasitoids of terrestrial gastropods, with emphasis on Phoridae, Calliphoridae, Sarcophagidae, Muscidae and Fanniidae. In: Barker GM (Ed), *Natural Enemies of Terrestrial Molluscs*. CABI, Wallingford, 85–158.

Criddle N (1931) Grasshopper control in Canada east of the Rocky Mountains. *Bulletin of the Dominion of Canada Department of Agriculture, New Series* 143: 1–18.

Crosskey RW (1965) The immature stages and affinities of the tachinid fly *Glaurocara flava*, a parasite of the African bush-cricket *Homorocoryphus nitidulus vicinus*. *Proceedings of the Zoological Society of London* 144: 203–218. doi: 10.1111/j.1469-7998.1965.tb05173.x

Crumb SE (1929) Tobacco cutworms. *Technical Bulletin, United States Department of Agriculture* 88: 1–179.

Dahlem GA, Downes WL Jr (1996) Revision of the genus *Boettcheria* in America North of Mexico (Diptera: Sarcophagidae). *Insecta Mundi* 10: 77–103.

Danyk T, Johnson DL, Mackauer M (2000) Parasitism of the grasshopper *Melanoplus sanguinipes* by a sarcophagid fly, *Blaesoxipha atlanis*: influence of solitary and gregarious development on host and parasitoid. *Entomologia Experimentalis et Applicata* 94: 259–268. doi: 10.1046/j.1570-7458.2000.00628.x

Davis EJ III, Turner WJ (1978) Biology, distribution and abundance of flesh flies (Diptera: Sarcophagidae) of the Wallowa-Whitman National Forest in northeastern Oregon. *Melanderia* 30: 111–153.

Davis JJ (1919) Contributions to a knowledge of the natural enemies of *Phyllophaga*. *Bulletin of the Illinois State Natural History Survey* 13: 53–138.

Davis JJ, Luginbill P (1921) The green June beetle or fig eater. *Bulletin of the North Carolina Agricultural Experiment Station* 242: 1–35.

Davis R (1960) Parasites of the elm spanworm, *Ennomos subsignarius*, in Georgia. *Proceedings of the Entomological Society of Washington* 62: 247–248.

Dean GA (1916) Insects injurious to alfalfa. *Report of the Kansas State Board of Agriculture* 35: 367–400.

DeBry RW, Timm AE, Dahlem GA, Stamper T (2010) mtDNA-based identification of *Lucilia cuprina* (Wiedemann) and *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) in the continental United States. *Forensic Science International* 202: 102–109. doi: 10.1016/j.forsciint.2010.04.038

Decker GC (1931) The biology of the stalk borer *Papaipema nebris* (Gn.). *Research Bulletin, Iowa State Agricultural Experiment Station* 143: 290–351.

Decker GC (1932) Biology of the bidens borer, *Epiblema otiosana* (Clemens) (Lepidoptera, Olethreutidæ). *Journal of the New York Entomological Society* 40: 503–509.

Decker GC (1935) The parasites of some lepidopterous stalk borers in Iowa. *Iowa State College Journal of Science* 9: 567–580.

Decker UM, Powell W, Clark SJ (1993) Sex pheromones in the cereal aphid parasitoids *Praon volucre* and *Aphidius rhopalosiphi*. *Entomologia Experimentalis et Applicata* 69: 33–39. doi: 10.1111/j.1570-7458.1993.tb01725.x

Deisler J (1987) The ecology of the Stock Island tree snail *Orthalicus reses reses* (Say). *Bulletin of the Florida State Museum, Biological Sciences* 31: 107–145.

Denno RF, Cothran WR (1976) Competitive interactions and ecological strategies of sarcophagid and calliphorid flies inhabiting rabbit carrion. *Annals of the Entomological Society of America* 69: 109–113. doi: 10.1093/aesa/69.1.109

Dessimoz C, Gil M (2010) Phylogenetic assessment of alignments reveals neglected tree signal in gaps. *Genome Biology* 11: R37. doi: 10.1186/gb-2010-11-4-r37

Dew JA (1913) Fall army worm. *Journal of Economic Entomology* 6: 361–366. doi: 10.1093/jee/6.4.361

Distant WL (1897a) Stridulation and habits of Cicadidae. *The Zoologist, Fourth Series* 1: 520–521.

Distant WL (1897b) Zoological rambles in and around the Transvaal. *The Zoologist, Fourth Series* 1: 157–163.

Dixon WN, Payne TL (1980) Attraction of entomophagous and associate insects of the southern pine beetle to bettle- and host tree-produced volatiles. *Journal of the Georgia Entomological Society* 15: 378–389.

Dobson AJ, Barnett AG (2008) *An Introduction to Generalized Linear Models*. 3rd ed. CRC Press, Boca Raton, 307 pp.

Dodge HR (1955) Sarcophagid flies parasitic on reptiles. *Proceedings of the Entomological Society of Washington* 57: 183–187.

Dodge HR (1956) New North American Sarcophagidae, with some new synonymy (Diptera). *Annals of the Entomological Society of America* 49: 182–190. doi: 10.1093/aesa/49.2.182

Dodge HR (1966) *Sarcophaga utilis* Aldrich and allies (Diptera, Sarcophagidae). *Entomological News* 77: 85–97.

Dodge HR (1968) The Sarcophagidae of Barro Colorado Island, Panama (Diptera). *Annals of the Entomological Society of America* 61: 421–450. doi: 10.1093/aesa/61.2.421

Dodge HR, Seago JM (1954) Sarcophagidae and other Diptera taken by trap and net on Georgia mountain summits in 1952. *Ecology* 35: 50. doi: 10.2307/1931403

Donisthorpe HSJK (1927) *The Guests of British Ants: Their Habits and Life-Histories*. George Routledge and Sons, London, 244 pp.

Doolan JM, MacNally RC (1981) Spatial dynamics and breeding ecology in the cicada *Cystosoma saundersii*: the interaction between distributions of resources and intraspecific behaviour. *The Journal of Animal Ecology* 50: 925. doi: 10.2307/4147

Dorn S, Beckage NE (2007) Superparasitism in gregarious hymenopteran parasitoids: ecological, behavioural and physiological perspectives. *Physiological Entomology* 32: 199–211. doi: 10.1111/j.1365-3032.2007.00576.x

Dove WE (1937) Myiasis of man. *Journal of Economic Entomology* 30: 29–39. doi: 10.1093/jee/30.1.29

Downes WL (1955) Notes on the morphology and classification of the Sarcophagidae and other calyptrates (Diptera). *Proceedings of the Iowa Academy of Science* 62: 514–538.

Downes WL Jr (1965) Family Sarcophagidae. In: *A Catalog of the Diptera of America North of Mexico*. *Agricultural Handbooks*. United States Department of Agriculture, Washington, D.C., 933–961.

Drake CJ (1920) The southern green stink-bug in Florida. *The Quarterly Bulletin of the State Plant Board of Florida* 4: 41–94.

Dress AW, Flamm C, Fritzsch G, Grünewald S, Kruspe M, Prohaska SJ, Stadler PF (2008) Noisy: identification of problematic columns in multiple sequence alignments. *Algorithms for Molecular Biology* 3: 7. doi: 10.1186/1748-7188-3-7

Dweck HKM, Svensson GP, Gündüz EA, Anderbrant O (2010) Kairomonal response of the parasitoid, *Bracon hebetor* Say, to the male-produced sex pheromone of its host, the greater waxmoth, *Galleria mellonella* (L.). *Journal of Chemical Ecology* 36: 171–178. doi: 10.1007/s10886-010-9746-x

Early M, Goff ML (1986) Arthropod succession patterns in exposed carrion on the island of O'ahu, Hawaiian Islands, USA. *Journal of Medical Entomology* 23: 520–531. doi: 10.1093/jmedent/23.5.520

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797. doi: 10.1093/nar/gkh340

Efron B, Tibshirani R (1993) *An Introduction to the Bootstrap*. Chapman & Hall, New York, 436 pp.

Eggleton P, Belshaw R (1992) Insect Parasitoids: An Evolutionary Overview. *Philosophical Transactions of the Royal Society B: Biological Sciences* 337: 1–20. doi: 10.1098/rstb.1992.0079

Eisner T, Eisner M, Attygalle AB, Deyrup M, Meinwald J (1998) Rendering the inedible edible: circumvention of a millipede's chemical defense by a predaceous beetle larva. *Proceedings of the National Academy of Sciences of the United States of America* 95: 1108–1113.

Elliott L, Hershberger W (2007) *The Songs of Insects*. Houghton Mifflin Co, Boston, 227 pp.

van Emden FI (1950) Dipterous parasites of Coleoptera. *Entomologist's Monthly Magazine* 86: 182–206.

Essig EO (1926) *Insects of Western North America*. Macmillan, New York, 1035 pp.

Evans HE (1987) Observations on the prey and nests of *Podalonia occidentalis* Murray (Hymenoptera: Sphecidae). *Pan-Pacific Entomologist* 63: 130–134.

Farris HE, Oshinsky ML, Forrest TG, Hoy RR (2008) Auditory sensitivity of an acoustic parasitoid (*Emblemasoma* sp., Sarcophagidae, Diptera) and the calling behavior of potential hosts. *Brain, Behavior and Evolution* 72: 16–26. doi: 10.1159/000139458

Fatouros NE, Dicke M, Mumm R, Meiners T, Hilker M (2008) Foraging behavior of egg parasitoids exploiting chemical information. *Behavioral Ecology* 19: 677–689. doi: 10.1093/beheco/arn011

Fatouros NE, Huigens ME, van Loon JJA, Dicke M, Hilker M (2005) Butterfly anti-aphrodisiac lures parasitic wasps. *Nature* 433: 704–704. doi: 10.1038/433704a

Fatouros NE, van Loon JJA, Hordijk KA, Smid HM, Dicke M (2005) Herbivore-induced plant volatiles mediate in-flight host discrimination by parasitoids. *Journal of Chemical Ecology* 31: 2033–2047. doi: 10.1007/s10886-005-6076-5

Feder JL, Bush GL (1989) A field test of differential host-plant usage between two sibling species of *Rhagoletis pomonella* fruit flies (Diptera: Tephritidae) and its consequences for sympatric models of speciation. *Evolution* 43: 1813–1819.

Feener DH, Brown BV (1997) Diptera as parasitoids. *Annual Review of Entomology* 42: 73–97. doi: 10.1146/annurev.ento.42.1.73

Feener DH Jr, Jacobs LF, Schmidt JO (1996) Specialized parasitoid attracted to a pheromone of ants. *Animal Behaviour* 51: 61–66. doi: 10.1006/anbe.1996.0005

Felland CM (1990) Habitat-specific parasitism of the stalk borer (Lepidoptera: Noctuidae) in northern Ohio. *Environmental Entomology* 19: 162–166. doi: 10.1093/ee/19.1.162

Feng-Yi Su K, Narayanan Kutty S, Meier R (2008) Morphology versus molecules: the phylogenetic relationships of Sepsidae (Diptera: Cyclorrhapha) based on morphology and DNA sequence data from ten genes. *Cladistics* 24: 902–916. doi: 10.1111/j.1096-0031.2008.00222.x

Fernandes LF, Pimenta FC, Fernandes FF (2009) First report of human myiasis in Goiás State, Brazil: frequency of different types of myiasis, their various etiological agents, and associated factors. *Journal of Parasitology* 95: 32–38. doi: 10.1645/GE-1103.1

Ferrar P (1979) The Immature stages of dung-breeding muscoid flies in Australia, with notes on the species, and keys to larvae and puparia. *Australian Journal of Zoology Supplementary Series* 27: 1–106. doi: 10.1071/AJZS073

Ferrar P (1987) 8 *A Guide to the Breeding Habits and Immature Stages of Diptera Cyclorrhapha, Part 1*. Brill [u.a.], Leiden, 478 pp.

Fiaboe MK, Chabi-Olaye A, Gounou S, Smith H, Borgemeister C, Schulthess F (2003) *Sesamia calamistis* calling behavior and Its role in host finding of egg parasitoids *Telenomus busseolae*, *Telenomus isis*, and *Lathromeris ovicida*. *Journal of Chemical Ecology* 29: 921–929. doi: 10.1023/A:1022935917260

Figg DE, Hall RD, Thomas GD (1983) Insect parasites associated with Diptera developing in bovine dung pats on central Missouri pastures. *Environmental Entomology* 12: 961–966. doi: 10.1093/ee/12.3.961

Forbush EH (1924) Gulls and terns feeding on the seventeen-year cicada. *The Auk* 41: 468–470. doi: 10.2307/4074498

Forrest TG, Raspet R (1994) Models of female choice in acoustic communication. *Behavioral Ecology* 5: 293–303. doi: 10.1093/beheco/5.3.293

Foster SP, Denholm I, Thompson R, Poppy GM, Powell W (2005) Reduced response of insecticide-resistant aphids and attraction of parasitoids to aphid alarm pheromone; a potential fitness trade-off. *Bulletin of Entomological Research* 95. doi: 10.1079/BER2004336

Fowler HG (1987) Field behavior of *Euphasiopteryx depleta* (Diptera: Tachinidae): phonotactically orienting parasitoids of mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus*). *Journal of the New York Entomological Society* 95: 474–480.

Fowler HG (1988) Scientific notes: traps for collecting live *Euphasiopteryx depleta* (Diptera: Tachinidae) at a sound source. *Florida Entomologist* 71: 654–656.

Fowler HG, Kochalka JN (1985) Scientific notes: new record of *Euphasiopteryx depleta* (Diptera: Tachinidae) from Paraguay: attraction to broadcast calls of *Scapteriscus acletus* (Orthoptera: Gryllotalpidae). *Florida Entomologist* 68: 225–226.

Fox J, Weisberg S (2011) *An R Companion to Applied Regression*. 2nd ed. SAGE Publications, Thousand Oaks, Calif, 449 pp.

Franco JC, Silva EB, Cortegano E, Campos L, Branco M, Zada A, Mendel Z (2008) Kairomonal response of the parasitoid *Anagyrus* spec. nov. near *pseudococci* to the sex pheromone of the vine mealybug. *Entomologia Experimentalis et Applicata* 126: 122–130. doi: 10.1111/j.1570-7458.2007.00643.x

Frenoy C, Durier C, Hawlitzky N (1992) Effect of kairomones from egg and female adult stages of *Ostrinia nubilalis* (Hübner) (Lepidoptera, Pyralidae) on *Trichogramma brassicae* Bezdenko (Hymenoptera, Trichogrammatidae) female kinesis. *Journal of Chemical Ecology* 18: 761–773. doi: 10.1007/BF00994613

Friend RB, Turner N (1931) The Mexican bean beetle in Connecticut. *Bulletin of the Connecticut Agricultural Experiment Station* 332: 72–108.

Fuller ME (1938) On the biology and early stages of *Helicobia australis* (Sarcophaginae), a dipterous insect associated with grasshoppers. *Proceedings of the Linnean Society of New South Wales* 63: 133–138.

Fu X, Che K, Zhu Z, Liu J, Guo Y (2014) The complete mitochondria genome of *Sarcophaga africa* (Diptera: Sarcophagidae). *Mitochondrial DNA*: 1–2. doi: 10.3109/19401736.2014.982582

Gabryś BJ, Gadomski HJ, Klukowski Z, Pickett JA, Sobota GT, Wadhams LJ, Woodcock CM (1997) Sex pheromone of cabbage aphid *Brevicoryne brassicae*: identification and field trapping of male aphids and parasitoids. *Journal of Chemical Ecology* 23: 1881–1890. doi: 10.1023/B:JOEC.0000006457.28372.48

Gardner PP (2005) A benchmark of multiple sequence alignment programs upon structural RNAs. *Nucleic Acids Research* 33: 2433–2439. doi: 10.1093/nar/gki541

Gatesy J, DeSalle R, Wheeler W (1993) Alignment-ambiguous nucleotide sites and the exclusion of systematic data. *Molecular Phylogenetics and Evolution* 2: 152–157. doi: 10.1006/mpev.1993.1015

Gazal V, Bailez O, Viana-Bailez AM (2009) Mechanism of host recognition in *Neodohrniphora elongata* (Brown) (Diptera: Phoridae). *Animal Behaviour* 78: 1177–1182. doi: 10.1016/j.anbehav.2009.07.036

Gee NG (1930) Some further notes on the elm moth. *Bulletin of the Department of Biology of Yenching University* 1: 9–10.

Gelman A, Rubin DB (1992) Inference from iterative simulation using multiple sequences. *Statistical Science* 7: 457–472.

George KA, Archer MS, Toop T (2012) Effects of bait age, larval chemical cues and nutrient depletion on colonization by forensically important calliphorid and sarcophagid flies. *Medical and Veterinary Entomology* 26: 188–193. doi: 10.1111/j.1365-2915.2011.00996.x

Gerhardt HC, Huber F (2002) *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. University of Chicago Press, Chicago, 531 pp.

Geweke J (1992) Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. In: Bernardo JM, Berger JO, Dawid AP, Smith AFM (Eds), *Bayesian statistics 4*. *Oxford science publications*. Clarendon Press, Oxford, 169–193.

Geyer CJ (1992) Practical Markov chain Monte Carlo. *Statistical Science* 7: 473–483. doi: 10.1214/ss/1177011137

Gilbert C, Kim MP (2007) Effects of male age and cervical proprioceptors on sexual aerial pursuit by male flesh flies, *Neobellieria bullata* (Diptera: Sarcophagidae). *Journal of Insect Behavior* 20: 427–435. doi: 10.1007/s10905-007-9088-x

Gilmore JU (1938) Observations on the hornworms attacking tobacco in Tennessee and Kentucky. *Journal of Economic Entomology* 31: 706–712. doi: 10.1093/jee/31.6.706

Girard JE, Budris JP (1975) Sex pheromone in the flesh fly, *Sarcophaga bullata*. *Melsheimer Entomological Series* 20: 1–5.

Girard JE, Germino FJ, Budris JP, Vita RA, Garrity MP (1979) Pheromone of the male flesh fly, *Sarcophaga bullata*. *Journal of Chemical Ecology* 5: 125–130. doi: 10.1007/BF00987693

Giroux M, Pape T, Wheeler TA (2010) Towards a phylogeny of the flesh flies (Diptera: Sarcophagidae): morphology and phylogenetic implications of the acrophallus in the subfamily Sarcophaginae. *Zoological Journal of the Linnean Society* 158: 740–778. doi: 10.1111/j.1096-3642.2009.00561.x

Giroux M, Wheeler TA (2009) Systematics and phylogeny of the subgenus *Sarcophaga* (*Neobellieria*) (Diptera: Sarcophagidae). *Annals of the Entomological Society of America* 102: 567–587. doi: 10.1603/008.102.0401

Giroux M, Wheeler TA (2010) Systematics of *Bulbostyla*, a new subgenus of *Sarcophaga* Meigen, and change of status for *Robackina* Lopes (Diptera: Sarcophagidae). *Zootaxa* 2553: 35–59.

Glinwood RT, Du Y-J, Powell W (1999) Responses to aphid sex pheromones by the pea aphid parasitoids *Aphidius ervi* and *Aphidius eadyi*. *Entomologia Experimentalis et Applicata* 92: 227–232. doi: 10.1046/j.1570-7458.1999.00541.x

Glinwood RT, Powell W, Tripathi CPM (1998) Increased parasitization of aphids on trap plants alongside vials releasing synthetic aphid sex pheromone and effective range of the pheromone. *Biocontrol Science and Technology* 8: 607–614. doi: 10.1080/09583159830126

Godan D (1983) *Pest Slugs and Snails: Biology and Control*. Springer-Verlag, Berlin, 445 pp.

Godfray HCJ (1994) *Parasitoids: behavioral and evolutionary ecology*. Princeton University Press, Princeton, NJ, 473 pp.

Godfray HCJ, Cook JM (1997) Mating systems of parasitoid wasps. In: Choe JC, Crespi BJ (Eds), *The Evolution of Mating Systems in Insects and Arachnids*. Cambridge University Press, Cambridge, 211–225.

Gomes L, Gomes G, Desuó IC (2009) A preliminary study of insect fauna on pig carcasses located in sugarcane in winter in southeastern Brazil. *Medical and Veterinary Entomology* 23: 155–159. doi: 10.1111/j.1365-2915.2009.00796.x

Gosper CR (1999) Birds foraging for cicadas at Primbee, NSW. *Australian Birds* 31: 142–144.

Gould SJ, Vrba ES (1982) Exaptation–a missing term in the science of form. *Paleobiology* 8: 4–15. doi: 10.2307/2400563

Graenicher S (1931) Some observations on the biology of the Sarcophaginae (Diptera: Sarcophagidae). *Entomological News* 42: 227–230.

Graenicher S (1935) Some biological notes on *Sarcophaga bullata* Park. (Diptera: Sarcophagidae). *Entomological News* 46: 193–196.

Gray DA, Banuelos C, Walker S, Cade W, Zuk M (2007) Behavioural specialization among populations of the acoustically orienting parasitoid fly *Ormia ochracea* utilizing different cricket species as hosts. *Animal Behaviour* 73: 99–104. doi: 10.1016/j.anbehav.2006.07.005

Gray DA, Cade WH (1999) Sex, death, and genetic variation: natural and sexual selection on cricket song. *Proceedings of the Royal Society B: Biological Sciences* 266: 707–709. doi: 10.1098/rspb.1999.0693

Greathead DJ (1963) A review of the insect enemies of Acridoidea (Orthoptera). *Transactions of the Royal Entomological Society of London* 114: 437–517. doi: 10.1111/j.1365-2311.1963.tb01073.x

Greene CT (1925) The puparia and larvae of sarcophagid flies. *Proceedings of the United States National Museum* 66: 1–26.

Greenfield MD (2002) *Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication*. Oxford University Press, Oxford; New York, 414 pp.

Grimaldi DA, Engel MS (2005) *Evolution of the Insects*. Cambridge University Press, Cambridge (U.K.); New York, 755 pp.

Grissell EE (1981) The identity of Nearctic *Cerocephala* Westwood (Hymenoptera: Pteromalidae). *Proceedings of the Entomological Society of Washington* 83: 620–624.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321. doi: 10.1093/sysbio/syq010

Guo J, Xie K, Che K, Hu Z, Guo Y (2014) The complete mitochondria genome of *Ravinia pernix* (Diptera: Sarcophagidae). *Mitochondrial DNA*: 1–2. doi: 10.3109/19401736.2014.982560

Hagman M, Pape T, Schulte R (2005) Flesh fly myiasis (Diptera, Sarcophagidae) in Peruvian poison frogs genus *Epipedobates* (Anura, Dendrobatidae). *Phyllomedusa: Journal of Herpetology* 4: 69. doi: 10.11606/issn.2316-9079.v4i1p69-73

Hall DG (1929) An annotated list of the Sarcophaginae which have been collected in Kansas. *Journal of the Kansas Entomological Society* 2: 83–90. doi: 10.2307/25081273

Hall DG (1933) The Sarcophaginae of Panama (Diptera: Calliphoridae). *Bulletin of the American Museum of Natural History* 66: 251–285.

Hall DG, Bohart GE (1948) The Sarcophagidae of Guam (Diptera). *Proceedings of the Entomological Society of Washington* 50: 127–135.

Hall MJR, Adams ZJO, Wyatt NP, Testa JM, Edge W, Nikolausz M, Farkas R, Ready PD (2009) Morphological and mitochondrial DNA characters for identification and phylogenetic analysis of the myiasis-causing flesh fly *Wohlfahrtia magnifica* and its relatives, with a description of *Wohlfahrtia monegrosensis* sp. n. Wyatt & Hall. *Medical and Veterinary Entomology* 23: 59–71. doi: 10.1111/j.1365-2915.2008.00779.x

Hallock HC (1929) Notes on methods of rearing Sarcophaginae (Diptera), and the biology of *Sarcophaga latisterna* Park. *Annals of the Entomological Society of America* 22: 246–250. doi: 10.1093/aesa/22.2.246

Hallock HC (1940a) The Sarcophaginæ and their relatives in New York: II. *Journal of the New York Entomological Society* 48: 201–231. doi: 10.2307/25004862

Hallock HC (1940b) The Sarcophaginæ and their relatives in New York, part I. *Journal of the New York Entomological Society* 48: 127–153. doi: 10.2307/25004856

Hallock HC (1942) The Sarcophaginæ and their relatives in New York: II. *Journal of the New York Entomological Society* 50: 215–241. doi: 10.2307/25004967

Hall SCB, Parmenter CJ (2008) Necrotic egg and hatchling remains are key factors attracting dipterans to sea turtle (*Caretta caretta*, *Chelonia mydas*, *Natator depressus*) nests in central Queensland, Australia. *Copeia* 2008: 75–81. doi: 10.1643/CH-06-151

Hanski I (1976) Breeding experiments with carrion flies (Diptera) natural conditions. *Annales Entomologici Fennici* 42: 113–121.

Hardie J, Nottingham SF, Powell W, Wadhams LJ (1991) Synthetic aphid sex pheromone lures female parasitoids. *Entomologia Experimentalis et Applicata* 61: 97–99. doi: 10.1111/j.1570-7458.1991.tb02401.x

Harris FJ (1978) On the use of windows for harmonic analysis with the discrete Fourier transform. *Proceedings of the IEEE* 66: 51–83. doi: 10.1109/PROC.1978.10837

Harrison JF, Woods HA, Roberts SP (2012) 3 *Ecological and Environmental Physiology of Insects*. Oxford University Press, Oxford, 378 pp.

Harrison JO (1963) On the biology of three banana pests in Costa Rica (Lepidoptera: Limacodidae, Nymphalidae). *Annals of the Entomological Society of America* 56: 87–94. doi: 10.1093/aesa/56.1.87

Harris VE, Todd JW (1980) Male-mediated aggregation of male, female and 5th-instar southern green stink bugs and concomitant attraction of a tachinid parasite, *Trichopoda pennipes*. *Entomologia Experimentalis et Applicata* 27: 117–126. doi: 10.1111/j.1570-7458.1980.tb02955.x

Haseman L (1917) *Sarcophaga haemorrhoidalis* larvae as parasites of the human intestine (Dipt.). *Entomological News* 28: 343–346.

Hastings JM, Toolson EC (1991) Thermoregulation and activity patterns of two syntopic cicadas, Tibicen chiricahua and T. duryi (Homoptera: Cicadidae), in central New Mexico. *Oecologia* 85: 513–520.

Hatsushika R, Ushirogawa H, Iwanaga Y (2002) A case study of human otomyiasis caused by *Parasarcophaga similis* (Meade, 1876) (Diptera: Sarcophagidae) found in Hiroshima, Japan. *Kawasaki Medical Journal* 28: 33–41.

Hayes WP (1917) Studies on the life-history of *Ligyrus gibbosus* Deg. (Coleoptera). *Journal of Economic Entomology* 10: 253–261. doi: 10.1093/jee/10.2.253

Hayes WP, DeCoursey JD (1938) Observations of grasshopper parasitism in 1937. *Journal of Economic Entomology* 31: 519–522. doi: 10.1093/jee/31.4.519

Haynes KF, Yeargan KV (1999) Exploitation of intraspecific communication systems: illicit signalers and receivers. *Annals of the Entomological Society of America* 92: 960–970. doi: 10.1093/aesa/92.6.960

Hedlund K, Vet LEM, Dicke M (1996) Generalist and specialist parasitoid strategies of using odours of adult drosophilid flies when searching for larval hosts. *Oikos* 77: 390–398. doi: 10.2307/3545929

Hedrick AV, Kortet R (2006) Hiding behaviour in two cricket populations that differ in predation pressure. *Animal Behaviour* 72: 1111–1118. doi: 10.1016/j.anbehav.2006.03.018

Hedwig B, Robert D (2014) Auditory parasitoid flies exploiting acoustic communication of insects. In: Hedwig B (Ed), *Insect Hearing and Acoustic Communication*. Springer Berlin Heidelberg, Berlin, Heidelberg, 45–63.

Hermann HR, Blum MS, Wheeler JW, Overal WL, Schmidt JO, Chao J-T (1984) Comparative Anatomy and Chemistry of the Venom Apparatus and Mandibular Glands in *Dinoponera grandis* (Guérin) and *Paraponera clavata* (F.) (Hymenoptera: Formicidae: Ponerinae). *Annals of the Entomological Society of America* 77: 272–279.

Hewitt GB, Rees NE (1974) Abundance of grasshoppers in relation to rangeland renovation practices. *Journal of Range Management* 27: 156. doi: 10.2307/3896756

Higaki M, Adachi I (2011) Response of a parasitoid fly, *Gymnosoma rotundatum* (Linnaeus) (Diptera: Tachinidae) to the aggregation pheromone of *Plautia stali* Scott (Hemiptera: Pentatomidae) and its parasitism of hosts under field conditions. *Biological Control* 58: 215–221. doi: 10.1016/j.biocontrol.2011.05.009

Hilker M, Bläske V, Kobs C, Dippel C (2000) Kairomonal effects of sawfly sex pheromones on egg parasitoids. *Journal of Chemical Ecology* 26: 2591–2601. doi: 10.1023/A:1005592930772

Hinchliff CE, Roalson EH (2013) Using supermatrices for phylogenetic inquiry: an example using the sedges. *Systematic Biology* 62: 205–219. doi: 10.1093/sysbio/sys088

Hinds WE, Dew JA (1915) The grass worm or fall army worm. *Bulletin of the Alabama Agricultural Experiment Station* 186: 61–92.

Hinds WE, Osterberger BA (1931) The soybean caterpillar in Louisiana. *Journal of Economic Entomology* 24: 1168–1173. doi: 10.1093/jee/24.6.1168

Hoffmeister TS, Gienapp P (1999) Exploitation of the host's chemical communication in a parasitoid searching for concealed host larvae. *Ethology* 105: 223–232. doi: 10.1046/j.1439-0310.1999.00361.x

Honěk A (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* 66: 483. doi: 10.2307/3544943

Hosken D, Bailey W, Oshea J, Roberts J (1994) Localization of insect calls by the bat *Nyctophilus geoffroyi* (Chiroptera, Vespertilionidae): a laboratory study. *Australian Journal of Zoology* 42: 177. doi: 10.1071/ZO9940177

Howard NF (1930) The Mexican bean beetle in the East and its control. *Farmers' Bulletin, United States Department of Agriculture* 1624: 1–14.

Howard NF, English LL (1924) Studies of the Mexican bean beetle in the Southeast. *Bulletin of the United States Department of Agriculture* 1243: 1–50.

Howard WJ (1937) Bird behavior as a result of emergence of seventeen year locusts. *The Wilson Bulletin* 49: 43–44.

Huelsenbeck JP, Bollback JP, Levine AM (2002) Inferring the root of a phylogenetic tree. *Systematic Biology* 51: 32–43. doi: 10.1080/106351502753475862

Huggans JL, Blickenstaff CC (1966) Parasites and predators of grasshoppers in Missouri. *Research Bulletin, University of Missouri Agricultural Experiment Station* 903: 1–40.

Huigens ME, Pashalidou FG, Qian M-H, Bukovinszky T, Smid HM, van Loon JJA, Dicke M, Fatouros NE (2009) Hitch-hiking parasitic wasp learns to exploit butterfly antiaphrodisiac. *Proceedings of the National Academy of Sciences* 106: 820–825. doi: 10.1073/pnas.0812277106

van Huis A, Schiitte C, Cools MH, Fanget P, van der Hoek H, Piquet SP (1994) The role of semiochemicals in host location by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*. In: Highley E, Wright EJ, Banks HJ, Champ BR (Eds), *Stored Product Protection: Proceedings of the 6th International Working Conference on Stored-Product Protection: 17-23 April 1994, Canberra, Australia*. CAB International, Wallingford, Oxon, UK, 1158–1164.

Ingram JW, Bynum EK (1932) Observations on the sugarcane beetle in Louisiana. *Journal of Economic Entomology* 25: 844–849. doi: 10.1093/jee/25.4.844

Introna F Jr, Campobasso CP, Di Fazio A (1998) Three case studies in forensic entomology from southern Italy. *Journal of Forensic Sciences* 43: 210–214.

Irschick DJ, Gentry G, Herrel A, Vanhooydonck B (2006) Effects of sarcophagid fly infestations on green anole lizards (*Anolis carolinensis*): an analysis across seasons and age/sex classes. *Journal of Herpetology* 40: 107–112. doi: 10.2307/4092882

Ives AR (1991) Aggregation and coexistence in a carrion fly community. *Ecological Monographs* 61: 75. doi: 10.2307/1943000

Iwasa M, Maruyama M, Nakamura E, Yamashita N, Watanabe A (2005) Effects of ivermectin on target and non-target dung-breeding flies (Diptera) in cattle dung pats. *Medical Entomology and Zoology* 56: 191–199.

James MT (1947) The flies that cause myiasis in man. *United States Department of Agriculture Miscellaneous Publications* 631: 1–175.

Jang SA, Park CG (2010) *Gymnosoma rotundatum* (Diptera: Tachinidae) attracted to the aggregation pheromone of *Plautia stali* (Hemiptera: Pentatomidae). *Journal of Asia-Pacific Entomology* 13: 73–75. doi: 10.1016/j.aspen.2009.11.004

Johnson MT, Follett PA, Taylor AD, Jones VP (2005) Impacts of biological control and invasive species on a non-target native Hawaiian insect. *Oecologia* 142: 529–540. doi: 10.1007/s00442-004-1754-5

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL (2008) NCBI BLAST: a better web interface. *Nucleic Acids Research* 36: W5–W9. doi: 10.1093/nar/gkn201

Johnston TH, Tiegs OW (1922) New and little-known sarcophagid flies from southeastern Queensland. *Proceedings of the Royal Society of Queensland* 33: 46–90.

Johnston TH, Tiegs OW (1923) New and known Australian sarcophagid flies. *Proceedings of the Royal Society of Queensland* 34: 181–190.

Jones FM (1934) Bird-feeding experiments in 1934, on Martha's Vineyard island, Massachusetts: the return of the "17-year cicada" to the island: notes on two of its butterflies. *Proceedings of the Royal Entomological Society of London* 9: 80–82. doi: 10.1111/j.1365-3032.1934.tb00013.x

Jones PL, Page RA, Hartbauer M, Siemers BM (2011) Behavioral evidence for eavesdropping on prey song in two Palearctic sibling bat species. *Behavioral Ecology and Sociobiology* 65: 333–340. doi: 10.1007/s00265-010-1050-9

Jones TH (1918) Miscellaneous truck-crop insects in Louisiana. *Bulletin of the United States Department of Agriculture* 703: 1–14.

Jones TH, Wolcott GN (1922) The caterpillars which eat the leaves of sugar cane in Porto Rico. *Journal of the Department of Agriculture and Labor of Porto Rico* 6: 38–50.

Jordaens K, Sonet G, Richet R, Dupont E, Braet Y, Desmyter S (2013) Identification of forensically important *Sarcophaga* species (Diptera: Sarcophagidae) using the mitochondrial COI gene. *International Journal of Legal Medicine* 127: 491–504. doi: 10.1007/s00414-012-0767-6

Jumean Z, Unruh T, Gries R, Gries G (2005) *Mastrus ridibundus* parasitoids eavesdrop on cocoon-spinning codling moth, *Cydia pomonella*, larvae. *Naturwissenschaften* 92: 20–25. doi: 10.1007/s00114-004-0581-1

Kaiser L, Pham-Delegue MH, Bakchine E, Masson C (1989) Olfactory responses of *Trichogramma maidis* Pint, et Voeg.: Effects of chemical cues and behavioral plasticity. *Journal of Insect Behavior* 2: 701–712. doi: 10.1007/BF01065787

Kamal AS (1958) Comparative study of thirteen species of sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera) I. bionomics. *Annals of the Entomological Society of America* 51: 261–271. doi: 10.1093/aesa/51.3.261

Kankare M, Van Nouhuys S, Hanski I (2005) Genetic divergence among host-specific cryptic species in *Cotesia melitaearum* aggregate (Hymenoptera: Braconidae), parasitoids of checkerspot butterflies. *Annals of the Entomological Society of America* 98: 382–394. doi: 10.1603/0013-8746(2005)098[0382:GDAHCS]2.0.CO;2

Kano R, Field G, Shinonaga S (1967) *Fauna Japonica: Sarcophagidae (Insecta: Diptera)*. Biogeographical Society of Japan, Tokyo, 168 pp.

Kass RE, Carlin BP, Gelman A, Neal RM (1998) Markov chain Monte Carlo in practice: a roundtable discussion. *The American Statistician* 52: 93–100. doi: 10.1080/00031305.1998.10480547

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. doi: 10.1093/molbev/mst010

Katoh K, Toh H (2008) Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics* 9: 212. doi: 10.1186/1471-2105-9-212

Kelly EOG (1914) A new sarcophagid parasite of grasshoppers. *Journal of Agricultural Research* 2: 435–446.

Kelly JA, van der Linde TC, Anderson GS (2009) The influence of clothing and wrapping on carcass decomposition and arthropod succession during the warmer seasons in central South Africa. *Journal of Forensic Sciences* 54: 1105–1112. doi: 10.1111/j.1556-4029.2009.01113.x

Kennedy BH (1979) The effect of multilure on parasites of the European elm bark beetle, *Scolytus multistriatus*. *Bulletin of the Entomological Society of America* 25: 116–118. doi: 10.1093/besa/25.1.116

Kennedy BH (1984) Effect of multilure and its components on parasites of *Scolytus multistriatus* (Coleoptera: Scolytidae). *Journal of Chemical Ecology* 10: 373–385. doi: 10.1007/BF00987864

Kilpatrick JW, Schoof HF (1956) Fly production in treated and untreated privies. *Public Health Reports* 71: 787–796.

Kingsolver JG, Pfennig DW (2004) Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution* 58: 1608–1612.

Kirchberg E (1954) Zur Larvennahrung einiger heimischer Sarcophaga-Arten, insbesondere zur Frage, ob *S. carnaria* L. als obligatorischer Regenwurmparasit anzusehen sei (Diptera, Tachinidae). *Zeitschrift für Morphologie und Ökologie der Tiere* 43: 99–112.

Kirchberger PC, Sefc KM, Sturmbauer C, Koblmüller S (2014) Outgroup effects on root position and tree topology in the AFLP phylogeny of a rapidly radiating lineage of cichlid fish. *Molecular Phylogenetics and Evolution* 70: 57–62. doi: 10.1016/j.ympev.2013.09.005

Knipling EF (1936) A comparative study of the first-instar larvae of the genus *Sarcophaga* (Calliphoridae, Diptera), with notes on the biology. *The Journal of Parasitology* 22: 417. doi: 10.2307/3271688

Knipling EF, Rainwater HT (1937) Species and incidence of dipterous larvae concerned in wound myiasis. *The Journal of Parasitology* 23: 451. doi: 10.2307/3272391

Knowlton GF, Janes MJ (1931) Notes on some beneficial Utah Diptera. *Proceedings of the Utah Academy of Sciences* 8: 147–148.

Knutson L, Vala J-C eds. (2011) *Biology of Snail-Killing Sciomyzidae Flies*. Cambridge University Press, Cambridge; New York, 506 pp.

Koenig WD, Liebhold AM (2005) Effects of periodical cicada emergences on abundance and synchrony of avian populations. *Ecology* 86: 1873–1882. doi: 10.1890/04-1175

Köhler U, Lakes-Harlan R (2001) Auditory behaviour of a parasitoid fly (*Emblemasoma auditrix*, Sarcophagidae, Diptera). *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology* 187: 581–587. doi: 10.1007/s003590100230

Kolluru GR (1999) Variation and repeatability of calling behavior in crickets subject to a phonotactic parasitoid fly. *Journal of Insect Behavior* 12: 611–626. doi: 10.1023/A:1020923602780

Kolluru GR, Zuk M (2001) Parasitism patterns and the size-fecundity relationship in the acoustically orienting dipteran parasitoid *Ormia ochracea*. *Canadian Journal of Zoology* 79: 973–979. doi: 10.1139/z01-064

Kolluru GR, Zuk M, Chappell MA (2002) Reduced reproductive effort in male field crickets infested with parasitoid fly larvae. *Behavioral Ecology* 13: 607–614. doi: 10.1093/beheco/13.5.607

Krupke CH, Brunner JF (2003) Parasitoids of the consperse stink bug (Hemiptera: Pentatomidae) in North Central Washington and attractiveness of a host-produced pheromone component. *Journal of Entomological Science* 38: 84–92.

Kück P, Meusemann K, Dambach J, Thormann B, von Reumont BM, Wägele JW, Misof B (2010) Parametric and non-parametric masking of randomness in sequence alignments can be improved and leads to better resolved trees. *Frontiers in Zoology* 7: 10. doi: 10.1186/1742-9994-7-10

Kumazaki M, Matsuyama S, Suzuki T, Kuwahara Y, Fujii K (2000) Parasitic wasp, *Dinarmus basalis*, utilizes oviposition-marking pheromone of host azuki bean weevils as host-recognizing kairomone. *Journal of Chemical Ecology* 26: 2677–2695. doi: 10.1023/A:1026425407150

Kutty SN, Bernasconi MV, Šifner F, Meier R (2007) Sensitivity analysis, molecular systematics and natural history evolution of Scathophagidae (Diptera: Cyclorrhapha: Calyptratae). *Cladistics* 23: 64–83. doi: 10.1111/j.1096-0031.2006.00131.x

Kutty SN, Pape T, Pont A, Wiegmann BM, Meier R (2008) The Muscoidea (Diptera: Calyptratae) are paraphyletic: evidence from four mitochondrial and four nuclear genes. *Molecular Phylogenetics and Evolution* 49: 639–652. doi: 10.1016/j.ympev.2008.08.012

Kutty SN, Pape T, Wiegmann BM, Meier R (2010) Molecular phylogeny of the Calyptratae (Diptera: Cyclorrhapha) with an emphasis on the superfamily Oestroidea and the position of Mystacinobiidae and McAlpine's fly. *Systematic Entomology* 35: 614–635. doi: 10.1111/j.1365-3113.2010.00536.x

Laake EW, Cushing EC, Parish HE (1936) Biology of the primary screw worm fly, *Cochliomyia americana*, and a comparison of its stages with those of *C. macellaria*. *Technical Bulletin, United States Department of Agriculture* 500: 1–24.

Lake JA (1991) The order of sequence alignment can bias the selection of tree topology. *Molecular Biology and Evolution* 8: 378–385.

Lakes-Harlan R (2009) Monitoring species distribution of hearing Diptera with sound traps. *Fly Times* 43: 10–12.

Lakes-Harlan R, deVries T, Stölting H, Stumpner A (2014) Useless hearing in male *Emblemasoma auditrix* (Diptera, Sarcophagidae) – a case of intralocus sexual conflict during evolution of a complex sense organ? *PLoS ONE* 9: e87211. doi: 10.1371/journal.pone.0087211

Lakes-Harlan R, Heller K-G (1992) Ultrasound-sensitive ears in a parasitoid fly. *Naturwissenschaften* 79: 224–226. doi: 10.1007/BF01227133

Lakes-Harlan R, Kohler U (2003) Influence of habitat structure on the phonotactic strategy of a parasitoid fly *Emblemasoma auditrix*. *Ecological Entomology* 28: 758–765. doi: 10.1111/j.1365-2311.2003.00562.x

Lakes-Harlan R, Lehmann GUC (2015) Parasitoid flies exploiting acoustic communication of insects—comparative aspects of independent functional adaptations. *Journal of Comparative Physiology A* 201: 123–132. doi: 10.1007/s00359-014-0958-3

Lakes-Harlan R, Stölting H, Moore TE (2000) Phonotactic behaviour of a parasitoid fly (*Emblemasoma auditrix*, Diptera, Sarcophagidae) in response to the calling song of its host cicada (*Okanagana rimosa*, Homoptera, Cicadidae). *Zoology* 103: 31–39.

Lakes-Harlan R, Stolting H, Stumpner A (1999) Convergent evolution of insect hearing organs from a preadaptive structure. *Proceedings of the Royal Society B: Biological Sciences* 266: 1161–1167. doi: 10.1098/rspb.1999.0758

Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701. doi: 10.1093/molbev/mss020

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30: 3276–3278. doi: 10.1093/bioinformatics/btu531

Lassmann T, Sonnhammer ELL (2002) Quality assessment of multiple alignment programs. *FEBS Letters* 529: 126–130. doi: 10.1016/S0014-5793(02)03189-7

Lassmann T, Sonnhammer ELL (2005) Automatic assessment of alignment quality. *Nucleic Acids Research* 33: 7120–7128. doi: 10.1093/nar/gki1020

Laumann RA, Moraes MCB, Čokl A, Borges M (2007) Eavesdropping on sexual vibratory signals of stink bugs (Hemiptera: Pentatomidae) by the egg parasitoid *Telenomus podisi*. *Animal Behaviour* 73: 637–649. doi: 10.1016/j.anbehav.2006.09.011

Leal WS, Higuchi H, Mizutani N, Nakamori H, Kadosawa T, Ono M (1995) Multifunctional communication in *Riptortus clavatus* (Heteroptera: Alydidae): conspecific nymphs and egg parasitoid *Ooencyrtus nezarae* use the same adult attractant pheromone as chemical cue. *Journal of Chemical Ecology* 21: 973–985. doi: 10.1007/BF02033802

Lederhouse RC, Morse RA, Ambrose JT, Burgett DM, Conner WE, Edwards L, Fell RD, Rutowski R, Turell M (1976) Crepuscular mating aggregations in certain *Ormia* and *Sitophaga*. *Annals of the Entomological Society of America* 69: 656–658. doi: 10.1093/aesa/69.4.656

Lederhouse RC, Morse RA, Ambrose JT, Burgett DM, Conner WE, Edwards L, Fell RD, Rutowski R, Turell M (1976) Crepuscular mating aggregations in certain *Ormia* and *Sitophaga*. *Annals of the Entomological Society of America* 69: 656–658.

Lee Y-T, Chen T-L, Lin Y-C, Fung C-P, Cho W-L (2011) Nosocomial nasal myiasis in an intubated patient. *Journal of the Chinese Medical Association* 74: 369–371. doi: 10.1016/j.jcma.2011.06.001

Lehmann GUC (2003) Review of biogeography, host range and evolution of acoustic hunting in Ormiini (Insecta, Diptera, Tachinidae), parasitoids of night-calling bushcrickets and crickets (Insecta, Orthoptera, Ensifera). *Zoologischer Anzeiger - A Journal of Comparative Zoology* 242: 107–120. doi: 10.1078/0044-5231-00091

Lehmann GUC (2008) How different host species influence parasitism patterns and larval competition of acoustically-orienting parasitoid flies (Tachinidae: Ormiini). In: Weber EA, Krause LH (Eds), *Animal Behavior: New Research*. Nova Science Publishers, New York, 93–132.

Lehmann GUC, Heller K-G (1998) Bushcricket song structure and predation by the acoustically orienting parasitoid fly *Therobia leonidei* (Diptera: Tachinidae: Ormiini). *Behavioral Ecology and Sociobiology* 43: 239–245. doi: 10.1007/s002650050488

Lehmann GUC, Lehmann AW (2006) Potential lifetime reproductive success of male bushcrickets parasitized by a phonotactic fly. *Animal Behaviour* 71: 1103–1110. doi: 10.1016/j.anbehav.2005.08.009

Lehrer AZ, Luciano P (1979) Sarcophagides (Diptera) parasites de *Porthetria dispar* (L.) en Sardaigne et leur cartographie dans le réseau U. T. M. *Studi Sassaresi* 27: 161–173.

Lemmon AR, Brown JM, Stanger-Hall K, Lemmon EM (2009) The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. *Systematic Biology* 58: 130–145. doi: 10.1093/sysbio/syp017

van Lenteren JC (1981) Host discrimination by parasitoids. In: Nordlund DA, Jones RL, Lewis WJ (Eds), *Semiochemicals: Their Role in Pest Control*. Wiley, New York, 153–179.

Letsch HO, Kuck P, Stocsits RR, Misof B (2010) The impact of rRNA secondary structure consideration in alignment and tree reconstruction: simulated data and a case study on the phylogeny of hexapods. *Molecular Biology and Evolution* 27: 2507–2521. doi: 10.1093/molbev/msq140

Lewis T ed. (1984) *Insect Communication*. Academic Press, London, 414 pp.

Lewis WJ, Nordlund DA, Gueldner RC, Teal PEA, Tumlinson JH (1982) Kairomones and their use for management of entomophagous insects: XIII. kairomonal activity for *Trichogramma* spp. of abdominal tips, excretion, and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. *Journal of Chemical Ecology* 8: 1323–1331. doi: 10.1007/BF00987765

Lloyd DC (1951) A survey for grasshopper parasites in temperate South America. *The Canadian Entomologist* 83: 213–230. doi: 10.4039/Ent83213-9

Lloyd JE (1973) Firefly parasites and predators. *The Coleopterists Bulletin* 27: 91–106. doi: 10.2307/3999442

Lopes H de S (1940) Contribuição ao conhecimento do genero *Udamopyga* Hall e de outros Sarcophagideos que vivem em molluscos ao Brasil (Diptera). *Revista de Entomologia* 11: 924–954.

Lopes H de S (1946) Sarcophagidae do México, capturados pelo professor A. Dampf. (Diptera). *Memórias do Instituto Oswaldo Cruz* 44: 119–146. doi: 10.1590/S0074-02761946000100004

Lopes H de S (1950) Sôbre os gêneros *Boettcheria* Parker, 1914 e *Boettcherimima* n. gen. (Diptera Sarcophagidae). *Memórias do Instituto Oswaldo Cruz* 48: 687–732. doi: 10.1590/S0074-02761950000100026

Lopes H de S (1969a) Family Sarcophagidae. In: *A Catalogue of the Diptera of the Americas South of the United States*. Departamento de Zoologia, Secretaria da Agricultura do Estado de São Paulo, São Paulo, Brazil, 103.1–103.88.

Lopes H de S (1969b) Neotropical Sarcophagidae reared from Gastropoda by Dr. W. Weyrauch (Diptera). *Studia Entomologica* 12: 133–160.

Lopes H de S (1973) Collecting and rearing sarcophagid flies (Diptera) in Brazil during forty years. *Anais da Academia Brasileira de Ciências* 45: 279–291.

Lopes H de S (1974a) On *Engelimyia* new genus for *Sarcophaga cassidifera* Engel (Diptera, Sarcophagidae). *Revista Brasileira de Biologia* 34: 425–430.

Lopes H de S (1974b) On the types of some Sarcophagidae from Panama, described by H. R. Dodge (Diptera). *Revista Brasileira de Biologia* 34: 511–522.

Lopes H de S (1975) On some North American Sarcophagidae with red legs (Diptera). *Revista Brasileira de Biologia* 35: 155–164.

Lopes H de S (1981) Notes on American Sarcophagidae (Diptera). *Revista Brasileira de Biologia* 41: 149–152.

Lopes H de S (1982) The importance of the mandible and clypeal arch of the first instar larvae in the classification of the Sarcophagidae (Diptera). *Revista Brasileira de Entomologia* 26: 293–326.

Lopes H de S (1988) On Emblemasomini (Diptera, Sarcophagidae), with descriptions of four new species. *Memórias do Instituto Oswaldo Cruz* 83: 17–27. doi: 10.1590/S0074-02761988000100003

Lord JK (1866) A new species of cicada, from the Cascade Mountains. *The Intellectual Observer* 8: 428–435.

Lozano C, Gonzalez E, Peña A, Campos M, Plaza MT, Rodriguez M, Izquierdo I, Tamayo J (2000) Response of parasitoids *Dendrosoter protuberans* and *Cheiropachus quadrum* to attractants of *Phloeotribus scarabaeoides* in an olfactometer. *Journal of Chemical Ecology* 26: 791–799. doi: 10.1023/A:1005492608748

Luginbill P (1928) The fall army worm. *Technical Bulletin, United States Department of Agriculture* 34: 1–91.

Luo A-R, Zhang Y-Z, Qiao H-J, Shi W-F, Murphy RW, Zhu C-D (2010) Outgroup selection in tree reconstruction: a case study of the family Halictidae(Hymenoptera: Apoidea). *ACTA ENTOMOLOGICA SINICA* 53: 192.

MacNally R, Young D (1981) Song energetics of the bladder cicada, *Cystosoma saundersii*. *Journal of Experimental Biology* 90: 185–196.

Madden AH, Chamberlin FS (1945) Biology of the tobacco hornworm in the southern cigar-tobacco district. *Technical Bulletin, United States Department of Agriculture* 896: 1–51.

Maddison WP, Maddison DR (2015) *Mesquite: a modular system for evolutionary analysis*. Available from: http://mesquiteproject.org.

Maier CT (1982) Observations on the seventeen-year periodical cicada, *Magicicada septendecim* (Hemiptera: Homoptera: Cicadidae). *Annals of the Entomological Society of America* 75: 14–23.

Mangold JR (1978) Attraction of *Euphasiopteryx ochracea*, *Corethrella* sp. and gryllids to broadcast songs of the southern mole cricket. *Florida Entomologist* 61: 57–61.

Marchiori CH, Castro MEV, Paiva TCG, Teixeira FF, Silva CG (2000) Dípteros muscóides de importância médica e veterinária e seus parasitóides em Goiás. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 52: 350–353. doi: 10.1590/S0102-09352000000400010

Marchiori CH, Pereira LA, Silva Filho OM, Ribeiro LCS, Borges VR (2002) *Brachymeria podagrica* (Fabricius) (Hymenoptera: Chalcididae) as parasitoids of *Sarcodexia lambens* (Wiedemann) (Diptera: Sarcophagidae) in Brasil. *Arquivos do Instituto Biológico* 69: 121–122.

Marchiori CH, Pereira LA, Silva Filho OM, Silva Ribeiro LC, Borges VR (2003) Parasitoids of flies collected on human faeces in Itumbiara County, Goias State, Brasil. *Biotemas* 16: 121–128.

Marinho MAT, Junqueira ACM, Paulo DF, Esposito MC, Villet MH, Azeredo-Espin AML (2012) Molecular phylogenetics of Oestroidea (Diptera: Calyptratae) with emphasis on Calliphoridae: insights into the inter-familial relationships and additional evidence for paraphyly among blowflies. *Molecular Phylogenetics and Evolution* 65: 840–854. doi: 10.1016/j.ympev.2012.08.007

Martín-Vega D, Baz A (2013) Sex-biased captures of sarcosaprophagous diptera in carrion-baited traps. *Journal of Insect Science* 13: 1–12. doi: 10.1673/031.013.1401

Maschwitz U, Weissflog A, Seebauer S, Disney RHL, Witte V (2008) 51 *Studies on European ant decapitating flies (Diptera: Phoridae): I. Releasers and phenology of parasitism of* Pseudacteon formicarum. California State University, Chico, CA, ETATS-UNIS.

Mathis KA, Philpott SM, Moreira RF (2011) Parasite lost: chemical and visual cues used by *Pseudacteon* in search of *Azteca instabilis*. *Journal of Insect Behavior* 24: 186–199. doi: 10.1007/s10905-010-9247-3

Matuhira K, Kouzaki Y (2001) Biological control of the mulberry scale, *Pseudaulacaspis pentagona* (Targion), using synthetic sex pheromone and insecticide sensitivity of its natural enemy parasitoid wasp. *Bulletin of the Kagoshima Tea Experiment Station* 15: 13–21.

Matuszewski S, Frątczak K, Konwerski S, Bajerlein D, Szpila K, Jarmusz M, Szafałowicz M, Grzywacz A, Mądra A (2015) Effect of body mass and clothing on carrion entomofauna. *International Journal of Legal Medicine*. doi: 10.1007/s00414-015-1145-y

Mazen NAM, Aal AAAA (2007) A study on some sarcophagid flies (Diptera), causing myiasis in Assiut, Egypt. *The Egyptian Journal of Experimental Biology (Zoology)* 3: 9–18.

Mbata GN, Shu S, Phillips TW, Ramaswamy SB (2004) Semiochemical cues used by *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) to locate its host, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Annals of the Entomological Society of America* 97: 353–360. doi: 10.1093/aesa/97.2.353

McAlpine JF (1981) Morphology and terminology–adults. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds), *Manual of Nearctic Diptera, Volume 1*. Canadian Government Publishing Centre, Hull, Quebec, Canada, 9–63.

McAlpine JF (1989) Phylogeny and classification of the Muscomorpha. In: McAlpine JF, Wood DM (Eds), *Manual of Nearctic Diptera, Volume 3*. *Monograph*. Canadian Gov. Publ. Centre, Supply and Services Canada, Hull, Quebec, 1397–1518.

McClure M, Whistlecraft J, McNeil JN (2007) Courtship behavior in relation to the female sex pheromone in the parasitoid, *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Chemical Ecology* 33: 1946–1959. doi: 10.1007/s10886-007-9355-5

McDonagh LM, Stevens JR (2011) The molecular systematics of blowflies and screwworm flies (Diptera: Calliphoridae) using 28S rRNA, COX1 and EF-1α: insights into the evolution of dipteran parasitism. *Parasitology* 138: 1760–1777. doi: 10.1017/S0031182011001089

McFadden D (1974) Conditional logit analysis of qualitative choice behavior. In: Zarembka P (Ed), *Frontiers in Econometrics*. *Economic Theory and Mathematical Economics*. Academic Press, New York, 105–142.

McGregor R, Henderson D (1998) The influence of oviposition experience on response to host pheromone in *Trichogramma sibericum* (Hymenoptera: Trichogrammatidae). *Journal of Insect Behavior* 11: 621–632. doi: 10.1023/A:1022390608064

Vander Meer RK, Porter SD (2002) Fire ant, *Solenopsis invicta*, worker alarm pheromones attract *Pseudacteon* phorid flies. In: *Proceedings of the 2002 Imported Fire Ant Conference*. Athens, Georgia, 77–80.

Menard S (2000) Coefficients of determination for multiple logistic regression analysis. *The American Statistician* 54: 17–24. doi: 10.1080/00031305.2000.10474502

Méndez J, Pape T (2002) Biology and immature stages of *Peckia gulo* (Fabricius, 1805) (Diptera: Sarcophagidae). *Studia Dipterologica* 9: 371–374.

Micha SG, Wyss U (1996) Aphid alarm pheromone (E)-*β*-farnesene: A host finding kairomone for the aphid primary parasitoid *Aphidius uzbekistanicus* (Hymenoptera: Aphidiinae). *Chemoecology* 7: 132–139. doi: 10.1007/BF01245965

Michelsen A, Fonseca P (2000) Spherical sound radiation patterns of singing grass cicadas, *Tympanistalna gastrica*. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology* 186: 163–168. doi: 10.1007/s003590050016

Michelsen A, Nocke H (1974) Biophysical Aspects of Sound Communication in Insects. In: Treherne JE, Berridge MJ, Wigglesworth VB (Eds), *Advances in Insect Physiology*. Elsevier, 247–296.

Middlekauff WW (1959) Some biological observations on *Sarcophaga falciformis*, a parasite of grasshoppers (Diptera: Sarcophagidae). *Annals of the Entomological Society of America* 52: 724–728.

Mihályi F (1965) Rearing flies from faeces and meat, infected under natural condition. *Acta Zoologica Hungarica* 11: 153–164.

Milonas P, Mazomenos BE, Konstantopoulou MA (2009) Kairomonal effect of sex pheromone components of two lepidopteran olive pests on *Trichogramma* wasps. *Insect Science* 16: 131–136. doi: 10.1111/j.1744-7917.2009.00264.x

Misof B, Misof K (2009) A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: a more objective means of data exclusion. *Systematic Biology* 58: 21–34. doi: 10.1093/sysbio/syp006

Mitchell WC, Mau RFL (1971) Response of the female southern green stink bug and its parasite, *Trichopoda pennipes*, to male stink bug pheromones. *Journal of Economic Entomology* 64: 856–859. doi: 10.1093/jee/64.4.856

Mizutani N (2006) Pheromones of male stink bugs and their attractiveness to their parasitoids. *Japanese Journal of Applied Entomology and Zoology* 50: 87–99. doi: 10.1303/jjaez.2006.87

Mohr CO (1943) Cattle droppings as ecological units. *Ecological Monographs* 13: 275. doi: 10.2307/1943223

Moore TE (1966) The cicadas of Michigan (Homoptera: Cicadidae). *Papers of the Michigan Academy of Science, Arts, and Letters* 51: 75–96.

Moradeshaghi MJ, Bohart GE (1968) The biology of *Euphytomima nomiivora* (Diptera: Sarcophagidae), a parasite of the alkali bee, *Nomia melanderi* (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society* 41: 456–473.

Morehead SA, Feener DH (2000) An experimental test of potential host range in the ant parasitoid *Apocephalus paraponerae*. *Ecological Entomology* 25: 332–340. doi: 10.1046/j.1365-2311.2000.00261.x

Moretti TDC, Ribeiro OB, Thyssen PJ, Solis DR (2008) Insects on decomposing carcasses of small rodents in a secondary forest in southeastern Brazil. *European Journal of Entomology* 105: 691–696. doi: 10.14411/eje.2008.094

Morgan DJW, Hare JD (1998) Volatile cues used by the parasitoid, *Aphytis melinus*, for host location: California red scale revisited. *Entomologia Experimentalis et Applicata* 88: 235–245. doi: 10.1046/j.1570-7458.1998.00368.x

Moriya S, Shiga M (1984) Attraction of the male brown-winged green bug, <i>Plautia stali Scott (Heteroptera: Pentatomidae) for males and females of the same species. *Applied Entomology and Zoology* 19: 317–322. doi: 10.1303/aez.19.317

Morrill AW (1917) Notes on important insects of the year. *Annual Report, Arizona Commission of Agriculture and Horticulture* 9: 33–61.

Morris GK, Walker TJ (1976) Calling songs of *Orchelimum* meadow katydids (Tettigoniidae): i. mechanism, terminology, and geographic distribution. *The Canadian Entomologist* 108: 785–800. doi: 10.4039/Ent108785-8

Moulds MS (1990) *Australian cicadas*. New South Wales University Press, Kensington, N.S.W., 217 pp.

Müller P, Robert D (2002) Death comes suddenly to the unprepared: singing crickets, call fragmentation, and parasitoid flies. *Behavioral Ecology* 13: 598–606. doi: 10.1093/beheco/13.5.598

Muma MH (1954) Predators and parasites of the citrus tree snail. *Citrus Magazine* 16: 8–9.

Muma MH (1955) Observations on the biology of the citrus tree snail. *The Citrus Industry* 36: 6–9, 21.

Myers JG (1929) *Insect Singers: A Natural History of the Cicadas*. George Routledge and Sons, London, 304 pp.

Nazni WA, Nooraidah H, Jeffery J, Azahari AH, Mohd Noor I, Sadiyah I, Lee HL (2007) Distribution and abundance of diurnal and nocturnal dipterous flies in the Federal Territory, Putrajaya. *Tropical Biomedicine* 24: 61–66.

Nazzi F, Powell W, Wadhams LJ, Woodcock CM (1996) Sex pheromone of aphid parasitoid *Praon volucre* (Hymenoptera, Braconidae). *Journal of Chemical Ecology* 22: 1169–1175. doi: 10.1007/BF02027952

Nelson LA, Cameron SL, Yeates DK (2012) The complete mitochondrial genome of the flesh fly, *Sarcophaga impatiens* Walker (Diptera: Sarcophagidae). *Mitochondrial DNA* 23: 42–43. doi: 10.3109/19401736.2011.644042

Nelson LA, Lambkin CL, Batterham P, Wallman JF, Dowton M, Whiting MF, Yeates DK, Cameron SL (2012) Beyond barcoding: A mitochondrial genomics approach to molecular

phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). *Gene* 511: 131–142. doi: 10.1016/j.gene.2012.09.103

Noble NS (1936) Fly parasites of grasshoppers. *Agricultural Gazette of New South Wales* 47: 383–385.

Noldus LPJJ, van Lenteren JC (1985) Kairomones for the egg parasite *Trichogramma evanescens* Westwood: I. effect of volatile substances released by two of its hosts, *Pieris brassicae* L. and *Mamestra brassicae*, L. *Journal of Chemical Ecology* 11: 781–791. doi: 10.1007/BF00988305

Nordlund DA, Lewis WJ, Gueldner RC (1983) Kairomones and their use for management of entomophagous insects: XIV. response of *Telenomus remus* to abdominal tips of *Spodoptera frugiperda*, (Z)-9-tetradecene-1-ol acetate and (Z)-9-dodecene-1-ol acetate. *Journal of Chemical Ecology* 9: 695–701. doi: 10.1007/BF00988776

Nufio CR, Papaj DR (2001) Host marking behavior in phytophagous insects and parasitoids. *Entomologia Experimentalis et Applicata* 99: 273–293. doi: 10.1046/j.1570-7458.2001.00827.x

Nutting WL (1953) The biology of *Euphasiopteryx brevicornis* (Townsend) (Diptera, Tachinidae), parasitic in the cone-headed grasshoppers (Orthoptera, Copiphorinae). *Psyche* 60: 69–81.

Oliva A (2008) Parasitoid wasps (Hymenoptera) from puparia of sarcosaprophagous flies (Diptera: Calliphoridae; Sarcophagidae) in Buenos Aires, Argentina. *Revista de la Sociedad Entomológica Argentina* 67: 139–141.

Onodera J, Matsuyama S, Suzuki T, Fujii K (2002) Host-recognizing kairomones for parasitic wasp, *Anisopteromalus calandrae*, from larvae of azuki bean weevil, *Callosobruchus chinensis*. *Journal of Chemical Ecology* 28: 1209–1220. doi: 10.1023/A:1016285801824

Orozco SX, Bertram SM (2004) Parasitized male field crickets exhibit reduced trilling bout rates and durations. *Ethology* 110: 909–917. doi: 10.1111/j.1439-0310.2004.01022.x

Orr DB, Russin JS, Boethel DJ (1986) Reproductive biology and behavior of *Telenomus calvus* (Hymenoptera: Scelionidae), a phoretic egg parasitoid of *Podisus maculiventris* (Hemiptera: Pentatomidae). *The Canadian Entomologist* 118: 1063–1072. doi: 10.4039/Ent1181063-10

Pagel M, Meade A, Barker D (2004) Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* 53: 673–684. doi: 10.1080/10635150490522232

Paik C-H, Kang S-M, Jeon Y-K, Lee G-H, Choi M-Y, Oh Y-J, Park C-G, Hwang C-Y (2009) Influence of aggregation pheromone of *Riptortus clavatus* (Heteroptera: Alydidae) on the occurrence of egg parasitoid, *Gryon japonicum* (Hymenoptera: Scelionidae). *Korean journal of applied entomology* 48: 67–72. doi: 10.5656/KSAE.2009.48.1.067

Pape T (1987) 19 *The Sarcophagidae (Diptera) of Fennoscandia and Denmark*. E. J. Brill/Scandinavian Science Press, Leiden, 203 pp.

Pape T (1990) Revisionary notes on American Sarcophaginae (Diptera: Sarcophagidae). *Tijdschrift voor Entomologie* 133: 43–74.

Pape T (1992) Phylogeny of the Tachinidae family-group (Diptera: Calyptratae). *Tijdschrift voor Entomologie* 135: 43–86.

Pape T (1994) The world *Blaesoxipha* Loew, 1861 (Diptera: Sarcophagidae). *Entomologica Scandinavica Supplements* 45: 1–247.

Pape T (1996) *Catalogue of the Sarcophagidae of the World (Insecta: Diptera)*. Associated Publishers, Gainesville, Fla, 558 pp.

Pape T, Dahlem GA (2010) Sarcophagidae (flesh flies). In: Brown BV, Borkent A, Cumming JM, Wood DM, Woodley NE, Zumbado M (Eds), *Manual of Central American Diptera, Volume 2*. NRC Research Press, Ottawa, 1313–1335.

Pape T, Dechmann D, Vonhof MJ (2002) A new species of *Sarcofahrtiopsis* Hall (Diptera: Sarcophagidae) living in roosts of Spix's disk-winged bat *Thyroptera tricolor* Spix (Chiroptera) in Costa Rica. *Journal of Natural History* 36: 991–998. doi: 10.1080/00222930110062291

Pape T, Mello-Patiu CA de (2006) Revision of *Engelimyia* Lopes, 1975 (Diptera: Sarcopahgidae). *Zootaxa* 1256: 21–47.

Pape T, Wolff M, Amat EC (2004) Los califóridos, éstridos, rinofóridos y sarcofágidos (Diptera: Calliphoridae, Oestridae, Rhinophoridae, Sarcophagidae) de Colombia. *Biota Colombiana* 5: 201–208.

Papp L (1971) Ecological and production biological data on the significance of flies breeding in cattle droppings. *Acta Zoologica Hungarica* 17: 91–105.

Parker RR (1914a) A new sarcophagid scavenger from Montana. *The Canadian Entomologist* 46: 417–423. doi: 10.4039/Ent46417-12

Parker RR (1914b) Summary of "Report to the Montana State Board of Entomology concerning fly investigations conducted in the Yellowstone Valley during the summer of 1914." *Reports of the Montana State Board of Entomology* 1: 35–50.

Parker RR (1916) New species of New England Sarcophagidæ. *The Canadian Entomologist* 48: 422–427. doi: 10.4039/Ent48422-12

Parker RR (1919) Concerning the subspecies of *Sarcophaga dux* Thomson. *Bulletin of the Brooklyn Entomological Society, New Series* 14: 41–46.

Park SH (1977) Studies on flies in Korea II. Taxonomical studies on sarcophagid flies (Diptera). *The Bulletin of Tokyo Medical and Dental University* 24: 249–284.

Pattengale ND, Aberer AJ, Swenson KM, Stamatakis A, Moret BME (2011) Uncovering hidden phylogenetic consensus in large data sets. *IEEE/ACM Transactions on Computational Biology and Bioinformatics* 8: 902–911. doi: 10.1109/TCBB.2011.28

Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A (2009) How many bootstrap replicates are necessary? In: Batzoglou S (Ed), *Research in Computational Molecular Biology*. Springer Berlin Heidelberg, Berlin, Heidelberg, 184–200.

Pedigo LP, Rice ME (2006) *Entomology and Pest Management*. 5th ed. Pearson Prentice Hall, Upper Saddle River, NJ, 749 pp.

Peri E, Cusumano A, Agrò A, Colazza S (2011) Behavioral response of the egg parasitoid *Ooencyrtus telenomicida* to host-related chemical cues in a tritrophic perspective. *BioControl* 56: 163–171. doi: 10.1007/s10526-010-9323-9

Peters JA (1948) The box turtle as a host for dipterous parasites. *American Midland Naturalist* 40: 472. doi: 10.2307/2421614

Peterson APG (1980) *Handbook of Noise Measurement*. 9th ed. GenRad, Inc, Concord, MA, 394 pp. Available from: http://www.ietlabs.com/pdf/Manuals/Handbook_Noise_Measurement.pdf.

Pickens LG (1981) The life history and predatory efficiency of *Ravinia lherminieri* (Diptera: Sarcophagidae) on the face fly (Diptera: Muscidae). *The Canadian Entomologist* 113: 523–526. doi: 10.4039/Ent113523-6

Piwczyński M, Szpila K, Grzywacz A, Pape T (2014) A large-scale molecular phylogeny of flesh flies (Diptera: Sarcophagidae). *Systematic Entomology* 39: 783–799. doi: 10.1111/syen.12086

Plank HK (1929) Natural enemies of the sugar cane moth stalkborer in Cuba. *Annals of the Entomological Society of America* 22: 621–640. doi: 10.1093/aesa/22.4.621

Poppy GM, Powell W, Pennacchio F (1997) Aphid parasitoid responses to semiochemicals – genetic, conditioned or learnt? *Entomophaga* 42: 193–199. doi: 10.1007/BF02769897

Povolný D, Verves J (1990) A preliminary list of Bulgarian Sarcophaginae (Diptera). *Acta Entomologica Musei Nationalis Pragae* 43: 283–329.

Povolný D, Verves Y (1997) 24 *The Flesh-Flies of Central Europe (Insecta, Diptera, Sarcophagidae)*. Verlag Dr. F. Pfeil, München, FRG, 260 pp.

Powell W, Hardie J, Hick AJ, Höller C, Mann J, Merritt L, Nottingham SF, Wadhams LJ, Witthinrich J, Wright AF (1993) Responses of the parasitoid *Praon volucre*

(Hymenoptera: Braconidae) to aphid sex pheromone lures in cereal fields in autumn: implications for parasitoid manipulation. *European Journal of Entomology* 90: 435–438.

Prado e Castro C, García MD, Arnaldos MI, González-Mora D (2010) Sarcophagidae (Diptera) attracted to piglet carcasses including new records for Portuguese fauna. *Graellsia* 66: 285–294. doi: 10.3989/graellsia.2010.v66.023

Prokopy RJ, Webster RP (1978) Oviposition-deterring pheromone of *Rhagoletis pomonella*: A kairomone for its parasitoid *Opius lectus*. *Journal of Chemical Ecology* 4: 481–494. doi: 10.1007/BF00989504

QGIS Development Team (2014) *QGIS Geographic Information System*. Open Source Geospatial Foundation Project. Available from: http://qgis.osgeo.org/.

Quartau JA, Simões PC (2006) Acoustic evolutionary divergence in cicadas: the species of *Cicada* L. in southern Europe. In: Drosopoulos S, Claridge M (Eds), *Insect Sounds and Communication: Physiology, Behaviour, Ecology and Evolution*. CRC Press, Boca Raton, FL, 227–237.

Ragge DR, Reynolds WJ (1998) *The Songs of the Grasshoppers and Crickets of Western Europe*. Harley Books, Colchester, Essex, 612 pp.

R Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Available from: http://www.R-project.org.

Reddy GVP, Holopainen JK, Guerrero A (2002) Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *Journal of Chemical Ecology* 28: 131–143. doi: 10.1023/A:1013519003944

Rees NE (1973) Arthropod and nematode parasites, parasitoids, and predators of Acrididae in America north of Mexico. *Technical Bulletin, Agricultural Research Service, U.S. Department of Agriculture* 1460: 1–288.

Reeves WK, Pape T, Adler PH (2000) Biological notes on New World Sarcophagidae (Diptera). *Studia Dipterologica* 7: 497–500.

Regnier FE, Wilson EO (1969) The alarm-defence system of the ant *Lasius alienus*. *Journal of Insect Physiology* 15: 893–898. doi: 10.1016/0022-1910(69)90129-2

Rice RE (1968) Observations on host selection by *Tomicobia tibialis* Ashmead (Hymenoptera: Pteromalidae). *Contributions from the Boyce Thompson Institute for Plant Research* 24: 53–56.

Rice RE (1969) Response of some predators and parasites of *Ips confusus* (LeC.) (Coleoptera: Scolytidae) to olfactory attractants. *Contributions from the Boyce Thompson Institute for Plant Research* 24: 189–194.

Rice RE, Jones RA (1982) Collections of *Prospaltella perniciosi* Tower (Hymenoptera: Aphelinidae) on San Jose Scale (Homoptera: Diaspididae) Pheromone Traps. *Environmental Entomology* 11: 876–880. doi: 10.1093/ee/11.4.876

Rivers D, Dahlem G (2013) *The Science of Forensic Entomology*. John Wiley & Sons, Hoboken, NJ, 400 pp.

Roback SS (1954) The evolution and taxonomy of the Sarcophaginae. *Illinois Biological Monographs* 23: 1–181.

Robert D (2005) Directional Hearing in Insects. In: Popper AN, Fay RR (Eds), *Sound Source Localization*. Springer-Verlag, New York, 6–35.

Robert D, Amoroso J, Hoy R (1992) The evolutionary convergence of hearing in a parasitoid fly and its cricket host. *Science* 258: 1135–1137. doi: 10.1126/science.1439820

Robert D, Edgecomb RS, Read MP, Hoy RR (1996) Tympanal hearing in tachinid flies (Diptera, Tachinidae, Ormiini): the comparative morphology of an innovation. *Cell and Tissue Research* 284: 435–448. doi: 10.1007/s004410050604

Robert D, Miles RN, Hoy RR (1999) Tympanal hearing in the sarcophagid parasitoid fly *Emblemasoma* sp.: the biomechanics of directional hearing. *The Journal of Experimental Biology* 202: 1865–1876.

Roberts RA (1933a) Additional notes on myiasis in rabbits (Dipt.: Calliphoridae, Sarcophagidae). *Entomological News* 44: 157–159.

Roberts RA (1933b) Biology of *Brachymeria fonscolombei* (Dufour), a hymenopterous parasite of blowfly larvae. *Technical Bulletin, United States Department of Agriculture* 365: 1–21.

Roberts RA (1934) Some insects collected in Mexico, mostly in association with man and animals or animal products. *Journal of the New York Entomological Society* 42: 249–262.

Rogers CE (1974) Bionomics of the carrot beetle in the Texas Rolling Plains. *Environmental Entomology* 3: 969–974. doi: 10.1093/ee/3.6.969

Rognes K (1997) The Calliphoridae (blowflies) (Diptera: Oestroidea) are not a monophyletic group. *Cladistics* 13: 27–66. doi: 10.1111/j.1096-0031.1997.tb00240.x

Roitberg BD, Lalonde RG (1991) Host marking enhances parasitism risk for a fruit-infesting fly *Rhagoletis basiola*. *Oikos* 61: 389. doi: 10.2307/3545246

Romera E, Arnaldos MI, García MD, González-Mora D (2003) Los Sarcophagidae (Insecta, Diptera) de un ecosistema cadavérico en el sureste de la Península Ibérica. *Anales de Biología* 25: 49–63.

Ronquist F, van der Mark P, Huelsenbeck JP (2009) Bayesian phylogenetic analysis using MrBayes. In: Salemi M, Vandamme A-M, Lemey P (Eds), *The Phylogenetic Handbook:*

*A Practical Approach to Phylogenetic Analysis and Hypothesis Testing*. Cambridge University Press, Cambridge, UK ; New York.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. doi: 10.1093/sysbio/sys029

Rosen MJ, Levin EC, Hoy RR (2009) The cost of assuming the life history of a host: acoustic startle in the parasitoid fly *Ormia ochracea*. *Journal of Experimental Biology* 212: 4056–4064. doi: 10.1242/jeb.033183

Rotundo G, Tremblay E (1975) Sull'attrattività delle femmine vergini di due specie di Pseudococcidi (Homoptera Coccoidea) per un Imenottero parassita (Hymenoptera Chalcidoidea). *Bollettino del Laboratorio di Entomologia Agraria "Filippo Silvestri" Portici* 32: 172–179.

Roure B, Baurain D, Philippe H (2013) Impact of missing data on phylogenies inferred from empirical phylogenomic data sets. *Molecular Biology and Evolution* 30: 197–214. doi: 10.1093/molbev/mss208

Ruther J (2013) Novel insights into pheromone-mediated communication in parasitic hymenopterans. In: Wajnberg E, Colazza S (Eds), *Chemical Ecology of Insect Parasitoids*. John Wiley & Sons, Chichester, UK, 112–144.

Sabrosky CW (1953a) Taxonomy and host relations of the tribe Ormiini in the Western Hemisphere (Diptera, Larvaevoridae). *Proceedings of the Entomological Society of Washington* 55: 167–183.

Sabrosky CW (1953b) Taxonomy and host relations of the tribe Ormiini in the Western Hemisphere, II (Diptera, Larvaevoridae). *Proceedings of the Entomological Society of Washington* 55: 289–305.

Sakaluk SK, Belwood JJ (1984) Gecko phonotaxis to cricket calling song: A case of satellite predation. *Animal Behaviour* 32: 659–662. doi: 10.1016/S0003-3472(84)80141-4

Salt G (1934) Experimental studies in insect parasitism. II. superparasitism. *Proceedings of the Royal Society B: Biological Sciences* 114: 455–476. doi: 10.1098/rspb.1934.0019

Salt G (1935) Experimental studies in insect parasitism III–host selection. *Proceedings of the Royal Society B: Biological Sciences* 117: 413–435. doi: 10.1098/rspb.1935.0037

Salt G (1938) Experimental studies in insect parasitism. VI.—host suitability. *Bulletin of Entomological Research* 29: 223. doi: 10.1017/S0007485300035574

Salt G (1961) Competition among insect parasitoids. *Symposia of the Society for Experimental Biology* 15: 96–119.

Samways MJ (1988) Comparative monitoring of red scale *Aonidiella aurantii* (Mask.) (Hom., Diaspididae) and its *Aphytis* spp. (Hym., Aphelinidae) parasitoids. *Journal of Applied Entomology* 105: 483–489. doi: 10.1111/j.1439-0418.1988.tb00214.x

Sanborn AF, Breitbarth JH, Heath JE, Heath MS (2002) Temperature responses and habitat sharing in two sympatric species of *Okanagana* (Homoptera: Cicadoidea). *Western North American Naturalist* 62: 437–450.

Sanborn AF, Heath JE, Heath MS (2009) Long-range sound distribution and the calling song of the cicada *Beameria venosa* (Uhler) (Hemiptera: Cicadidae). *The Southwestern Naturalist* 54: 24–30. doi: 10.1894/JC-23.1

Sanborn AF, Heath MS, Phillips PK, Heath JE (2011) A new species of the genus *Beameria* (Hemiptera: Cicadidae) from North America. *Journal of Natural History* 45: 1589–1605. doi: 10.1080/00222933.2011.559597

Sanborn AF, Heath MS, Phillips PK, Heath JE (2012) The genus *Pacarina* Distant, 1905 (Hemiptera: Cicadidae) with the description of a new species. *Journal of Natural History* 46: 923–941. doi: 10.1080/00222933.2011.651647

Sanborn AF, Heath MS, Sueur J, Phillips PK (2005) The genus *Neocicada* Kato, 1932 (Hemiptera: Cicadomorpha: Cicadidae), with descriptions of three new species. *Systematic Entomology* 30: 191–207. doi: 10.1111/j.1365-3113.2005.00266.x

Sanborn AF, Phillips PK (1992) Observations on the effect of a partial solar eclipse on calling in some desert cicadas (Homoptera: Cicadidae). *The Florida Entomologist* 75: 285–287. doi: 10.2307/3495634

Sanborn AF, Phillips PK (1995) Scaling of sound pressure level and body size in cicadas (Homoptera: Cicadidae; Tibicinidae). *Annals of the Entomological Society of America* 88: 479–484. doi: 10.1093/aesa/88.4.479

Sanchez NE, Onsager JA (1994) Effects of dipterous parasitoids on reproduction of *Melanoplus sanguinipes* (Orthoptera: Acrididae). *Journal of Orthoptera Research*: 65. doi: 10.2307/3503410

Sanders DP, Dobson RC (1966) The insect complex associated with bovine manure in Indiana. *Annals of the Entomological Society of America* 59: 955–959. doi: 10.1093/aesa/59.5.955

Sanderson MJ, Shaffer HB (2002) Troubleshooting molecular phylogenetic analyses. *Annual Review of Ecology and Systematics* 33: 49–72.

Schmidt JO (1986) Chemistry, pharmacology, and chemical ecology of ant venoms. In: Piek T (Ed), *Venoms of the Hymenoptera: Biochemical, Pharmacological, and Behavioural Aspects*. Academic Press, London; Orlando, 425–508.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675. doi: 10.1038/nmeth.2089

Schniederkötter K, Lakes-Harlan R (2004) Infection behavior of a parasitoid fly, *Emblemasoma auditrix*, and its host cicada *Okanagana rimosa*. *Journal of Insect Science* 4: 36–36. doi: 10.1093/jis/4.1.36

Schöller M, Prozell S (2002) Response of *Trichogramma evanescens* to the main sex pheromone component of *Ephestia* spp. and *Plodia interpunctella*, (Z,E)-9,12-tetra-decadenyl acetate (ZETA). *Journal of Stored Products Research* 38: 177–184. doi: 10.1016/S0022-474X(01)00015-7

Schröder D (1974) A study of the interactions between the internal larval parasites of *Rhyacionia buoliana* [Lepidoptera: Olethreutidae]. *Entomophaga* 19: 145–171. doi: 10.1007/BF02373274

Schwarz G (1978) Estimating the dimension of a model. *The Annals of Statistics* 6: 461–464. doi: 10.1214/aos/1176344136

Seabra SG, Pinto-Juma G, Quartau JA (2006) Calling songs of sympatric and allopatric populations of *Cicada barbara* and *C. orni* (Hemiptera: Cicadidae) on the Iberian Peninsula. *European Journal of Entomology* 103: 843–852. doi: 10.14411/eje.2006.115

Segal DB, Humphrey JM, Edwards SJ, Kirby MD (1968) Parasites of man and domestic animals in Vietnam, Thailand, Laos, and Cambodia: host list and bibliography. *Experimental Parasitology* 23: 412–464.

Senior-White R (1924) A revision of the sub-family Sarcophaginae in the Oriental Region. *Records of the Indian Museum* 26: 193–283.

Senior-White R, Aubertin D, Smart J (1940) *The Fauna of British India, Including the Remainder of the Oriental Region: Diptera. Volume VI. Family Calliphoridae*. Sewell RBS (Ed). Taylor and Francis, London, 288 pp.

Shannon RC (1914) Habits of some Tachinidae. *Proceedings of the Entomological Society of Washington* 16: 182.

Sharma SK (1975) Some observations on the mating behaviour of flesh fly, *Sarcophaga bravicornis* [sic]. *Zeitschrift für Angewandte Zoologie* 62: 35–38.

Shavit Grievink L, Penny D, Holland BR (2013) Missing data and influential sites: choice of sites for phylogenetic analysis can be as important as taxon sampling and model choice. *Genome Biology and Evolution* 5: 681–687. doi: 10.1093/gbe/evt032

Shaw MR (1994) Parasitoid host ranges. In: Hawkins BA, Sheehan W (Eds), *Parasitoid Community Ecology*. Oxford University Press, Oxford; New York, 111–144.

Sherman F (1920) The green clover worm (*Plathypena scabra* Fabr.) as a pest on soy beans. *Journal of Economic Entomology* 13: 295–303. doi: 10.1093/jee/13.3.295

Shewell GE (1987) Sarcophagidae. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds), *Manual of Nearctic Diptera, Volume 2*. Canadian Government Publishing Centre, Hull, Quebec, Canada, 1159–1186.

Shi C-M, Zhang X-S, Zhang D-X (2015) Parasitoidism of the *Sarcophaga dux* (Diptera: Sarcophagidae) on the *Mesobuthus martensii* (Scorpiones: Buthidae) and Its Implications. *Annals of the Entomological Society of America*: sav090. doi: 10.1093/aesa/sav090

Shi J, Guo Y, Zhang C, Zhou Y, Yan J, Liao H, Lagabaiyila Z (2014) The complete mitochondrial genome of the flesh fly, *Parasarcophaga portschinskyi* (Diptera: Sarcophagidae). *Mitochondrial DNA*: 1–2. doi: 10.3109/19401736.2014.971282

Shiota T, Yoshida Y, Hirai S, Torii S (1990) Intestinal myiasis caused by *Parasarcophaga crassipalpis* (Diptera: Sarcophagidae). *Pediatrics* 85: 215–217.

da Silva ÂZ, Hoffmeister CH, Anjos VA dos, Ribeiro PB, Krüger RF (2014) Necrophagous diptera associated with wild animal carcasses in southern Brazil. *Revista Brasileira de Entomologia* 58: 337–342. doi: 10.1590/S0085-56262014000400001

Silva CC, Moraes MCB, Laumann RA, Borges M (2006) Sensory response of the egg parasitoid *Telenomus podisi* to stimuli from the bug *Euschistus heros*. *Pesquisa Agropecuária Brasileira* 41: 1093–1098. doi: 10.1590/S0100-204X2006000700004

Simmons JA, Wever EG, Pylka JM (1971) Periodical cicada: sound production and hearing. *Science* 171: 212–213. doi: 10.1126/science.171.3967.212

Simmons MP (2012) Radical instability and spurious branch support by likelihood when applied to matrices with non-random distributions of missing data. *Molecular Phylogenetics and Evolution* 62: 472–484. doi: 10.1016/j.ympev.2011.10.017

Simmons MP (2014) A confounding effect of missing data on character conflict in maximum likelihood and Bayesian MCMC phylogenetic analyses. *Molecular Phylogenetics and Evolution* 80: 267–280. doi: 10.1016/j.ympev.2014.08.021

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651–701. doi: 10.1093/aesa/87.6.651

Singer MS, Mace KC, Bernays EA (2009) Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars May RC (Ed). *PLoS ONE* 4: e4796. doi: 10.1371/journal.pone.0004796

Singh B, Wells JD (2013) Molecular systematics of the Calliphoridae (Diptera: Oestroidea): evidence from one mitochondrial and three nuclear genes. *Journal of Medical Entomology* 50: 15–23. doi: 10.1603/ME11288

Singh D, Bharti M (2008) Some notes on the nocturnal larviposition by two species of *Sarcophaga* (Diptera: Sarcophagidae). *Forensic Science International* 177: e19–e20. doi: 10.1016/j.forsciint.2007.08.007

Smith CW (1940) An exchange of grasshopper parasites between Argentina and Canada with notes on parasitism of native grasshoppers. *Annual Report of the Entomological Society of Ontario* 70: 57–62.

Smith HE (1915) The grasshopper outbreak in New Mexico during the summer of 1913. *Bulletin of the United States Department of Agriculture* 293: 1–12.

Smith KGV (1989) 10 *An Introduction to the Immature Stages of British Flies: Diptera Larvae, with Notes on Eggs, Puparia and Pupae*. British Museum of Natural History, London, 280 pp.

Smith MA, Rodriguez JJ, Whitfield JB, Deans AR, Janzen DH, Hallwachs W, Hebert PDN (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences* 105: 12359–12364. doi: 10.1073/pnas.0805319105

Smith MA, Wood DM, Janzen DH, Hallwachs W, Hebert PDN (2007) DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proceedings of the National Academy of Sciences* 104: 4967–4972. doi: 10.1073/pnas.0700050104

Smith RC, Kelly EG, Dean GA, Bryson HR, Parker RL (1943) Common insects of Kansas. *Report of the Kansas State Board of Agriculture* 62: 1–440.

Smith RJ (2009) Use and misuse of the reduced major axis for line-fitting. *American Journal of Physical Anthropology* 140: 476–486. doi: 10.1002/ajpa.21090

Smith RL, Langley WM (1978) Cicada stress sound: an assay of its effectiveness as a predator defense mechanism. *The Southwestern Naturalist* 23: 187. doi: 10.2307/3669767

Sonet G, Jordaens K, Braet Y, Bourguignon L, Dupont E, Backeljau T, de Meyer M, Desmyter S (2013) Utility of GenBank and the Barcode of Life Data Systems (BOLD) for the identification of forensically important Diptera from Belgium and France. *ZooKeys* 365: 307–328. doi: 10.3897/zookeys.365.6027

Soper RS, Shewell GE, Tyrrell D (1976) *Colcondamyia auditrix* nov. sp. (Diptera: Sarcophagidae), a parasite which is attracted by the mating song of its host, *Okanagana rimosa* (Homoptera: Cicadidae). *The Canadian Entomologist* 108: 61–68. doi: 10.4039/Ent10861-1

So P-M, Dudgeon D (1989) Life-history responses of larviparous *Boettcherisca formosensis* (Diptera: Sarcophagidae) to larval competition for food, including comparisons with oviparous *Hemipyrellia ligurriens* (Calliphoridae). *Ecological Entomology* 14: 349–356. doi: 10.1111/j.1365-2311.1989.tb00964.x

Souza AM de, Linhares AX (1997) Diptera and Coleoptera of potential forensic importance in southeastern Brazil: relative abundance and seasonality. *Medical and Veterinary Entomology* 11: 8–12. doi: 10.1111/j.1365-2915.1997.tb00284.x

Sparks AN, Jackson RD, Carpenter JE, Muller RA (1986) Insects captured in light traps in the Gulf of Mexico. *Annals of the Entomological Society of America* 79: 132–139. doi: 10.1093/aesa/79.1.132

Spencer GJ, Buckell ER (1957) On the acridiophagous Sarcophagidae of British Columbia with records of all others taken in the province. *Proceedings of the Entomological Society of British Columbia* 54: 29–36.

Spofford MG, Kurczewski FE (1985) Courtship and mating behavior of *Phrosinella aurifacies* Downes (Diptera: Sarcophagidae: Miltogramminae). *Proceedings of the Entomological Society of Washington* 87: 273–282.

Spofford MG, Kurczewski FE, Downes WL (1989) Nearctic species of Miltogrammini (Diptera: Sarcophagidae) associated with species of Aculeata (Hymenoptera: Vespoidea, Pompiloidea, Sphecoidea, Apoidea). *Journal of the Kansas Entomological Society* 62: 254–267.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. doi: 10.1093/bioinformatics/btu033

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* 57: 758–771. doi: 10.1080/10635150802429642

Stamper T, Dahlem GA, Cookman C, Debry RW (2013) Phylogenetic relationships of flesh flies in the subfamily Sarcophaginae based on three mtDNA fragments (Diptera: Sarcophagidae). *Systematic Entomology* 38: 35–44. doi: 10.1111/j.1365-3113.2012.00646.x

Stamper T, Davis P, DeBry RW (2009) The nocturnal ovipositing behavior of carrion flies in Cincinnati, Ohio. *Journal of Forensic Sciences* 54: 1450–1452. doi: 10.1111/j.1556-4029.2009.01198.x

Stark EJ, Harper JD (1982) Pupal mortality in forest tent caterpillar (Lepidoptera: Lasiocampidae): causes and impact on populations in southwestern Alabama. *Environmental Entomology* 11: 1071–1077. doi: 10.1093/ee/11.5.1071

Stegmaier CE (1972) Notes on some Sarcophagidae (Diptera) reared from snails (Mollusca) in Florida. *The Florida Entomologist* 55: 237. doi: 10.2307/3493372

Steidle JM, Steppuhn A, Ruther J (2003) Specific foraging kairomones used by a generalist parasitoid. *Journal of Chemical Ecology* 29: 131–143. doi: 10.1023/A:1021932731350

Stelinski LL, Liburd OE (2005) Behavioral evidence for host fidelity among populations of the parasitic wasp, *Diachasma alloeum* (Muesebeck). *Naturwissenschaften* 92: 65–68. doi: 10.1007/s00114-004-0593-x

Stephens CS (1962) *Oiketicus kirbyi* (Lepidoptera: Psychidae) a pest of bananas In Costa Rica. *Journal of Economic Entomology* 55: 381–386. doi: 10.1093/jee/55.3.381

Sternlicht M (1973) Parasitic wasps attracted by the sex pheromone of their coccid host. *Entomophaga* 18: 339–342. doi: 10.1007/BF02371011

Stevens JR (2003) The evolution of myiasis in blowflies (Calliphoridae). *International Journal for Parasitology* 33: 1105–1113. doi: 10.1016/S0020-7519(03)00136-X

Stevens JR, West H, Wall R (2008) Mitochondrial genomes of the sheep blowfly, *Lucilia sericata*, and the secondary blowfly, *Chrysomya megacephala*. *Medical and Veterinary Entomology* 22: 89–91. doi: 10.1111/j.1365-2915.2008.00710.x

Stewart KE (1934) Insects affecting animals and man. *Canadian Insect Pest Review* 12: 156–159.

Stireman JO, O'Hara JE, Wood DM (2006) Tachinidae: evolution, behavior, and ecology. *Annual Review of Entomology* 51: 525–555. doi: 10.1146/annurev.ento.51.110104.151133

Stireman JO, Singer MS (2003) What determines host range in parasitoids? An analysis of a tachinid parasitoid community. *Oecologia* 135: 629–638. doi: 10.1007/s00442-003-1235-2

Strauß J, Lakes-Harlan R (2014) Evolutionary and phylogenetic origins of tympanal hearing organs in insects. In: Hedwig B (Ed), *Insect Hearing and Acoustic Communication*. Springer Berlin Heidelberg, Berlin, Heidelberg, 5–26.

Strehl CE, White J (1986) Effects of superabundant food on breeding success and behavior of the red-winged blackbird. *Oecologia* 70: 178–186. doi: 10.1007/BF00379237

Stucky BJ (2012) SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. *Journal of Biomolecular Techniques : JBT* 23: 90–93. doi: 10.7171/jbt.12-2303-004

Stucky BJ (2013) Morphology, bioacoustics, and ecology of *Tibicen neomexicensis* sp. n., a new species of cicada from the Sacramento Mountains in New Mexico, U.S.A. (Hemiptera, Cicadidae, *Tibicen*). *ZooKeys* 337: 49–71. doi: 10.3897/zookeys.337.5950

Stucky BJ (2015) Infection behavior, life history, and host parasitism rates of *Emblemasoma erro* (Diptera: Sarcophagidae), an acoustically hunting parasitoid of the cicada *Tibicen dorsatus* (Hemiptera: Cicadidae). *Zoological Studies* 54: 1–17. doi: 10.1186/s40555-015-0105-z

Stumpner A, von Helversen D (2001) Evolution and function of auditory systems in insects. *Naturwissenschaften* 88: 159–170. doi: 10.1007/s001140100223

Stumpner A, Lakes-Harlan R (1996) Auditory interneurons in a hearing fly (*Therobia leonidei*, Ormiini, Tachinidae, Diptera). *Journal of Comparative Physiology A* 178. doi: 10.1007/BF00188164

Sugden JW (1940) Characteristics of certain western cicadas. *Journal of the New York Entomological Society* 48: 117–125.

Sullivan J, Joyce P (2005) Model selection in phylogenetics. *Annual Review of Ecology, Evolution, and Systematics* 36: 445–466. doi: 10.1146/annurev.ecolsys.36.102003.152633

Swinton AH (1880) *Insect Variety: Its Propagation and Distribution*. Cassell, Petter, Galpin & Co., London, 326 pp.

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56: 564–577. doi: 10.1080/10635150701472164

Tamaki G, Chauvin RL, Burditt AK (1983) Field evaluation of *Doryphorophaga doryphorae* (Diptera: Tachinidae), a parasite, and its host the Colorado potato beetle (Coleoptera: Chrysomelidae). *Environmental Entomology* 12: 386–389.

Tanaka S, Guardia M, Denlinger DL, Wolda H (1990) Relationships between body size, reproductive traits, and food resource in three species of tropical flesh flies. *Researches on Population Ecology* 32: 303–317. doi: 10.1007/BF02512565

Tantawi TI, El-Kady EM, Greenberg B, El-Ghaffar HA (1996) Arthropod succession on exposed rabbit carrion in Alexandria, Egypt. *Journal of Medical Entomology* 33: 566–580. doi: 10.1093/jmedent/33.4.566

Tawfik MFS, Ibrahim AMA, Afifi AI, El-Deeb HI, Azzam KM (2000) Biological studies on the malacophagous insect, *Ravinia striata* (Fabricius) (Sarcophagidae: Diptera). *Egyptian Journal of Agricultural Research* 78: 91–96.

Temerak SA, Whitcomb WH (1984) Parasitoids of predaceous and phytophagous pentatomid bugs in soybean fields at two sites of Alachua County, Florida. *Zeitschrift für Angewandte Entomologie* 97: 279–282. doi: 10.1111/j.1439-0418.1984.tb03749.x

Tessmer JW, Meek CL, Wright VL (1995) Circadian patterns of oviposition by necrophilous flies (Diptera: Calliphoridae) in southern Louisiana. *Southwestern Entomologist* 20: 439–445.

Thai H-T, Mentré F, Holford NHG, Veyrat-Follet C, Comets E (2013) A comparison of bootstrap approaches for estimating uncertainty of parameters in linear mixed-effects models. *Pharmaceutical Statistics* 12: 129–140. doi: 10.1002/pst.1561

Thomas HT (1950) Field notes on the mating habits of *Sarcophaga* Meigen (Diptera). *Proceedings of the Royal Entomological Society of London. Series A, General Entomology* 25: 93–98. doi: 10.1111/j.1365-3032.1950.tb00101.x

Thompson WR (1943) *Section 1: Parasite Host Catalogue, Part 1: Parasites of the Arachnida and Coleoptera*. Imperial Parasite Service, Belleville, Ontario, Canada, 151 pp.

Thompson WR (1944) *Section 1: Parasite Host Catalogue, Part 5: Parasites of the Lepidoptera, (A-Ch)*. Imperial Parasite Service, Belleville, Ontario, Canada, 130 pp.

Thompson WR (1946) *Section 1: Parasite Host Catalogue, Part 7: Parasites of the Lepidoptera, (G-M)*. Imperial Parasite Service, Belleville, Ontario, Canada, 385 pp.

Thompson WR (1950) *Section 1: Parasite Host Catalogue, Part 11: Parasites of the Neuroptera, Odonata, Orthoptera, Psocoptera, Siphonaptera and Thysanoptera*. Commonwealth Bureau of Biological Control, Ottawa, Ontario, Canada, 35 pp.

Thompson WR (1951) *Section 2: Host Parasite Catalogue, Part 1: Hosts of the Coleoptera and Diptera*. Commonwealth Institute of Biological Control, Ottawa, Ontario, Canada, 147 pp.

Thomson IR, Vincent CM, Bertram SM (2012) Success of the parasitoid fly *Ormia ochracea* (Diptera: Tachinidae) on natural and unnatural cricket hosts. *Florida Entomologist* 95: 43–48. doi: 10.1653/024.095.0108

Thomson RC, Shaffer HB (2010) Sparse supermatrices for phylogenetic inference: taxonomy, alignment, rogue taxa, and the phylogeny of living turtles. *Systematic Biology* 59: 42–58. doi: 10.1093/sysbio/syp075

Thorley JL, Wilkinson M (1999) Testing the phylogenetic stability of early tetrapods. *Journal of Theoretical Biology* 200: 343–344. doi: 10.1006/jtbi.1999.0999

Thornhill R, Alcock J (1983) *The Evolution of Insect Mating Systems*. Harvard University Press, Cambridge, MA, 547 pp.

Thornton PE, Running SW, White MA (1997) Generating surfaces of daily meteorological variables over large regions of complex terrain. *Journal of Hydrology* 190: 214–251. doi: 10.1016/S0022-1694(96)03128-9

Thornton PE, Thornton MM, Mayer BW, Wilhelmi N, Wei Y, Devarakonda R, Cook RB (2014) Daymet: Daily Surface Weather Data on a 1-km Grid for North America, Version 2. Available from: http://daac.ornl.gov (September 26, 2014).

Tiensuu L (1939) Die sarcophagiden (Dipt.) Finnlands. *Suomen Hyönteistieteellinen Aikakauskirja* 5: 255–266.

Tillman PG, Aldrich JR, Khrimian A, Cottrell TE (2010) Pheromone attraction and cross-attraction of *Nezara*, *Acrosternum*, and *Euschistus* spp. stink bugs (Heteroptera:

Pentatomidae) in the field. *Environmental Entomology* 39: 610–617. doi: 10.1603/EN09114

Tormos J, Asís JD, Gayubo SF, Portillo M, Torres F (2000) Nesting behavior of *Oxybelus lamellatus* Olivier (Hymenoptera: Sphecidae). *Annals of the Entomological Society of America* 93: 326–332. doi: 10.1603/0013-8746(2000)093[0326:NBOOLO]2.0.CO;2

Townsend CHT (1917) New genera and species of American muscoid Diptera. *Proceedings of the Biological Society of Washington* 30: 43–50.

Treherne RC, Buckell ER (1924) Grasshoppers of British Columbia. *Bulletin of the Dominion of Canada Department of Agriculture, New Series* 39: 1–47.

Tsueda H (2014) Response of two parasitoid wasps to the sex pheromone of Japanese mealybug, *Planococcus kraunhiae*. *Japanese Journal of Applied Entomology and Zoology* 58: 147–152. doi: 10.1303/jjaez.2014.147

Tukey JW (1967) An introduction to the calculations of numerical spectrum analysis. In: Harris B (Ed), *Spectral Analysis of Time Series*. John Wiley & Sons, New York, 25–46.

Tuttle MD, Ryan MJ, Belwood JJ (1985) Acoustical resource partitioning by two species of phyllostomid bats (*Trachops cirrhosus* and *Tonatia sylvicola*). *Animal Behaviour* 33: 1369–1371. doi: 10.1016/S0003-3472(85)80204-9

Udgaonkar US, Dharamsi R, Kulkarni SA, Shah SR, Patil SS, Bhosale AL, Gadgil SA, Mohite RS (2012) Intestinal myiasis. *Indian Journal of Medical Microbiology* 30: 332. doi: 10.4103/0255-0857.99496

Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R, Leunissen JAM (2007) Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research* 35: W71–W74. doi: 10.1093/nar/gkm306

US Environmental Protection Agency (2013) Level III Ecoregions of the Conterminous United States. Available from: http://www.epa.gov/wed/pages/ecoregions/level_iii_iv.htm (April 16, 2014).

Verves YG (1980) Some Sarcophaginae (Diptera) from Afghanistan. *Folia Entomologica Hungarica* 41: 355–357.

Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37: 141–172. doi: 10.1146/annurev.en.37.010192.001041

Vet LEM, Dicke M, Wäckers FL (1991) How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. *Netherlands Journal of Zoology* 41: 202–213. doi: 10.1163/156854291X00144

Vincent CM, Bertram SM (2010) Crickets groom to avoid lethal parasitoids. *Animal Behaviour* 79: 51–56. doi: 10.1016/j.anbehav.2009.10.001

Vinson SB (1976) Host selection by insect parasitoids. *Annual Review of Entomology* 21: 109–133. doi: 10.1146/annurev.en.21.010176.000545

Vinson SB (1984) How parasitoids locate their hosts: a case of insect espionage. In: Lewis T (Ed), *Insect Communication*. *Commemorations of the Royal Entomological Society of London*. Academic Press, London, 325–348.

Vinson SB (1998) The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological Control* 11: 79–96. doi: 10.1006/bcon.1997.0601

Vinson SB, Iwantsch GF (1980a) Host regulation by insect parasitoids. *The Quarterly Review of Biology* 55: 143–165.

Vinson SB, Iwantsch GF (1980b) Host suitability for insect parasitoids. *Annual Review of Entomology* 25: 397–419. doi: 10.1146/annurev.en.25.010180.002145

Virant-Doberlet M, Čokl A (2004) Vibrational communication in insects. *Neotropical Entomology* 33: 121–134. doi: 10.1590/S1519-566X2004000200001

de Vries T, Lakes-Harlan R (2005) Phonotaxis of the female parasitoid *Emblemasoma auditrix* (Diptera, Sarcophagidae) in relation to number of larvae and age. *Zoology* 108: 239–246. doi: 10.1016/j.zool.2005.04.004

Wagner WE (1996) Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. *Behavioral Ecology* 7: 279–285. doi: 10.1093/beheco/7.3.279

Wagner WE, Basolo AL (2007) Host preferences in a phonotactic parasitoid of field crickets: the relative importance of host song characters. *Ecological Entomology* 32: 478–484. doi: 10.1111/j.1365-2311.2007.00898.x

Walker EM (1931) Cutaneous myiasis in Canada. *Canadian Public Health Journal* 22: 504–508.

Walker TJ (1957) Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. *Annals of the Entomological Society of America* 50: 626–636.

Walker TJ (1964a) Cryptic species among sound-producing ensiferan Orthoptera (Gryllidae and Tettigoniidae). *The Quarterly Review of Biology* 39: 345–355.

Walker TJ (1964b) Experimental demonstration of a cat locating orthopteran prey by the prey's calling song. *Florida Entomologist* 47: 163–165.

Walker TJ (1973) Systematics and acoustic behavior of United States and Caribbean short-tailed crickets (Orthoptera: Gryllidae: *Anurogryllus*). *Annals of the Entomological Society of America* 66: 1269–1277.

Walker TJ (1979) Calling crickets (*Anurogryllus arboreus*) over pitfalls: females, males, and predators. *Environmental Entomology* 8: 441–443. doi: 10.1093/ee/8.3.441

Walker TJ (1986) Monitoring the flights of field crickets (*Gryllus* spp.) and a tachinid fly (*Euphasiopteryx ochracea*) in north Florida. *Florida Entomologist* 69: 678–685.

Walker TJ (1989) A live trap for monitoring *Euphasiopteryx* and tests with *E. ochracea* (Diptera: Tachinidae). *Florida Entomologist* 72: 314–319.

Walker TJ (1993) Phonotaxis in female *Ormia ochracea* (Diptera: Tachinidae), a parasitoid of field crickets. *Journal of Insect Behavior* 6: 389–410. doi: 10.1007/BF01048119

Walker TJ (2004) The uhleri group of the genus *Amblycorypha* (Orthoptera: Tettigoniidae): extraordinarily complex songs and new species. *Journal of Orthoptera Research* 13: 169–183. doi: 10.1665/1082-6467(2004)013[0169:TUGOTG]2.0.CO;2

Walker TJ, Forrest TG, Spooner JD (2003) The *rotundifolia* complex of the genus *Amblycorypha* (Orthoptera: Tettigoniidae): songs reveal new species. *Annals of the Entomological Society of America* 96: 433–447. doi: 10.1603/0013-8746(2003)096[0433:TRCOTG]2.0.CO;2

Walker TJ, Parkman JP, Frank JH, Schuster DJ (1996) Seasonality of *Ormia depleta* and limits to its spread. *Biological Control* 6: 378–383. doi: 10.1006/bcon.1996.0048

Walker TJ, Wineriter SA (1991) Hosts of a phonotactic parasitoid and levels of parasitism (Diptera: Tachinidae: *Ormia ochracea*). *Florida Entomologist* 74: 554–559.

Walton WR (1915) A new nocturnal species of Tachinidae. *Proceedings of the Entomological Society of Washington* 17: 162–164.

Watson JR (1942) *Sarcophaga bullata* Parker as a cause of intestinal myiasis. *Florida Entomologist* 25: 5–6.

Watts KJ, Combs RL (1977) Parasites of *Haematobia irritans* and other flies breeding in bovine feces in northeast Mississippi. *Environmental Entomology* 6: 823–826. doi: 10.1093/ee/6.6.823

Webster FM (1907) The value of parasites in cereal and forage crop production. *Bulletin of the Bureau of Entomology of the U.S. Department of Agriculture* 67: 94–100.

Weddell JA (1937) The grasshopper outbreak in Queensland, 1934-35. *Queensland Agricultural Journal* 47: 246–259, 354–364, 451–462.

Welch P (1967) The use of fast Fourier transform for the estimation of power spectra: a method based on time averaging over short, modified periodograms. *IEEE Transactions on Audio and Electroacoustics* 15: 70–73. doi: 10.1109/TAU.1967.1161901

Wells JD, Greenberg B (1994) Resource use by an introduced and native carrion flies. *Oecologia* 99: 181–187. doi: 10.1007/BF00317099

Wells JD, Pape T, Sperling FA (2001) DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). *Journal of Forensic Sciences* 46: 1098–1102.

West-Eberhard MJ (1984) Sexual selection, competitive communication and species-specific signals in insects. In: Lewis T (Ed), *Insect Communication*. *Commemorations of the Royal Entomological Society of London*. Academic Press, London, 283–324.

Wheeler WC (1990) Nucleic acid sequence phylogeny and random outgroups. *Cladistics* 6: 363–367. doi: 10.1111/j.1096-0031.1990.tb00550.x

Whiteside RC, Burbutis PP, Kelsey LP (1967) Insect parasites of the green clover worm in Delaware. *Journal of Economic Entomology* 60: 326–328. doi: 10.1093/jee/60.2.326

Whitlock M, Schluter D (2009) *The analysis of biological data*. Roberts and Co. Publishers, Greenwood Village, Colo, 700 pp.

Wiegmann BM, Trautwein MD, Winkler IS, Barr NB, Kim J-W, Lambkin C, Bertone MA, Cassel BK, Bayless KM, Heimberg AM, Wheeler BM, Peterson KJ, Pape T, Sinclair BJ, Skevington JH, Blagoderov V, Caravas J, Kutty SN, Schmidt-Ott U, Kampmeier GE, Thompson FC, Grimaldi DA, Beckenbach AT, Courtney GW, Friedrich M, Meier R, Yeates DK (2011) Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences* 108: 5690–5695. doi: 10.1073/pnas.1012675108

Wiens JJ, Morrill MC (2011) Missing data in phylogenetic analysis: reconciling results from simulations and empirical data. *Systematic Biology* 60: 719–731. doi: 10.1093/sysbio/syr025

Wilkinson M (1996) Majority-rule reduced consensus trees and their use in bootstrapping. *Molecular Biology and Evolution* 13: 437–444.

Wilm A, Mainz I, Steger G (2006) An enhanced RNA alignment benchmark for sequence alignment programs. *Algorithms for Molecular Biology: AMB* 1: 19. doi: 10.1186/1748-7188-1-19

Wilson EB (1927) Probable inference, the law of succession, and statistical inference. *Journal of the American Statistical Association* 22: 209. doi: 10.2307/2276774

Wilson JW (1932) Coleoptera and Diptera collected from a New Jersey sheep pasture. *Journal of the New York Entomological Society* 40: 77–93.

Wineriter SA, Walker TJ (1990) Rearing phonotactic parasitoid flies [Diptera: Tachinidae, Ormiini, *Ormia* spp.]. *Entomophaga* 35: 621–632. doi: 10.1007/BF02375096

Wiskerke JSC, Dicke M, Vet LEM (1993) Larval parasitoid uses aggregation pheromone of adult hosts in foraging behaviour: a solution to the reliability-detectability problem. *Oecologia* 93: 145–148. doi: 10.1007/BF00321204

Witte V, Disney RHL, Weissflog A, Maschwitz U (2010) Studies in European ant-decapitating flies (Diptera: Phoridae): ant alarm pheromone as host finding cue in *Pseudacteon brevicauda*, a parasite of *Myrmica rubra* (Formicidae: Myrmicinae). *Journal of Natural History* 44: 905–912. doi: 10.1080/00222930903437366

Wong ES, Dahlem GA, Stamper TI, DeBry RW (2015) Discordance between morphological species identification and mtDNA phylogeny in the flesh fly genus *Ravinia* (Diptera: Sarcophagidae). *Invertebrate Systematics* 29: 1. doi: 10.1071/IS14018

Wood DM (1987) Tachinidae. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds), *Manual of Nearctic Diptera, Volume 2*. Canadian Government Publishing Centre, Hull, Quebec, Canada, 1193–1269.

Xue W-Q, Verves YG, Du J (2011) A review of subtribe Boettcheriscina Verves 1990 (Diptera: Sarcophagidae), with descriptions of a new species and genus from China. *Annales de la Société Entomologique de France* 47: 303–329. doi: 10.1080/00379271.2011.10697723

Yack JE (2004) The structure and function of auditory chordotonal organs in insects. *Microscopy Research and Technique* 63: 315–337. doi: 10.1002/jemt.20051

Yager DD (1999) Structure, development, and evolution of insect auditory systems. *Microscopy Research and Technique* 47: 380–400. doi: 10.1002/(SICI)1097-0029(19991215)47:6<380::AID-JEMT3>3.0.CO;2-P

Yan J, Liao H, Zhu Z, Xie K, Guo Y, Cai J (2014) The complete mitochondria genome of *Parasarcophaga similis* (Diptera: Sarcophagidae). *Mitochondrial DNA*: 1–2. doi: 10.3109/19401736.2014.958708

Yasuda K, Tsurumachi M (1995) Influence of male adults of the leaf-footed plant bug, *Leptoglossus australis* (Fabricius) (Heteroptera: Coreidae), on host-searching of the egg parasitoid, *Gryon pennsylvanicum* (Ashmead) (Hymenoptera: Scelionidae). *Applied Entomology and Zoology* 30: 139–144. doi: 10.1303/aez.30.139

Young D (1990) Do cicadas radiate sound through their ear-drums? *Journal of Experimental Biology* 151: 41–56.

Yun J-H, Roh SW, Whon TW, Jung M-J, Kim M-S, Park D-S, Yoon C, Nam Y-D, Kim Y-J, Choi J-H, Kim J-Y, Shin N-R, Kim S-H, Lee W-J, Bae J-W (2014) Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied and Environmental Microbiology* 80: 5254–5264. doi: 10.1128/AEM.01226-14

Zaki FN (1985) Reactions of the egg parasitoid *Trichogramma evanescens* Westw. to certain insect sex pheromones. *Zeitschrift für Angewandte Entomologie* 99: 448–453. doi: 10.1111/j.1439-0418.1985.tb02010.x

Zhang C, Fu X, Zhu Z, Xie K, Guo Y (2014) The complete mitochondrial genome sequence of *Helicophagella melanura* (Diptera: Sarcophagidae). *Mitochondrial DNA*: 1–2. doi: 10.3109/19401736.2014.987258

Zheng B, Agresti A (2000) Summarizing the predictive power of a generalized linear model. *Statistics in Medicine* 19: 1771–1781. doi: 10.1002/1097-0258(20000715)19:13<1771::AID-SIM485>3.0.CO;2-P

Zhong M, Wang X, Liu Q, Luo B, Wu C, Wen J (2014) The complete mitochondrial genome of the flesh fly, *Boettcherisca peregrine* (Diptera: Sarcophagidae). *Mitochondrial DNA*: 1–3. doi: 10.3109/19401736.2013.873925

Zuk M, Kolluru GR (1998) Exploitation of Sexual Signals by Predators and Parasitoids. *The Quarterly Review of Biology* 73: 415–438.

Zuk M, Rotenberry JT, Tinghitella RM (2006) Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biology Letters* 2: 521–524. doi: 10.1098/rsbl.2006.0539

Zuk M, Simmons L, Cupp L (1993) Calling characteristics of parasitized and unparasitized populations of the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology and Sociobiology* 33. doi: 10.1007/BF00172933

Zuk M, Simmons LW, Rotenberry JT (1995) Acoustically-orienting parasitoids in calling and silent males of the field cricket *Teleogryllus oceanicus*. *Ecological Entomology* 20: 380–383. doi: 10.1111/j.1365-2311.1995.tb00471.x

# APPENDIX A

## A NEW LIVE TRAP DESIGN FOR ACOUSTICALLY ORIENTING PARASITOID FLIES

### A.1 Introduction

Ever since the discovery that some species of parasitoid flies are attracted to the acoustic mating calls of their host insects (Cade 1975, Soper et al. 1976), field broadcasts of acoustic signals have been an important means for studying these "acoustic parasitoids". The general strategy is to use one or more loudspeakers to broadcast an audio recording or synthesized signal that mimics the sounds of the host in order to attract searching parasitoids to the sound source. Once flies arrive at the acoustic bait, a variety of techniques can be used to capture them, including simple collecting by hand (e.g., Soper et al. 1976, Fowler and Kochalka 1985, Wagner 1996, Lakes-Harlan et al. 2000, Köhler and Lakes-Harlan 2001, de Vries and Lakes-Harlan 2005, Wagner and Basolo 2007), sticky traps (e.g., Fowler 1987, Walker 1993, Allen 1998, Kolluru and Zuk 2001), electrified wire grids (Mangold 1978, Walker 1986), and custom-built live traps (e.g., Cade 1975, 1979, Fowler 1988, Walker 1989, Allen et al. 1999). However, if the researcher's goals include non-destructive sampling or capturing healthy specimens for further study, then the only viable options are collecting by hand or automatic live traps.

Detailed plans have been published for acoustic live traps designed to catch two species of acoustic parasitoids of the tachinid tribe Ormiini, *Ormia ochracea* (Bigot) (Cade 1979, Walker 1989) and *O. depleta* (Wiedemann) (Fowler 1988). All of these traps use the same basic principle of a box with an internal loudspeaker at one end and one or more inverted funnels or slits that allow a fly easy access to the interior of the box but make it difficult for the fly to find its way back out. The slit trap design of Walker (1989) (or variations on his design) has been the most widely used, usually for capturing *O. ochracea* but also, in at least one case, for collecting

an unidentified species of the sarcophagid genus *Emblemasoma* (e.g., Walker 1993, Gray and Cade 1999, Gray et al. 2007, Farris et al. 2008, Vincent and Bertram 2010).

In all of these designs, the entrances to the funnels or tapered slits leading to the interior of the trap occupy a rather small portion of the outer area of the trap (in Fowler's most successful design, for instance, the entrances to the funnels leading to the trap's interior account for only about 3% of the outer surface area) or are restricted to only one side of the trap, as with Cade's and Walker's designs. As a consequence, flies that land on the exterior surface of the trap might have to spend considerable time exploring the trap's exterior before they find a surface that leads them directly to the trap's interior and the broadcasting loudspeaker. This evidently works well for some ormiine tachinid species, such as *Homotrixa alleni* Barraclough and *O. ochracea*, because these species will often spend several minutes at a sound source searching for possible larviposition sites (Cade 1975, Walker 1989, Allen et al. 1999).

During the summer of 2010, I began a series of field studies of the sarcophagid acoustic parasitoid *Emblemasoma erro* Aldrich, which parasitizes *Neotibicen dorsatus* and other species of cicadas (Hemiptera: Cicadidae) in the central United States (Stucky 2015). I initially experimented with attracting flies to a loudspeaker and collecting them by hand, and although this was an effective way to obtain live flies, I eventually also needed a means of trapping flies that did not depend on the fly-catching skills of the researcher.

After observing the behaviors of many *E. erro* that were attracted to a loudspeaker in the field, I concluded that existing acoustic live trap designs for ormiine tachinids were unlikely to work well for *Emblemasoma erro*. Unlike *O. ochracea*, which often remains at a sound source for several minutes, many of the *E. erro* that I observed made an initial phonotactic flight to the loudspeaker, landed near it, quickly walked towards the sound source, then departed only a few

seconds later. In cases where the flies remained on the box for longer periods of time, they often engaged in very little or no exploratory walking after their initial landing and approach towards the speaker. Consequently, traps that require the flies to persistently search the trap's exterior surface in order to find an entrance appeared to be unsuitable for *E. erro*'s typical phonotactic behavior. Furthermore, the most widely used acoustic trap design, that of Walker (1989), has its only entrance at the top of the trap box with the loudspeaker at the bottom. Thus, any flies landing on the side of the trap do not have a direct route toward the loudspeaker and the trap's interior. They must instead walk up the side of the trap, away from the sound source, in order to find the entrance at the top. I noticed that many *E. erro* seemed to approach the loudspeaker from the sides rather than from directly above, indicating they would be likely to land on the sides of a trap. This, in combination with their observed behavior after landing near a speaker, suggested that a trap with only a top entrance would perform poorly for *E. erro*.

To address these concerns, I developed a new acoustic live trap that was specifically designed for flies such as *Emblemasoma erro* whose behaviors at a sound source differ considerably from *Ormia ochracea*. This paper describes the design and construction of this trap and discusses its effectiveness in capturing *E. erro*.

## A.2 Methods

### *Trap design*

Considering the phonotactic behavior of *E. erro* as observed during field broadcasts of cicada calls in 2010, along with a desire to make the traps easy to deploy and useful for a variety of applications, I identified four major design goals for the traps.

1. To minimize the time flies must spend searching the exterior of the trap, a large percentage of the outer surface of the trap box should be occupied by the funnels or slits that channel the flies directly to the trap's interior.
2. As much as possible, flies that land on the trap should not have to walk further from the sound source in any direction in order to find an entrance to the trap's interior; that is, from nearly all positions on the trap's surface, the most direct route to the trap's interior should also lead the fly directly toward the speaker.
3. The trap should follow a modular design that allows the loudspeaker component to be easily used by itself for manually collecting flies by hand.
4. The trap should be lightweight for easy portability and inexpensive to construct.

The first two goals were perhaps the most important, because they were intended to minimize the time required for a fly to find and pass through an entrance to the interior of the trap, thus maximizing trap efficiency for flies such as *E. erro* that often leave a sound source shortly after making their initial approach.

***Testing the trap***

The initial trap design was completed during the summer of 2011, and extensive field observations of the trap's performance were made during that time to fine-tune aspects of the design. This initial work was conducted primarily at field sites in McPherson County in central Kansas.

The most unique feature of the final trap design was the inclusion of oblique entrance cones on all vertical trap surfaces in addition to the top entrance found in most other designs. The side entrances were also the most complex aspect of trap construction. To assess the effectiveness of this design, a series of tests was completed in August of 2012 to evaluate

whether flies showed a preference for the side or top entrances of the trap. For each test, either 1 or 3 traps were deployed at field sites in Geary, McPherson, and Reno counties in central Kansas where the presence of *E. erro* had previously been documented. Cicada calling songs were broadcast from the traps for between 8 to 30 minutes. During the broadcast, all flies that entered the traps were carefully observed, and whether each fly entered from the top or one of the sides was recorded. To prevent pseudoreplication caused by counting single flies more than once, no captured flies were released until testing was completed at a field site.

The count data from this experiment were analyzed using an exact two-tailed binomial test. Confidence intervals (CIs) were constructed using the method of Wilson (Wilson 1927, Agresti and Coull 1998). Statistical analyses were conducted using R version 3.1.3 (R Core Team 2015).

## A.3 Results

### *Description of the trap*

The final, complete trap is illustrated in Figures A.1 and A.2. The trap consists of two main parts. The "speaker box" is a simple wooden box with an upward-facing loudspeaker mounted in the middle of the top face and a piece of aluminum window screening covering the aperture of the speaker to prevent flies from contacting the loudspeaker's components. The second part is a "trap box" that fits over the top of the speaker box and is described in more detail below. Both the speaker box and the trap box were constructed with 1/4" plywood for their outer surfaces and 1.9 cm × 1.9 cm (0.75" × 0.75") wooden braces for the internal frame. At least two coats of varnish were applied to all exterior wood surfaces.

PylePro model PH44 loudspeakers were used for trap design and testing, but most loudspeakers with an appropriate frequency response range should work for the speaker box.

For insect sounds, so-called “tweeter” speakers usually provide the best frequency response, and horn-loaded loudspeakers are often a good choice for field use due to their efficiency. Tweeters should generally be used with a high-pass filter of some sort (such as an in-line capacitor) to avoid audio distortion or speaker damage caused by low frequencies.

The trap box is an approximate cube with extensions on the bottom to fit securely over the speaker box. Each vertical side of the trap box has an oblique circular screen cone leading toward the loudspeaker, and the top of the trap box has a right circular screen cone leading directly downward toward the center of the loudspeaker. The cones were constructed from aluminum window screen stitched together along the seams with braided fishing line.

**Figure A.1** The complete trap with the loudspeaker box on the bottom, trapping unit above the loudspeaker box, and holding jar assembly on top.

**Figure A.2** The live trap deployed in the field. Captured flies are visible in the holding jar assembly at the top of the trap.

Custom-written software was used to generate two-dimensional patterns for cutting the cones out of sheets of window screen; the patterns are provided as supplementary data for this paper in Scalable Vector Graphics (SVG) format (http://www.w3.org/Graphics/SVG/). Once complete, the cones were attached to the interior of the trap box with clear silicone caulk.

The top of the trap box has a jar assembly for holding captured flies similar in design to that of Walker (1989). This was constructed from two 8 fluid ounce (≈ 0.24 liter) clear polystyrene plastic jars with a window screen cone in the mouth of the bottom jar. Hot melt glue was used to attach the two jars together and to affix the entire unit to the top of the trap box.

***Test results***

A total of 85 flies were observed entering the traps during the design performance tests. Of these, 81 (95.3%) entered through one of the side cones while 4 entered from the top. If flies have no preference for either the sides or the top and instead choose a trap entrance randomly, then the expected probability of a fly entering through any one of the cones would be 0.2 (the opening diameter of all five entrance cones are the same). Under this null model, the probability of entering through the top is 0.2 and the total probability of entering through any one of the sides is 0.8. A two-tailed binomial test of the data rejected this null hypothesis ($p < 0.001$), indicating that flies preferentially enter the trap through the sides rather than the top (95% CI for the proportion of flies that enter through one of the sides: 0.885 – 0.982).

## A.4 Discussion

This new trap includes two design elements that set it apart from previous acoustic traps. First, unlike other trap designs, a large portion of the external area of the trap box is occupied by the outer entrances of the cones leading to the trap's interior (just over 46%). Second, the oblique cones on the sides give flies direct paths to the loudspeaker from most locations on the outside of the trap box. Together, these two features were intended to allow *E. erro* to rapidly locate an entrance to the trap's interior simply by moving toward the sound source. Observations in the field confirmed that many flies were able to access the interior of the trap within a few seconds of their initial arrival.

The field tests also verified the importance of the side entrances in the trap design, with *E. erro* showing a strong preference for entering the trap through the sides. Fowler (1988) observed a similar pattern in his tests of trap designs for the tachinid *Ormia depleta*. Top-entrance-only traps have been proven very effective for capturing *O. ochracea* (Walker

1989), but these results suggest that they would not work as well for *E. erro*. It should be noted, though, that the top opening in Walker's slit trap design differs from the funnel-shaped opening tested here, and it would be interesting to test whether different shapes have an effect on trap efficiency.

Two other advantages of this trap design are its light weight and modularity. The mean combined mass of the speaker box and trap box (not including the mass of the speaker and mounting hardware, which depends on the speaker brand and model) for three complete traps was 2.88 kg (6.35 pounds), so the traps are quite portable. The modular design of the trap, with separate speaker box and trap box components, makes the speaker box convenient to use by itself for manual collecting, simple presence/absence surveys, or behavioral observations.

Perhaps the most serious drawback to this trap design is its relative complexity. The presence of five entrance funnels makes this trap considerably more labor-intensive to construct than designs with only a single top entrance. Nevertheless, the use of multiple entrances clearly improves the trap's efficiency, so the extra labor seems to be justified.

Another potential problem is that five entrances, rather than one, might make it easier for flies to escape from the trap simply because they have more places to get out. In my use of these traps over the past few years, on at least one occasion a fly that had been in the holding jars at the top of the trap managed to find its way back down into the trap box and out of the trap. Many hours spent observing trap performance suggest that such losses are quite rare, though. I also never operated the traps for more than one hour before removing the captured flies, which might have helped minimize the number of escapes. The probability of a fly escaping would surely increase the longer the traps are left unattended.

If the traps are to be operated unattended for long periods of time (e.g., more than an hour or two), then a more spacious holding jar assembly at the top of trap would be advisable. Flies that entered the trap usually found their way into the holding jars after a few minutes and seldom returned to the main trap box, so the system worked as intended, but when many flies arrived at the trap, the jar assembly could become rather crowded. Crowding appeared to agitate the flies and increase the chances that they found their way back into the main trap box, possibly increasing the chances of an escape.

Finally, it should be noted that despite this trap's overall effectiveness, it certainly does not catch all of the *E. erro* that are attracted to the loudspeaker. I observed many flies that either landed on the outside of the trap and then failed to move closer to the sound source or simply left before they found one of the trap's entrances. Both Fowler (1988) and Walker (1989) reported similar results when testing their trap designs. Because flies that initially leave the trap sometimes make one or more return visits (B. Stucky, personal observation), it is difficult to accurately estimate the percentage of flies attracted to the trap that ultimately evade capture. Regardless, flies that leave before entering the trap often depart only a few seconds after arrival, and it is not obvious how the trap design could be further improved to more consistently capture such brief visitors.

Overall, though, this new trap design appears to be relatively efficient for capturing *E. erro*. Traps built from this design are lightweight, portable, and can be constructed from inexpensive components. Furthermore, my use of these traps over the last several field seasons demonstrates their suitability for routine field work. After dozens of hours of use, these traps have so far required virtually no maintenance, and I have used them to capture hundreds of individuals of *E. erro* from a variety of habitats. As a next step, it would be useful to assay the

performance of this trap for species of acoustic parasitoids besides *E. erro*. Tests with *O. depleta* would be especially interesting since this species proved difficult to capture with previous live trap designs (Fowler 1988).

# APPENDIX B

## GENBANK ACCESSION NUMBERS AND REFERENCES FOR DNA SEQUENCE DATA

**Table B.1** GenBank accession numbers and literature references for all previously published DNA sequences used for the phylogenetic analysis in Chapter 6.

| | Gene | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Taxon** | **CO1** | **CytB** | **12S** | **16S** | **18S** | **28S** | **EF1a** | **References** |
| *Argoravinia rufiventris* | GQ409315.1 | GQ409385.1 | GQ409053.1 | – | GQ409171.1 | GQ409230.1 | GQ409451.1 | 1 |
| *Blaesoxipha plinthopyga* | AF259514.1 | GQ409387.1 | GQ409055.1 | GQ409120.1 | GQ409173.1 | GQ409232.1 | GQ409453.1 | 1, 2 |
| *Boettcheria cimbicis* | GQ223324.1 | GQ409388.1 | GQ409057.1 | – | GQ409174.1 | GQ409234.1 | GQ409455.1 | 1, 3 |
| *Dexosarcophaga transita* | GQ409322.1 | GQ409393.1 | – | GQ409126.1 | GQ409177.1 | GQ409236.1 | – | 1 |
| *Engelimyia inops* | GQ409324.1 | GQ409395.1 | GQ409063.1 | GQ409128.1 | GQ409179.1 | GQ409238.1 | – | 1 |
| *Helicobia pilipleura* | GQ409329.1 | GQ409399.1 | GQ409066.1 | GQ409131.1 | GQ409182.1 | GQ409242.1 | GQ409464.1 | 1 |
| *Helicobia rapax* | GQ223319.1 | GQ409400.1 | GQ409067.1 | – | GQ409183.1 | GQ409243.1 | GQ409465.1 | 1, 3 |
| *Helicobia resinata* | GQ223334.1 | GQ409401.1 | GQ409068.1 | – | GQ409184.1 | GQ409244.1 | – | 1, 3 |
| *Lepidodexia latifrons* | GQ409323.1 | GQ409394.1 | GQ409062.1 | GQ409127.1 | GQ409178.1 | GQ409237.1 | GQ409460.1 | 1 |
| *L.* (*Notochaeta*) sp. | GQ409339.1 | GQ409407.1 | GQ409076.1 | GQ409137.1 | GQ409192.1 | GQ409252.1 | GQ409473.1 | 1 |
| *Nyctia lugubris* | GQ409340.1 | GQ409408.1 | GQ409077.1 | GQ409138.1 | GQ409193.1 | GQ409253.1 | GQ409474.1 | 1 |
| *Peckia alvarengai* | GQ409342.1 | GQ409410.1 | GQ409079.1 | GQ409139.1 | – | GQ409254.1 | – | 1 |
| *Peckia chrysostoma* | GQ223333.1 | GQ409411.1 | GQ409080.1 | – | GQ409195.1 | GQ409255.1 | – | 1, 3 |
| *Peckia intermutans* | GQ223335.1 | GQ409413.1 | GQ409082.1 | – | GQ409196.1 | GQ409256.1 | GQ409475.1 | 1, 3 |
| *Peckia lambens* | GQ409357.1 | GQ409424.1 | GQ409093.1 | GQ409148.1 | – | GQ409269.1 | – | 1 |
| *Peckia uncinata* | GQ223332.1 | GQ409414.1 | GQ409083.1 | GQ409141.1 | GQ409197.1 | GQ409257.1 | – | 1, 3 |
| *Peckiamyia minutipenis* | GQ409347.1 | GQ409415.1 | GQ409084.1 | GQ409142.1 | GQ409198.1 | GQ409258.1 | GQ409476.1 | 1 |
| *Pterella nigrofasciata* | GQ409353.1 | GQ409421.1 | GQ409090.1 | – | GQ409204.1 | GQ409265.1 | GQ409480.1 | 1 |
| *Ravinia pernix* | KM676414.1 | KM676414.1 | KM676414.1 | KM676414.1 | – | – | – | 4 |

| Taxon | Gene | | | | | | | References |
|---|---|---|---|---|---|---|---|---|
| | CO1 | CytB | 12S | 16S | 18S | 28S | EF1a | |
| *Sarcophaga africa* | KM881633.1 | KM881633.1 | KM881633.1 | KM881633.1 | – | – | – | 5 |
| *Sarcophaga arizonica* | FJ025655.2 | FJ025749.1 | FJ025413.1 | FJ025473.1 | FJ025498.1 | FJ025559.1 | FJ025696.1 | 6 |
| *Sarcophaga australis* | GQ409360.1 | GQ409427.1 | GQ409096.1 | GQ409151.1 | – | GQ409270.1 | GQ409483.1 | 1 |
| *Sarcophaga bullata* | KC192975.1 | KC177598.1 | KC177482.1 | KC177446.1 | KC177314.1 | KC177829.1 | – | 7 |
| *Sarcophaga crassipalpis* | JQ582121.1 | GQ409428.1 | GQ409097.1 | GQ409152.1 | GQ409208.1 | GQ409271.1 | GQ409484.1 | 1, 8 |
| *Sarcophaga fertoni* | GQ409362.1 | GQ409429.1 | GQ409098.1 | GQ409153.1 | GQ409209.1 | GQ409272.1 | GQ409485.1 | 1 |
| *Sarcophaga forma* | GQ409363.1 | GQ409430.1 | GQ409099.1 | GQ409154.1 | GQ409210.1 | GQ409273.1 | GQ409486.1 | 1 |
| *Sarcophaga impatiens* | JN859549.1 | JN859549.1 | JN859549.1 | JN859549.1 | – | – | – | 9 |
| *Sarcophaga marshalli* | JQ582059.1 | GQ409431.1 | | GQ409155.1 | GQ409211.1 | GQ409274.1 | – | 1, 8 |
| *Sarcophaga melanura* | KP091687.1 | KP091687.1 | KP091687.1 | KP091687.1 | – | – | – | 10 |
| *Sarcophaga omikron* | GQ409365.1 | GQ409432.1 | GQ409100.1 | GQ409156.1 | GQ409212.1 | GQ409275.1 | GQ409487.1 | 1 |
| *Sarcophaga peregrina* | KF921296.1 | KF921296.1 | KF921296.1 | KF921296.1 | – | – | – | 11 |
| *Sarcophaga portschinskyi* | KM287570.1 | KM287570.1 | KM287570.1 | KM287570.1 | – | – | – | 12 |
| *Sarcophaga similis* | KM287431.1 | KM287431.1 | KM287431.1 | KM287431.1 | – | – | – | 13 |
| *Sarcophaga triplasia* | GQ223317.1 | GQ409433.1 | GQ409102.1 | – | GQ409214.1 | – | GQ409488.1 | 1, 3 |
| *Sarcophila meridionalis* | JQ686223.1 | GQ409434.1 | GQ409103.1 | – | GQ409215.1 | GQ409277.1 | GQ409489.1 | 1, 8 |
| *Sarcophila sp.* | GQ409369.1 | GQ409435.1 | GQ409104.1 | GQ409157.1 | GQ409216.1 | GQ409278.1 | – | 1 |
| *Sinopiella rufopilosa* | GQ409370.1 | GQ409436.1 | GQ409105.1 | GQ409158.1 | GQ409217.1 | GQ409279.1 | – | 1 |
| *Sphenometopa claripennis* | GQ409371.1 | GQ409437.1 | GQ409106.1 | GQ409159.1 | GQ409218.1 | – | GQ409490.1 | 1 |
| *Spirobolomyia flavipalpis* | GQ409372.1 | GQ409438.1 | GQ409107.1 | GQ409160.1 | GQ409219.1 | GQ409280.1 | GQ409491.1 | 1 |
| *Taxigramma multipunctata* | GQ409377.1 | GQ409444.1 | GQ409112.1 | – | GQ409224.1 | GQ409284.1 | – | 1 |
| *Titanogrypa luculenta* | GQ223331.1 | GQ409445.1 | GQ409113.1 | GQ409165.1 | GQ409225.1 | GQ409287.1 | GQ409495.1 | 1, 3 |
| *Villegasia postuncinata* | GQ409381.1 | GQ409448.1 | GQ409116.1 | GQ409168.1 | GQ409228.1 | GQ409289.1 | GQ409497.1 | 1 |
| *Wohlfahrtia nuba* | GQ409383.1 | GQ409449.1 | GQ409117.1 | GQ409169.1 | GQ409229.1 | – | – | 1 |
| *Wohlfahrtia trina* | GQ409384.1 | GQ409450.1 | GQ409118.1 | GQ409170.1 | – | – | GQ409498.1 | 1 |

| Taxon | Gene | | | | | | | References |
|---|---|---|---|---|---|---|---|---|
| | CO1 | CytB | 12S | 16S | 18S | 28S | EF1a | |
| *Calliphora vomitoria* | KF918996.1 | FJ025705.1 | FJ025365.1 | JQ246722.1 | FJ025482.1 | AJ300134.1 | FR719220.1 | 6, 14, 15, 16 |
| *Chrysomya megacephala* | AJ426041.2 | AJ426041.2 | AJ426041.2 | AJ426041.2 | FJ025483.1 | FR719281.1 | FR719225.1 | 6, 14, 17 |
| *Lucilia sericata* | AJ422212.1 | AJ422212.1 | AJ422212.1 | AJ422212.1 | KC413865.1 | FJ650537.1 | FR719257.1 | 14, 17, 18, 19 |
| *Pollenia rudis* | JX913761.1 | JX913761.1 | JX913761.1 | JX913761.1 | GQ409202.1 | AJ558192.1 | FR719265.1 | 1, 14, 20, 21 |

References for Table B.1: **1)** Kutty et al. 2010, **2)** Wells et al. 2001, **3)** Stamper et al. 2013, **4)** Guo et al. 2014, **5)** Fu et al. 2014, **6)** Kutty et al. 2008, **7)** Wiegmann et al. 2011, **8)** Jordaens et al. 2013, **9)** Nelson Cameron et al. 2012, **10)** Zhang et al. 2014, **11)** Zhong et al. 2014, **12)** Shi et al. 2014, **13)** Yan et al. 2014, **14)** McDonagh and Stevens 2011, **15)** Marinho et al. 2012, **16)** Sonet et al. 2013, **17)** Stevens et al. 2008, **18)** DeBry et al. 2010, **19)** Yun et al. 2014, **20)** Stevens 2003, **21)** Nelson Lambkin et al. 2012.

## APPENDIX C

LARVAL FEEDING STRATEGIES OF SARCOPHAGID FLIES

This appendix provides summaries of the known larval feeding strategies of all of the sarcophagid species included in the phylogenetic analysis of Chapter 6. These summaries were primarily derived from an extensive literature review; see Chapter 6 for a detailed description of the review methods.

### Subfamily Miltogramminae

***Pterella nigrofasciata* (Rohdendorf)**

At least three species of *Pterella* – *P. convergens* (Pandellé), *P. grisea* (Meigen), and *P. melanura* (Meigen) – have been reported as cleptoparasites of Hymenoptera (Pape 1987, Povolný and Verves 1997, Tormos et al. 2000), but I was unable to locate any records for *P. nigrofasciata*.

***Sphenometopa claripennis* (Villeneuve)**

At least two Nearctic species of *Sphenometopa* – *S.* "sp. nr *nebulosa* (Coquillett)" and *S. tergata* (Coquillett) – have been reported as cleptoparasites of Hymenoptera (Evans 1987, Spofford et al. 1989), but I was unable to locate any records for the Palaearctic *S. claripennis*.

### Subfamily Paramacronychiinae

***Nyctia lugubris* (Macquart)**

*Nyctia lugubris* has often been considered synonymous with *N. halterata* (Panzer) (e.g., Povolný and Verves 1997), and nearly all available literature records for *Nyctia* refer to *N. halterata*. *N. halterata* has repeatedly been reported as a parasitoid of snails (Pape 1987, 1996,

Povolný and Verves 1997, Coupland and Barker 2004) or weevils (Smith 1989), but none of these sources provided enough detail to confirm the status of this species as a parasitoid. Pape (1987, 1996) mentioned several non-English references that I did not examine.

***Sarcophila meridionalis* Verves**

Pape (1996) stated that *Sarcophila* includes "scavengers and insect predators", but I was unable to locate relevant information for *S. meridionalis*. The Food and Agriculture Organization of the United Nations' AGRIS database (http://agris.fao.org) includes an abstract for a paper by F. Haqiqiyan titled "The study on biology of *Sarcophila meridionalis* (Diptera: Sarcophagidae) parasitoid of *Bradyporus latipes* (Orthoptera: Tettigoniidae) in Chaharmahal & Bakhtiary province", which suggests that this species is a parasitoid. The AGRIS record did not include any additional publication details, and I was unable to obtain Haqiqiyan's full paper.

***Wohlfahrtia nuba* (Wiedemann)**

Larvae of this species have been reared from vertebrate carrion (Tantawi et al. 1996, Mazen and Aal 2007), and *W. nuba* evidently is also a facultative parasite of vertebrates, having been reared from wounds on domestic livestock (El-Azazy and El-Metenawy 2004).

***W. trina* (Wiedemann)**

*W. trina* has been reared from vertebrate carrion (Mazen and Aal 2007). Hall et al. (2009) stated that *W. trina* is a "facultative parasite" but provided no further details.

## Subfamily Sarcophaginae

***Blaesoxipha* (*Acanthodotheca*) sp.**

There is considerable evidence that members of the subgenus *Acanthodotheca* are, in general, parasitoids that mostly use beetles as hosts. Pape (1994) reported rearing data for 14 of

the 71 described species, and these records suggest all 14 species are parasitoids, with 13 using beetles as hosts and 1 using cockroaches. Reeves et al. (2000) provided rearing data for *B.* (*A.*) "spec. near *enotah* Pape", a species with no rearing data in Pape (1994), that suggest it is also a parasitoid of beetles. Reeves et al. also reported 5 specimens of *B.* (*A.*) *dampfi* (Hall) that were reared "from a dead rodent" in Costa Rica; this was the only definite scavenging record I found for any species of *Acanthodotheca*.

The specimen of *Acanthodotheca* included in the phylogenetic analysis could not be definitively identified to species, but it was most similar to *B.* (*A.*) *prohibita* (Aldrich) and *B.* (*A.*) *savoryi* (Parker). *B. prohibita* is clearly a parasitoid of beetles; females of this species have even been observed larvipositing on live beetles (Davis 1919, Hall 1929, Pape 1994). Much less information is available about *B. savoryi*, but Pape (1994) suggested that this species is also a beetle parasitoid.

***Blaesoxipha* (*Gigantotheca*) *impar* (Aldrich)**

*B. impar* has been reared on several occasions as a scavenger of vertebrate and invertebrate carrion (Aldrich 1916, Graenicher 1931, Roberts 1933b, Pape 1994). There are also multiple reports of this species developing as a true parasitoid on a variety of hosts (Aldrich 1916, Hall 1929, Pape 1994), at least some of which include sufficient detail to make scavenging unlikely as an alternative explanation.

***Blaesoxipha* (*Gigantotheca*) *plinthopyga* (Wiedemann)**

A large number of rearing records leave no doubt that *B. plinthopyga* is a scavenger of vertebrate and insect carrion (Aldrich 1916, Graenicher 1931, Roberts 1934, Spencer and Buckell 1957, Lopes 1973, Denno and Cothran 1976, Pape 1994, Wells and Greenberg 1994). *B. plinthopyga* can also sometimes act as a facultative parasite of mammals, usually by infesting

previous wounds (Roberts 1933a, Laake et al. 1936, James 1947). James (1947) stated that *B. plinthopyga* larvae can develop "as parasites in the bodies of insects", but he provided no references or further details, and I have not seen any evidence to corroborate this claim.

***Blaesoxipha* (*Kellymyia*) *kellyi* (Aldrich)**

All available evidence suggests that *B. kellyi* is exclusively a parasitoid under natural conditions. *B. kellyi* has been reared many times from a variety of grasshopper species and is considered to be beneficial in controlling agriculturally significant grasshopper outbreaks (Kelly 1914, Aldrich 1915, 1916, Smith 1915, 1940, Hall 1929, Criddle 1931, Hayes and DeCoursey 1938, Buckell 1945, Buckell and Spencer 1945, Spencer and Buckell 1957, Rees 1973, Hewitt and Rees 1974, Pape 1994, Sanchez and Onsager 1994). Kelly (1914) provided a detailed account of the larviposition behaviors of this fly. Rearing records strongly suggest that grasshoppers are the most important host insects, but *B. kellyi* has also been reared from several species of beetle (Aldrich 1916, Pape 1994).

***Boettcheria cimbicis* (Townsend)**

There are a large number of literature records documenting rearings of *B. cimbicis* from insects (Aldrich 1916, Hayes 1917, Davis 1919, Sherman 1920, Breakey 1929, Crumb 1929, Hall 1929, Hallock 1929, 1940a, Decker 1931, 1932, 1935, Smith et al. 1943, Rees 1973, Anderson and Kaya 1976), but many of these lack sufficient detail to determine whether *B. cimbicis* acted as a scavenger or a parasitoid. The best evidence that *B. cimbicis* is a true parasitoid comes from Hall (1929) and Anderson and Kaya (1976, with methods described in Anderson and Kaya 1974). In addition, the larviposition preferences of this fly suggest it is not a scavenger. Hallock (1940a) found that female *B. cimbicis* would not larviposit on dead insects, and Dahlem and Downes (1996) reported that female *B. cimbicis* do not voluntarily larviposit on

beef. It should also be noted that although Kelly (1914) reported observing female *B. cimbicis* larvipositing on live grasshoppers, Aldrich (1915) later determined that these specimens were misidentified.

***Emblemasoma auditrix* (Shewell)**

*E. auditrix* is well documented as an acoustically orienting, specialist parasitoid of the cicada *Okanagana rimosa* (Say) (Soper et al. 1976, Lakes-Harlan et al. 2000, Schniederkötter and Lakes-Harlan 2004).

***Emblemasoma emblemasoma* (Dodge)**

No information about the biology of this Central American species has previously been published. During field research in 2013 at Barro Colorado Island in Panama, I confirmed that *E. emblemasoma* is a parasitoid of cicadas by rearing it from individuals of several host cicada species that had been naturally parasitized in the field, primarily *Fidicina mannifera* (Fabricius). I also confirmed that female *E. emblemasoma* are attracted to the sounds of cicada mating calls.

***Emblemasoma erro* Aldrich**

As discussed in Chapter 6, *Emblemasoma erro*, as currently recognized, might actually be a complex of species. There is no question that *E. erro* from the central Great Plains are acoustic parasitoids of cicadas (Chapters 2 and 4, Stucky 2015). At all other localities where I observed or collected *E. erro*, female flies exhibited a strong, positive phonotactic response to broadcasts of cicada calls, and in some cases, flies were directly observed attacking calling male cicadas (Chapter 4 and B. Stucky, unpublished data). Thus, there can be little doubt that these flies are acoustically orienting parasitoids of cicadas, regardless of whether the taxon represents one or multiple species.

***Emblemasoma faciale* Aldrich**

No rearing records are available for *E. faciale*. However, at multiple field sites in Florida and Georgia, I confirmed that female *E. faciale* are attracted to broadcasts of cicada calls, and I also observed flies respond to calling male cicadas. *E. faciale* is undoubtedly an acoustic parasitoid of cicadas.

***Emblemasoma* sp. from Utah**

I was only able to collect females of this species and so, without male terminalia to examine, I could not determine its specific identity. The chaetotaxy of the specimens excluded the possibility that they were allied with the species formerly placed in *Colcondamyia*. This species was associated in the field with the cicada *Okanagana utahensis* Davis. Mature sarcophagid larvae emerged from several male *O. utahensis* that I collected alive in the field, and DNA sequence data obtained form one of these larvae confirmed that it was the same species as the unidentified adult *Emblemasoma* collected at the same locality. Therefore, this species is a parasitoid of cicadas.

***Engelimyia inops* (Walker)**

Lopes (1974a) successfully reared this species in the laboratory using human feces as a larval food source, but its feeding habits in the wild are evidently unknown (Pape and Mello-Patiu 2006).

***Helicobia pilipleura* Lopes**

The larval feeding habits of *H. pilipleura* are apparently unknown. Lopes (1973) reported rearing "*H. polipleura* [*sic*] Lopes", but he unfortunately did not indicate the rearing medium. Buenaventura R. et al. (2009) stated that *H. pilipleura* feed on feces and decomposing fish ("han sido halladas alimentándose de heces y de pescado descompuesto") and provided

Marchiori et al. (2000) as a reference, but Marchiori et al. did not identify *H. pilipleura* in their study. Although adult *H. pilipleura* have been collected from vertebrate carrion (Moretti et al. 2008, Barbosa et al. 2009), female *H. pilipleura* might not actually use vertebrate carrion as a larviposition substrate (Moretti et al. 2008).

***Helicobia rapax* (Walker)**

*H. rapax* is a common, widespread species of flesh fly in North America, and there are a large number of rearing records for *H. rapax* in the literature (Kelly 1914, Aldrich 1915, 1916, Hayes 1917, Morrill 1917, Jones 1918, Davis 1919, 1960, Davis and Luginbill 1921, Jones and Wolcott 1922, Howard and English 1924, Bequaert 1925, Greene 1925, Hall 1929, 1933, Plank 1929, Howard 1930, Breakey 1931, Decker 1931, 1935, Friend and Turner 1931, Hinds and Osterberger 1931, Ingram and Bynum 1932, Roberts 1934, Gilmore 1938, Buckell and Spencer 1945, Madden and Chamberlin 1945, Spencer and Buckell 1957, Huggans and Blickenstaff 1966, Whiteside et al. 1967, Dodge 1968, Stegmaier 1972, Lopes 1973, Rees 1973, Stark and Harper 1982, Godan 1983, Deisler 1987, Felland 1990, Reeves et al. 2000, Coupland and Barker 2004, Ceryngier et al. 2012). Collectively, these records suggest that *H. rapax* can act as both a scavenger and a true parasitoid with a very broad host range, but as with *Boettcheria cimbicis*, many records lack detail and are more or less ambiguous with regards to the mode of larval feeding. Nevertheless, several of these references provide convincing evidence that *H. rapax* can develop either as a parasitoid of living hosts (Hall 1929, Huggans and Blickenstaff 1966, Whiteside et al. 1967, Felland 1990) or as a scavenger (Roberts 1934).

***Helicobia resinata* (Hall)**

I did not locate any information about the larval feeding habits of *H. resinata*.

***Lepidodexia* (*Duckemyia*) *latifrons* (Kano and Lopes)**

I did not locate any information about the larval feeding habits of *L. latifrons*.

***Lepidodexia* (*Notochaeta*) sp.**

Lopes (1969a, 1973) suggested that all species of the subgenus *Notochaeta* might be parasitoids. However, Pape and Dahlem (2010) emphasized that the biology of species of *Lepidodexia* are, in general, very poorly known, so it would probably be premature to draw conclusions about the biology of an undetermined species of *Notochaeta*.

***Oxysarcodexia ventricosa* (van der Wulp)**

*O. ventricosa* is a coprophage that commonly scavenges on the dung of cattle and other large mammals (Aldrich 1915, 1916, Wilson 1932, Mohr 1943, Sanders and Dobson 1966, Figg et al. 1983, Blume 1986). Luginbill (1928) cited a correspondence with E. A. McGregor reporting that a single *O. ventricosa* was reared "from a pupa of the fall army worm at Batesburg, S. C., during 1912." No further details about this supposed rearing were provided, and given all other records for this species, it seems likely that the specimen in question was either misidentified or mistakenly associated with the presumed host.

***Peckia* (*Peckia*) *chrysostoma* (Wiedemann)**

This species is well documented as a scavenger of vertebrate carrion, invertebrate carrion, and mammalian excrement (Roberts 1934, Dodge 1968, Lopes 1969b, 1973, D'Almeida 1993, 1994, Méndez and Pape 2002, Marchiori et al. 2003, Moretti et al. 2008, Gomes et al. 2009). I did not find convincing evidence that *P. chrysostoma* naturally adopts other feeding strategies. Cleare (1926) reported specimens of *P. chrysostoma* that were reared from pupae of the moth *Mocis repanda* (Fabricius) and suggested that *P. chrysostoma* might help control outbreaks of *M. repanda*, but insufficient detail was provided to evaluate whether *P. chrysostoma* was a

scavenger or parasitoid in this case. I found no other evidence that *P. chrysostoma* can act as a true parasitoid, and given all other rearing data, it seems probable that Cleare's records can be attributed to *P. chrysostoma* scavenging on dead moth pupae.

***Peckia* (*Peckia*) *uncinata* (Hall)**

I did not locate any information about the larval feeding habits of *P. uncinata*.

***Peckia* (*Sarcodexia*) *lambens* (Wiedemann)**

There are many rearing records for *P. lambens* in the literature, and they suggest that this species has extraordinarily diverse feeding habits. *P. lambens* has been reported as a parasitoid, a scavenger of vertebrate and invertebrate carrion, a scavenger of mammalian feces, and a vertebrate parasite (Aldrich 1915, 1916, 1927, Drake 1920, Greene 1925, Plank 1929, Graenicher 1931, Hall 1933, Madden and Chamberlin 1945, Callan 1946, James 1947, Muma 1954, 1955, Stephens 1962, Harrison 1963, Dodge 1968, Lopes 1969b, 1973, Stegmaier 1972, Temerak and Whitcomb 1984, Deisler 1987, D'Almeida 1993, 1994, Marchiori et al. 2002, Coupland and Barker 2004, Hagman et al. 2005, Fernandes et al. 2009, Bermúdez C. et al. 2010). Although many of these records lack sufficient detail to distinguish between scavenging and parasitoidism, at least some do provide strong evidence that *P. lambens* can develop as a true parasitoid of both insect and amphibian hosts (Aldrich 1916, 1927, Drake 1920, Hagman et al. 2005), and its status as a scavenger is also well documented (Graenicher 1931, Lopes 1973, D'Almeida 1993, 1994, Marchiori et al. 2002).

***Peckia* (*Sarcodexia*) *tridentata* (Hall)**

I did not locate any information about the larval feeding habits of *P. tridentata*.

***Peckiamyia minutipenis* (Hall)**

The larval feeding habits of *P. minutipenis* are evidently unknown. Pape and Dahlem (2010) stated that "at least some species [of *Peckiamyia*] breed in carrion", but did not elaborate further. Coupland and Barker reported *P. expuncta* (Lopes) as a parasitoid of snails, but this conclusion seems to have been based on a misinterpretation of Lopes (1940).

***Ravinia derelicta* (Walker)**

*R. derelicta* has repeatedly been reared as a scavenger of cattle dung, and this species appears to be an obligate coprophage (Aldrich 1915, 1916, Greene 1925, Hall 1929, Knipling 1936, Sanders and Dobson 1966, Watts and Combs 1977, Figg et al. 1983, Blume 1986, Cervenka and Moon 1991).

***Ravinia lherminieri* (Robineau-Desvoidy)**

This species has an exceedingly complicated nomenclatural history, due to decades of confusion about the synonymies of species described by early European authors. This makes interpretation of literature records quite challenging. I briefly describe the major issues here.

The longest-standing difficulties have centered on the application of the names *R. anxia* (Walker), *R. lherminieri* (Robineau-Desvoidy), *R. ochracea* (Aldrich), and *R. querula* (Walker). Aldrich (1930) examined the type specimens for all of these names, and he concluded that *R. lherminieri* was synonymous with *R. anxia*, *R. querula*, and several additional species described by Walker and other taxonomists. Aldrich also concluded that *R. ochracea* was a junior synonym of *R. sueta* (van der Wulp).

Lopes (1946) recognized *R. ochracea* as distinct from *R. sueta* and provided characters to separate these two species. Dodge (1956) further improved the situation by describing characters to separate *R. lherminieri* from *R. querula*, but he mistakenly maintained the synonymy of *R.*

*anxia* with *R. lherminieri*. Dodge did not comment on his opinion of *ochracea*, but he implied in one paragraph (pg 189) that he recognized it as a valid species, and he did not include it in the synonymy of either *R. lherminieri* or *R. querula*.

G. Dahlem finally resolved the status of *R. lherminieri* in the 1980s when he examined the type of *lherminieri* and discovered that the name was synonymous with *R. ochracea*, not *R. anxia* (Wong et al. 2015). Unfortunately, this result was not published until Pape's catalogue (1996), which provided the correct synonymies, but without any explanation or comment.

Putting all of this together leads to the following conclusions about literature records that might pertain to what is now recognized as *R. lherminieri*. Any records for *R. ochracea* prior to Aldrich (1930) should be safe to assign to *R. lherminieri*, and any records for *R. ochracea* after Lopes (1946) are probably also safe to assign to *R. lherminieri*. Records for *R. lherminieri* after Dodge (1956) and before Pape (1996) very likely refer to *R. anxia*. Records for *R. lherminieri* prior to Dodge (1956) probably pertain to either *R. anxia*, *R. querula*, or both. I used these conclusions as guidelines for gathering literature records for *R. lherminieri*.

Rearing records for *R. lherminieri* in the literature document this species as a coprophage that feeds on cattle dung (Aldrich 1916, Greene 1925, Blume 1986). Mohr (1943) reared large numbers of "*Sarcophaga sueta"* from cattle dung in Illinois and also observed females larvipositing on dung. Unfortunately, given the state of the taxonomy at the time of Mohr's study, the flies he observed could have been either *R. sueta* or *R. lherminieri*. However, the geographic distribution of *R. sueta* reported by Pape (1996) does not come close to Illinois, so it is likely that Mohr studied *R. lherminieri*.

***Ravinia pernix* (Harris)**

*R. pernix*, the only Old World representative of the genus *Ravinia*, is a coprophage that feeds on the excrement of large mammals, and possibly is also a scavenger of vertebrate and invertebrate carrion (Bequaert 1925, Mihályi 1965, Kano et al. 1967, Papp 1971, Verves 1980, Pape 1987, Povolný and Verves 1990, 1997, Iwasa et al. 2005, Prado e Castro et al. 2010). However, its status as a scavenger of vertebrate carrion is somewhat questionable, because several studies have found that adult *R. pernix* will visit mammal carcasses but not larviposit on them (Tantawi et al. 1996, Anton et al. 2011).

Whether *R. pernix* can adopt any other larval feeding strategies is not clear. Many authors have stated that *R. pernix* can develop as a parasitoid (Baer 1921, Verves 1980, Smith 1989, Povolný and Verves 1990, 1997, Coupland and Barker 2004), but none of the sources I examined included sufficient detail to confirm this. Pape (1987) also seemed doubtful that *R. pernix* can act as a parasitoid. However, Thompson (1943), Roback (1954), and Povolný and Verves (1997) included additional, non-English references concerning *R. pernix* developing as a parasitoid that I was unable to review.

*R. pernix* has also frequently been described as a predator of other dipteran larvae (Pape 1987, Povolný and Verves 1997, Coupland and Barker 2004, Prado e Castro et al. 2010). These claims all appear to derive from Pickens (1981), but Pickens' study pertained only to *R. anxia* and he did not discuss *R. pernix* at all. Thus, there does not seem to be any strong evidence that *R. pernix* larvae prey on the larvae of other flies. Tawfik et al. (2000) published results suggesting that *R. pernix* can feed as a predator of the snail *Monacha obstructa* (Pfeiffer) in the laboratory, but they provided too little methodological detail to evaluate whether their results correspond with natural larviposition and feeding activities in the field.

***Ravinia vagabunda* (Walker)**

*R. vagabunda* has been reported as a coprophage and has been associated with mammal feces (Roback 1954, Kilpatrick and Schoof 1956, Blume 1985), but none of the sources I examined included sufficient detail to confirm this fly's larviposition and larval feeding habits.

***Sarcophaga* (*Australopierretia*) *australis* (Johnston and Tiegs)**

*S. australis* is a scavenger that feeds on various types of carrion and dung (Johnston and Tiegs 1922, Fuller 1938, Ferrar 1979, Hall and Parmenter 2008). *S. australis* was reported as a grasshopper parasitoid during an outbreak of the Australian plague locust, *Chortoicetes terminifera* (Walker), in the 1930s (Noble 1936, Weddell 1937), but these authors did not provide details of rearing conditions or methods. Fuller (1938), in a very thorough study, demonstrated that *S. australis* was most likely not a parasitoid and that records of its supposed parasitic habits were probably due to scavenging on dead grasshoppers.

***Sarcophaga* (*Bercaea*) *africa* (Wiedemann)**

This common, nearly cosmopolitan species of flesh fly is represented by a large number of rearing records in the literature, and it clearly is an opportunistic scavenger of both carrion and excrement (Parker 1914b, Aldrich 1915, 1916, Knipling 1936, Mihályi 1965, Kano et al. 1967, Early and Goff 1986, Povolný and Verves 1990, 1997, Introna et al. 1998, Bänziger and Pape 2004, Byrd and Castner 2009, Kelly et al. 2009). There is also ample evidence that *S. africa* can act as a facultative vertebrate parasite; adults of this species have been reared from cases of intestinal and wound myiasis in humans and other mammals (Aldrich 1916, Haseman 1917, Dove 1937, James 1947, Udgaonkar et al. 2012).

*S. africa* has repeatedly been reported as a parasitoid of insects or terrestrial molluscs (Webster 1907, Dew 1913, Hinds and Dew 1915, Dean 1916, Baer 1921, Smith et al. 1943,

Berner 1960, 1973, Rees 1973, Povolný and Verves 1997, Coupland and Barker 2004), but many of these records are highly doubtful. In particular, all New World literature records that I examined of *S. africa* developing as a parasitoid either lacked methodological detail or could be traced back to a series of early publications that reported rearing "*S. georgina*" Wiedemann [= *S. africa*] from various insects (Webster 1907, Dew 1913, Hinds and Dew 1915). The early date of these publications (prior to Aldrich's 1916 revision) means the species identifications for these records are questionable, as noted by both Aldrich (1916) and Hall (1929). Consequently, there does not seem to be strong evidence that New World *S. africa* are facultative parasitoids.

In contrast, there is much more convincing evidence that Old World *S. africa* can sometimes develop as parasitoids. Berner's (1960, 1973) records of rearing *S. africa* as a parasitoid of snails in western Europe are especially noteworthy. Baer (1921) reported *S. africa* as a parasitoid of grasshoppers, but provided no methodological details. Thompson (1946, 1950) provides several more non-English references that I did not review.

***Sarcophaga* (*Boettcherisca*) *peregrina* (Robineau-Desvoidy)**

This widespread Old World species is well documented as a scavenger of both vertebrate and invertebrate carrion and mammal excrement (Senior-White 1924, Greene 1925, Senior-White et al. 1940, Hall and Bohart 1948, Kano et al. 1967, Park 1977, Beaver 1986, Bänziger and Pape 2004). It has repeatedly been reared in cases of myiasis of humans and other mammals (Senior-White 1924, Segal et al. 1968, Chigusa et al. 1994, Hatsushika et al. 2002, Lee et al. 2011, Xue et al. 2011), and so can also act as a facultative mammalian parasite.

*S. peregrina* has sometimes been reported as a parasitoid (Johnston and Tiegs 1923, Senior-White 1924, Baranoff 1934, Corbett 1937, Thompson 1944, Xue et al. 2011), but most of these records lack sufficient detail to rule out scavenging on "hosts" that were already dead.

Senior-White's (1924) account of rearing *S. peregrina* from an earthworm certainly suggests that the worm was parasitized, but Senior-White did not state that the worm was definitely alive when collected or that the rearing conditions precluded secondary infestation. Overall, the evidence that *S. peregrina* can act as a facultative parasitoid is not very strong, and considering the other rearing records for this species, it seems likely that most, if not all, cases of supposed parasitoid development can be attributed to scavenging on invertebrate carrion.

### ***Sarcophaga* (*Liopygia*) *crassipalpis* Macquart**

*S. crassipalpis* is, like *S. africa*, another nearly cosmopolitan species that is primarily a scavenger but is also a facultative parasite of humans and other mammals (Greene 1925, Hallock 1942, James 1947, Kano et al. 1967, Povolný and Verves 1990, 1997, Shiota et al. 1990, Oliva 2008, Prado e Castro et al. 2010, da Silva et al. 2014). *S. crassipalpis* larvae might sometimes feed on grasshopper egg pods; see Povolný and Verves (1997) for references.

On a few occasions, *S. crassipalpis* has been reported as a parasitoid of insects. Gee (1930) reared two species of sarcophagid, *S. crassipalpis* and *S. dux* Thomson, from larvae of the moth *Euzora* sp., but it is not clear whether the caterpillars from which *S. crassipalpis* were reared were attacked before or after death. References for two additional non-English sources, which I did not include in this review, can be found in Greathead (1963) and Romera et al. (2003). Both of these sources supposedly report *S. crassipalpis* as a parasitoid of grasshoppers, although Greathead (1963) stated that the record he examined was doubtful.

### ***Sarcophaga* (*Liosarcophaga*) *marshalli* Parker**

Adult *S. marshalli* have been collected at vertebrate carrion (Romera et al. 2003, Arnaldos et al. 2004, Prado e Castro et al. 2010), but I did not locate any information about larval feeding habits.

***Sarcophaga* (*Liosarcophaga*) *portschinskyi* (Rohdendorf)**

Larval *S. portschinskyi* are probably scavengers of carrion (Pape 1987, Povolný and Verves 1990, 1997, Prado e Castro et al. 2010). They have also been reported as parasitoids and as predators of other fly larvae (Lehrer and Luciano 1979, Povolný and Verves 1990, 1997, Coupland and Barker 2004, Prado e Castro et al. 2010). However, none of the sources I reviewed provided enough detail to confirm any of these larval feeding habits. Coupland and Barker (2004) provided several additional references that I did not examine.

***Sarcophaga* (*Liosarcophaga*) *sarracenioides* Aldrich**

There are a large number of rearing records indicating that *S. sarracenioides* can develop both as a scavenger of vertebrate and invertebrate carrion and as a facultative parasitoid of insects, especially grasshoppers (Kelly 1914, Aldrich 1916, Davis 1919, Parker 1919, Treherne and Buckell 1924, Greene 1925, Essig 1926, Hall 1929, Roberts 1934, Hallock 1940a, Buckell and Spencer 1945, Madden and Chamberlin 1945, Spencer and Buckell 1957, Rees 1973, Rogers 1974, Ives 1991, Wells and Greenberg 1994). Female *S. sarracenioides* have been observed larvipositing on live grasshoppers (Kelly 1914, Aldrich 1916).

***Sarcophaga* (*Neobellieria*) *bullata* Parker**

*S. bullata* has been reared many times as a scavenger of vertebrate carrion, invertebrate carrion, and human excrement (Parker 1916, Greene 1925, Hall 1929, Graenicher 1931, 1935, Hallock 1942, Stegmaier 1972, Ives 1991, Wells and Greenberg 1994). *S. bullata* has also been documented as a facultative parasite of wounds in humans and other mammals and has occasionally been implicated in cases of human intestinal myiasis (Dove 1937, Knipling and Rainwater 1937, Watson 1942, James 1947). Davis and Turner (1978) stated that *S. bullata* was

a “documented parasitoid” and cited Rees (1973) to support this claim, but Rees (1973) did not list *S. bullata* as a parasitoid.

***Sarcophaga* (*Neobellieria*) *cooleyi* Parker**

*S. cooleyi* is closely related to *S. bullata* (Giroux and Wheeler 2009), and the larval feeding ecologies of these two species are similar. *S. cooleyi* is primarily a scavenger of carrion and mammal excrement (Parker 1914a, Aldrich 1915, Greene 1925, Denno and Cothran 1976, Giroux and Wheeler 2009), but has also been reared in at least one case of human myiasis (Stewart 1934). Coppel (1960) reported *S. cooleyi* as one of several parasitoid species that were reared from “larval and pupal collections” of the spruce budworm, *Christonuera fumiferana* (Clemens), but no details of the collecting or rearing methods were provided. Given all other rearing records for *S. cooleyi*, it seems likely that the flies reported by Coppel were scavenging on deceased larvae or pupae of *C. fumiferana*.

***Sarcophaga* (*Pandelleisca*) *similis* Meade**

*S. similis* is a scavenger of carrion and possibly also mammal excrement (Tiensuu 1939, Kirchberg 1954, Mihályi 1965, Kano et al. 1967, Hanski 1976, Povolný and Verves 1997, Matuszewski et al. 2015), and has been confirmed as a facultative parasite in cases of human myiasis (Kano et al. 1967, Chigusa et al. 1994, Hatsushika et al. 2002). Multiple authors have stated that *S. similis* is a parasitoid (Povolný and Verves 1990, 1997, Coupland and Barker 2004, Prado e Castro et al. 2010), but none of the sources I examined provided sufficient information to rule out the possibility of scavenging rather than true parasitoidism. Povolný and Verves (1997) referenced several Russian-language sources that I was not able to review.

***Sarcophaga* (*Robackina*) *triplasia* van der Wulp**

I did not locate any information about the larval feeding habits of *S. triplasia*.

***Sarcophaga* (*Sarcorohdendorfia*) *impatiens* Walker**

*S. impatiens* has been reared as a scavenger of vertebrate carrion (Johnston and Tiegs 1922, George et al. 2012).

***Sarcophaga* (*Sarcorohdendorfia*) *omikron* Johnston and Tiegs**

Johnston and Tiegs (1922) reported specimens of *S. omikron* that were "bred from wool" and "from rotten potato", both of which suggest that this species is a scavenger. However, these records did not include sufficient detail to confirm that they represented cases of voluntary larviposition in the field.

***Sarcophaga* (*Wohlfahrtiopsis*) *arizonica* (Townsend)**

Very little information is available about the biology of *S. arizonica*, and the larval feeding ecology of this species is essentially unknown. Knowlton and Janes (1931, see also Dodge 1966) reported that *S. arizonica* is a "dung beetle parasite" but they provided no further details or references to support their claim. Other species of the small subgenus *Wohlfahrtiopsis* are probably scavengers or facultative parasitoids (Dodge 1966, Ives 1991).

***Sinopiella rufopilosa* Lopes and Tibana**

I did not locate any information about the larval feeding habits of *S. rufopilosa*.

***Spirobolomyia flavipalpis* (Aldrich)**

Very few rearing records have been published for *S. flavipalpis* or any other species of *Spirobolomyia*. However, in every case, the flies were reared from large millipedes, presumably of the genus *Narceus* or similar spirobolid genera (Aldrich 1916, Townsend 1917, Pape 1990). These rearing records suggest that *S. flavipalpis* is a specialized parasitoid of millipedes, but none of the records include sufficient detail to definitively rule out scavenging. Nevertheless,

several lines of circumstantial evidence also support the hypothesis that *Spirobolomyia* are parasitoids of millipedes. First, the distribution of *Spirobolomyia* closely corresponds with the known distribution of *Narceus* millipedes (Pape 1990). Second, Eisner et al. (1998) observed that individuals of an undetermined species of *Spirobolomyia* in Florida were attracted to defensive secretions of the millipede *Floridobolus penneri* Causey. Although Lopes (1975) reported that three species of *Spirobolomyia* occur in Florida, Pape (1996) recorded *S. flavipalpis* as the only Florida representative of the genus. It therefore seems likely that the unidentified *Spirobolomyia* observed by Eisner et al. were *S. flavipalpis*.

On the other hand, Knutson and Vala (2011) stated that it was "questionable" that *Spirobolomyia* were millipede parasitoids, but they presented no contrary evidence other than a 2004 correspondence with T. Pape noting that *Spirobolomyia* are sometimes locally abundant, which caused Pape to wonder if they exclusively feed on millipedes. Yet Pape (along with G. Dahlem) later observed that, "The only biological information available suggests that the species [of *Spirobolomyia*] are parasitoids of large spirobolid millipedes" (Pape and Dahlem 2010). Indeed, rearing records, along with biogeographic and behavioral evidence, all suggest that *Spirobolomyia* are specialist parasitoids that primarily attack millipedes.

***Villegasia postuncinata* (Hall)**

*V. postuncinata* is a scavenger that has been reared from rotting snails and crabs (Lopes 1969b, Reeves et al. 2000, Méndez and Pape 2002).