

Is a Corpse a Corpse? Spatial Aspects of Necrophoresis in the Western Harvester Ant,  
*Pogonomyrmex occidentalis*

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# Abstract

Disease poses a threat to any society, and this threat is amplified for societies persisting in cramped quarters over extended periods of time. Highly social (eusocial) insects actively combat the danger of pathogen proliferation with a myriad of tactics, one of which is necrophoresis or the removal of corpses from the nest. This study examines the spatial patterns of the corpse depositions of several wild colonies of the western harvester ant, *Pogonmyrmex occidentalis*. These colonies were presented with nestmate as well as non-nestmate corpses to discern if this type of waste could be treated categorically. Specialized areas for corpse disposal, reported in the literature as “ant graveyards” were not observed, suggesting that observations of such accumulations in the species may be an artifact of laboratory conditions. Non-nestmate corpses were carried further away from the nest than were nestmate corpses, presumably reducing the chance of introduction of foreign pathogens to the colony. Factors external to the nest mound, such as slope and nearby neighbors, had no detectable effect on these depositions and failed to result in anything other than rather uniform dispersal of this particular waste. These findings shed light on the intricacies of a set of behaviors that are critical to the notable ecological success of these organisms. Research in recent years has increased our understanding of necrophoresis, but much remains to be discovered. Corpse removal is proving to be a dynamic activity in the world of eusocial insects, and investigation of this disease mitigation tactic, along with other such tactics employed by eusocial insects will aid in our understanding of topics such as immunity, division of labor, polyethism, and even the evolution of sociality itself.

# Introduction

## Sociality and the hazard of disease

Given that parasites are apt to target specific hosts (Schmid-Hempel, 2011), contact of one form or another among individuals of the same species forms the basis of virtually all infection.

Workers in eusocial insect colonies face this obstacle of disease because they are in constant contact with their colony-mates. Critical colony behaviors often involve repeated and intimate contact between involved individuals and provide ample opportunity for pathogen transmission.

In addition to this direct contact, high density living itself provides opportunity for disease to thrive. Pathogen transmission is potentially amplified in dense populations, and consequently infectious disease poses a great danger to highly social insects when compared to solitary insects (Schmid-Hempel, 1998). Members of these societies are closely related and may share genetic susceptibility to pathogens or parasites. This general relationship between sociality and sanitary risk has been documented in numerous social animals including lizards (Godfrey et al., 2009), birds (Brown and Brown, 1986), and mammals (Young et al., 2015). Disease risk is often amplified for highly social insects in comparison to other animal populations because of their shared nest site with stable temperature and humidity levels favorable for microorganism and parasite establishment. These factors create a situation in which waste management is crucial and threats from pathogens must be managed to prevent epidemic disease in colonies.

## Behaviors that reduce pathogen proliferation in social insects

The ability of eusocial insects to reduce mortality due to pathogens through collective defenses has been integral to their proliferation around the globe (Cremer et al., 2007). Prophylactic

measures and pathogen-specific responses are simultaneously carried out in many social insect colonies. Colony members may forage for antiseptic materials that facilitate pathogen suppression in the nest. For example, honeybees incorporate propolis, a viscous mixture collected from diverse botanical sources that possesses antimicrobial properties, into their nest architecture (Bankova et al., 2000; Wilson et al., 2015). Similarly, some ants collect and store coniferous tree resin to prevent the growth of potentially harmful fungi and bacteria within their nests (Chapuisat et al., 2007; Brüttsch and Chapuisat, 2014). Temporary manipulation of colony environment may also serve to counter spreading infection. Thermoregulation is used to combat pathogens in a coordinated response in honeybees known as social fever, in which individual honeybees within a colony synchronously raise their body temperatures to heat-kill bacteria within their hive (Starks et al., 2000). Altruistic self-removal has even been documented in several social insects in which diseased, dying, or otherwise unhealthy individuals willingly abandon their nest or hive. Some diseased ants leave their colony to die in solitude (Heinze and Walter, 2010; Bos et al., 2012) and honeybee workers of compromised health do the same (Rueppell et al., 2010). Eusocial insects thus employ a myriad of tactics to neutralize threats specific to the colony.

One challenge is universal to sedentary and long-lived groups; colony members die, possibly of infectious disease, within or close to the nest. Many strategies exist to deal with this particular problem. Termites display notably complex strategies in dealing with their dead, which include cannibalism, burial, and avoidance (Chouvenc et al. 2008; Chouvenc et al., 2012; Neoh et al., 2012). In contrast, hymenopterans rely almost exclusively on necrophoresis, or corpse removal, to deal with their dead. Insects such as honeybees that live in nests above ground level need only transport the corpse outside the confines of the nest and drop it to achieve a safe distance between the colony and corpse (Visscher, 1983). Corpse removal in ground-nesting ants is

necessarily more complex as there is no option to simply drop corpses to the ground below. Although there has been limited documentation of instances of cannibalism in some ants (Driessen et al., 1984; Howard and Tschinkel, 1976; Hölldobler and Wilson, 1990), as well as burial (Renucci et al. 2011), necrophoresis remains the predominant mode of corpse disposal for the vast majority of ants. This type of waste management, relying almost purely on spatial segregation between the living and dead, is critical to maintaining colony hygiene within the enclosed nests of ants.

## Segregation through social structure

Living in groups affords many benefits to insects that remain out of reach to those with a solitary lifestyle. Task specialization and cooperative effort both increase the efficiency and ability to perform necessary duties. These advantages are considered foundational to why social insects such as ants, termites, and some bees and wasps have become some of the most numerous and successful organisms in many of their habitats (Wilson, 1971). Predator defense, foraging efforts, nest construction, and brood care are some of the prominent examples of undertakings enhanced by cooperation among group members, but this is by no means the full extent of gains imparted by eusociality.

Task specialization itself can act as a barrier to disease transmission, as a finite subset of the workers in a colony engaged in a task may be repeatedly exposed to a risk. This is especially true for those ants that specialize in waste management. The limitation of exposure to a small subset of the workers helps to protect the overall colony from a risk such as the spread of a pathogen. Individuals performing duties with exceptionally high sanitary risk are often isolated from the rest of the colony through division of labor, and this partitioning may even be reinforced with hostility. Waste heap workers of leafcutter ants are met with heightened aggression from

their nestmates when attempting to leave their respective garbage chamber, presumably preventing or reducing the spread of detrimental pathogens from the decomposing waste heap (Hart and Ratnieks, 2001).

Younger workers tend to remain in the nucleus of the nest, closer to the queen, while older and more expendable individuals regularly specialize in more dangerous charges on the periphery of the nest and its territory (Hölldobler and Wilson, 1990; Schmid-Hempel and Schmid-Hempel, 1993; Pie et al., 2004). This unidirectional progression from inner to outer nest duties has been branded centrifugal polyethism, and it presumably diminishes the ability of pathogens to penetrate deep into the nest. Workers of the same age and/or morphological caste also tend to carry out their specialized duties in explicit areas, further compartmentalizing sanitary risks. In 2013 Mersch et al. tracked all physical interactions within several *Camponotus fellah* ant colonies. The experiment confirmed that interactions were almost exclusively within, and not between, functional groups, and that positions within these groups were assumed with increasing worker age. Findings such as these emphasize the intimate relationship between social and spatial structures in eusocial insects. As the vast majority of interactions between individuals occur within these subdivisions of the colony as opposed to between them, infections often stay localized (organizational immunity) and eventually wane (Naug and Smith, 2007). While division of labor alone has been shown to be rather ineffective in dampening the spread of infection (Naug and Camazine, 2002), when division of labor is coupled with the heterogeneity of interaction networks and gradients in demography that exist in eusocial insect societies pathogen transmission is effectively stifled.

## Segregation through necrophoresis

Necrophoresis itself is a powerful tool for keeping pathogens at bay because of the spatial separation that it achieves between the living and the dead. Compounding the effectiveness of corpse removal are other mechanisms that facilitate or speed the neutralization of the remains as a potential biological hazard. Corpses may be mounded in one location and tended much like a compost pile, which not only isolates the sanitary threat but presumably speeds decomposition (Bot et al., 2001). Contrastingly, corpses may be deposited in a highly dispersed manner outside the nest; this promotes desiccation and deterioration of the corpse due to exposure to open-air conditions and sunlight. Desiccation is, of course, inhibitory to many infectious pathogens and parasites, and a body isolated on the soil surface versus underground certainly dehydrates more quickly from reduced humidity levels relative to a subterranean setting. Sunlight is also suspected to assist in decontamination of infected corpses through exposure to ultraviolet light, and at least some ants show a statistical preference for choosing well-lit areas to deposit corpse over shady areas (Graham, 2007). Commonplace fungi capable of causing disease in insects such as *Beauveria bassiana* (Cagáň and Švercel, 2002) and *Paecilomyces fumosoroseus* (Smits et al. 1996) are severely hindered by ultraviolet radiation. Emerging propagules of these entomopathogenic fungi can be inhibited with lower doses, and rendered inert with high enough doses of ultraviolet light.

Temperature is also crucial to the development of disease causing agents, and diurnal temperature regimes differ in median temperature and amplitude outside of the nest. These increased fluctuations obstruct *B. bassiana* growth in the laboratory (Fargues and Luz, 2000). Similar to how ants transport brood or fungus to optimal temperature and humidity regimes to encourage growth (Bollazzi and Roces, 2002), corpses may be transported to areas that impede

infectious propagation. Denying pathogens a sheltered environment of high humidity and moderate temperature by removing waste from the nest seems to be a broadly operating countermeasure to disease.

External factors such as slope may play into the effectiveness of depositional patterns. Rain may remove corpses rejected into the surroundings altogether, or possibly reintroduce pathogens to the colony in the event of refuse situated uphill to the colony. Infectious waste placed in an area prone to foraging does not ultimately further the health of the colony, so location is important in both obvious and enigmatic ways. There are profound implications of dimensional patterns in the waste disposal of ants beyond the distance created between the living and dead. Unfortunately, details on the specifics of the spatial segregation created by necrophoric behavior are known for only a limited number of species.

## Corpse removal in ants

Ants are known for their rigorous approach to the hygiene of themselves as well as their dwellings, and necrophoresis is one of the behaviors in their repertoire deployed to maintain a meticulously clean nest. This behavior is so commonplace in ant species that it is considered typical of all ants. Even the remarkably primitive Australian ant *Nothomyrmecia macrops* removes the remains of adults and juveniles alike from inhabited sections of the nest (Taylor, 1978), suggesting that the behavior itself is not derived but instead is a fundamental predisposition that evolved early in the history of ants.

Corpses are not only removed by ants more promptly than other types of refuse, but are generally transported more rapidly and over considerably longer distances (Gordon, 1983; Wilson et al., 1958). Some ants almost completely ignore inert items while still transporting corpses substantial distances (Diez et al., 2012). This urgency towards the dead continually



sets necrophoresis apart from other duties in which worker ants regularly engage. As with behaviors such as foraging, nest construction, and colony defense, ant species vary in the particulars of their corpse removals. One of the most tangible of these intricacies is the difference in where the ants ultimately deposit corpses. These locations often differ within subfamily of ants and among ant species. Leafcutter ants may either accumulate corpses internally within specialized underground chambers for waste inside the nest such as *Atta cephalotes* (Bot et al., 2001), or external to the nest in piles actively avoided by the colonies' foragers such as seen in *Atta colombica* (Hart and Ratnieks, 2002). The red imported fire ant, *Solenopsis invicta*, scatters corpses around the nest in a manner heavily influenced by slope (Howard and Tschinkel, 1976). *Camponotus compressus* have displayed a specific disposal area for corpses while no aggregations of corpses were observed in *Diacamma vagans* (Banik et al., 2010). *Myrmica rubra* has likewise been documented dispersing their dead in a strewn fashion (Diez et al., 2012). The tiny and predatory *Strumigenys lopotyle* is prone to creating a tight ring of corpse fragments around its nest entrance (Wilson, 1971). There is undoubtedly much variation among species in the processing of dead individuals in ant colonies.



**Fig. 1**

**A.** Initial inspection of introduced nestmate corpse by pair of *Pogonomyrmex occidentalis* workers. Antennation is used to discern characteristics of the corpse through the presence or absence of chemical cues. **B.** A typical necrophoric carry of *P. occidentalis* with the undertaker ant grasping the corpse by the pronotum.

## Literature gaps, specific questions, and hypothesis

Since research up to this point has demonstrated that there is no typical necrophoric behavior for all ants, evaluation of species on an individual basis is needed for meaningful comprehension of the prophylactic strategies on which a species relies. This is the overarching reason why I chose to evaluate necrophoresis of the western harvester ant, *Pogonomyrmex occidentalis* (Cresson, 1865), for which previous work is lacking. Extensive work on several collective behaviors of this genus such as task allocation and foraging dynamics have been conducted, but very little work has focused on corpse removal in this species of harvester ant.

Studies of social insect corpse management to date have focused on either behavioral process, division of labor, or death recognition cues (Sun and Zhou, 2013). Detailed dimensional analysis of patterns resulting from corpse removal has been neglected. Only a select few species of ants have been evaluated in field settings for spatial patterns of their necrophoric behavior, notably members of the subfamily Myrmicinae belonging to the tribes Attini (Hart and Ratneiks, 2002) Solenopsidini (Howard and Tschinkel, 1976) and Myrmicini (Diez et al., 2012). Because *P. occidentalis* is a member of an additional tribe, tribe Pogonomyrmecini, within this hyperdiverse subfamily, there is added comparative interest in studying this species.

In terms of methodology, the work of Howard and Tschinkel (1976) and Diez (2012) is most closely aligned with my study. These describe the dispersion of nestmate corpses in areas around the nests for ants that do not have clearly defined areas for corpse disposal.

Much of what we do know today about corpse removal in ants comes from experiments carried out in laboratory conditions, not natural settings. This becomes problematic when the goal is to evaluate spatial distributions, as available space is often severely constrained in a laboratory trial and consequently grossly alters experimental outcomes. In addition to imposing constraints that do not naturally exist for ant colonies, other factors that may influence necrophoresis are absent in a laboratory setting. Numerous social insects live in temporally and spatially stable nests over spans of many years. Continuous habitation of this kind, in turn, alters the nest mound itself, such as imprinting colony scent on the nest area (Sturgis et al., 2011), and it is currently not known how factors such as these may regulate aspects of necrophoresis. While there are certainly advantages to research conducted in laboratory settings, field experiments and observation also have distinct advantages, namely viewing a particular behavior with all of its natural components in play. Moreover, excavation of wild colonies and transfer to artificial

settings unavoidably disturbs and stresses ants. This general reasoning led to my decision to conduct my experiments on established colonies in the field.

I was also interested in comparing the treatment of dead colony-mates (nestmates) with the treatment of non-nestmate corpses. While the response to cadavers of different origin has been explored in several studies of termites with interesting results (Neoh et al., 2012; Sun et al., 2013; Ulyshen and Shelton, 2012), ants have not received equivalent attention in this respect. Furthermore, searches for literature that combines ant corpses of different origin (nestmate or non-nestmate) with tendencies for corpse transport yielded no results.

In this paper, I investigate the natural distributions of corpses of established harvester ant colonies. I predicted to find dispersed as opposed to clumped or piled depositions. I also evaluate the influence that nest surroundings, specifically adjacent colonies and slope, have on these dispersals. I expected to see apparent geotaxis, or maneuvering in response to slope, in undertaker ants, with corpses being preferentially transported downhill. I was curious to see if the ants would treat nestmate corpses differently than non-nestmate corpses from foreign nests. I hypothesized that conspecific non-nestmates would be carried to greater distances given the fact that ants can consistently distinguish live nestmates from others based on cuticular hydrocarbon profiles (Wagner et al., 2000) and might associate foreign corpses with a higher probability for new pathogen introduction. A final goal was ascertaining whether corpse removal has any ties with territoriality for *P. occidentalis*, with either rival harvester ant colonies or other ant species regularly found in the vicinity of harvester ant nests.

The terms “conspecific” and “non-nestmate” will be used interchangeably throughout this paper, with both terms referring to a corpse of the same species that is not a nestmate of the necrophoric worker.

# Materials and Methods

## Site Descriptions

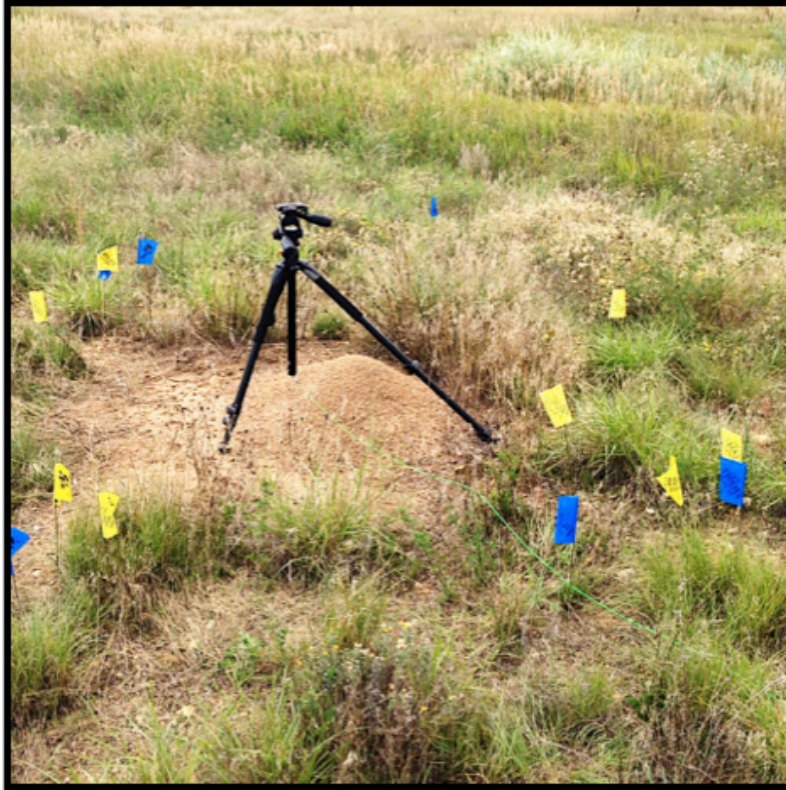
The harvester ant colonies observed for this study were distributed between two distinct sites within Boulder County, Colorado. Two different sites were utilized to ensure the opportunity to evaluate colonies with diverse attributes such as nest size, neighbor proximity, and slope. This approach also allowed for the ability to check for the existence of site-specific tendencies for corpse deposition patterns. Site #1 was located within a small open meadow on the University of Colorado at Boulder's East Campus (40.0121°N, 105.2499°W) at 1606m elevation. The surroundings included a mixed woodland thicket and a riparian zone. Given the terrain features surrounding the area the site may be considered somewhat naturally enclosed and segregated as opposed to functionally linked to adjacent spaces. The vegetation was largely composed of various grasses, sedges, and woody shrubs as is typical for many areas inhabited by the genus *Pogonomyrmex*. The overall topography was non-uniform with varying slope. Several prairie dog groups inhabited the immediate area. A total of 6 colonies were selected for use from this site. Site #2 was situated on the University of Colorado at Boulder's South Campus (39.9774°N, 105.2275°W) at 1636m elevation. In comparison to site #1, the vegetation there was comprised of a considerably higher proportion of grasses. Trees were absent and overall terrain was substantially flatter. Most colonies at this site were considerably larger than those at site #1 and colonies rarely had neighbors close enough to be considered within their foraging networks. Overall, site #2 possessed very mature and established colonies that appeared to have outcompeted their rivals years ago. A total of 4 colonies were selected for use from site #2.

Individual colonies within these sites were selected for use only if the nest mound had a single entrance (single origin for corpse carry measurements) and lacked nearby obstacles or obstructions that might impede corpse depositions.

## Field Protocol

I compared the deposition sites of nestmates and non-nestmates (conspecifics) by presenting wild colonies with corpses of the two different origins. All trials were conducted during fair weather in July or August 2014 while colonies were fully engaged in morning or afternoon term foraging efforts. Single colonies were presented with nine nestmate and nine conspecific corpses within one day. This was repeated for a total of 10 colonies totaling 180 depositions. Ants to be used as corpses were collected the day prior to trials and killed by freezing and stored at  $-22 \pm 1^{\circ}\text{C}$ . All ants utilized were collected from outgoing forager trunk trails, guaranteeing that all were of forager status. All conspecific corpses were collected from a single colony residing in a third separate site. Prior to initiating a trial, corpses were returned to ambient temperatures for one hour. This timing ensured that all corpses were presented to the worker ants within a 1 to 4 hour window, which is the time window that preliminary experiments had shown to elicit most rapid and consistent corpse removal. Corpses were placed within 5 cm from the nest entrance one at a time and in random order (in respect to corpse origin). Upon an ant initiating necrophoresis, the ant was monitored from a distance allowing visual tracking while ensuring that shadows were not cast over the carrying ant until the corpse was dropped and abandoned for a minimum of 30 seconds. This position would then be marked and the process repeated for subsequent corpses. After all 18 corpses for the colony had been transported the deposition sites were recorded by measuring the distance from the nest entrance with an accuracy of 1 cm and directions of the carry terminus relative to the nest entrance measured

with a compass. In addition, I gathered data on the structure of the nests themselves and their surroundings.



**Fig. 2**

Recording of corpse deposition sites. The tripod was used to take stable compass readings and provide an origin of measurements for carry distances. The marking flags designate where necrophoric workers abandoned corpses.

Nest cone dimensions were recorded for use in later estimating population size and relative maturity of the colonies. These particular measurements were recorded within one week to avoid general phenological changes of nest populations, nest size, or caste distributions. Nest sites were also surveyed for slope to address the possibility that geotaxis may play a role in corpse depositions. Direction and distance of nearby colonies was recorded to assess the possible influence of territorial dynamics on necrophoric behavior.

Preliminary experimentation began with additional parameters that quickly proved to be unproductive for my purposes and were consequently discarded. These parameters were marking individual ants and the transport of inert refuse. Initially, ants were captured after terminating necrophoresis and marked with paint to facilitate identification of repeating transporters with the aim of avoiding individual ants disproportionately influencing depositional patterns. This practice was removed from the procedure after preliminary experiments for several reasons. First, workers were very rarely observed conducting multiple corpse carries, which is precisely the event that this precaution was originally implemented to compensate. I suspect that individuals often failed to repeat corpse carries during our trials simply due to the large number of ants that seem to be at least somewhat involved in or recruited for necrophoric duties. Second, on the few occasions in which an individual ant was involved in more than one corpse transport each deposition site was original; the same ant did not deposit multiple corpses in an exclusive location. Although this was contrary to findings of similar studies of other species in which memory proved to cause repetitive depositions in similar sites by the same individual (Diez et al., 2011), this was not the case in this particular instance. Finally, given the apparent lack of value this extra measure of marking added to the study, it was deemed more imperative to present the corpses, which were time-sensitive in the sense of chemical cues once thawed (Diez et al., 2013), in the most streamlined manner possible.

Data collection on inert refuse depositions was not included in the trials since these data were unusable in my experimental context. The two types of inert refuse presented to worker ants during preliminary experiments were balls of vermiculite and toothpick sections comparable in size to the ants, both of which were met with overwhelming disinterest. Transports of these items were of such short distance that meaningful vectors could not be recorded, as ants would regularly move the objects out of very extremely high traffic areas and no further. The inert



refuse was usually abandoned on the nest mount itself, a testament to the utter lack of urgency in placement of this type of waste. This behavior towards inert refuse has been noted in previous studies of the European fire ant, *Myrmica rubra* (Diez et al., 2013).

## Data analysis

Data were collected from 10 separate colonies that were evaluated with linear and circular statistical methods. Because only colonies with one nest entrance were used in testing and corpses were presented at this one entrance, the resulting routes did not need to be normalized in any way. All instances of necrophoric transport for each colony were initiated from a single point and could thus be mapped uncorrected. Every necrophoric carry yielded a vector composed of a distance measured in centimeters and a heading measured in degrees (0°-360°). In addition to being categorized by colony, all depositions were grouped and analyzed as either nestmate or conspecific. All of this resulted in 10 pairs of nestmate and conspecific corpse location groupings that were evaluated independently for statistical values.

The circular data were analyzed by two tests to determine the level of randomness of the depositions. Batschelet (1981) gives a comprehensive overview of the fundamentals and application of each of these tests that are used to determine one-sidedness or directionality of circular data sets. The two tests used were chosen for their complementary and compensatory pairing in the context of the nature of the data evaluated. Circular data may have either zero, one, or multiple preferred directions, and be respectively considered randomly dispersed, unimodal, or multimodal. The first test used in this analysis was Rayleigh's test for uniform distribution. This test proves extremely useful in that as long as data are unimodal, this test not only generates a mean direction, but a meaningful measure of the concentration of data around the mean direction that can signify a preferred direction. This means that under the assumption

that parent data are unimodal, the Rayleigh test yields not only a measure of one-sidedness, but also a mean angle (azimuth), and a measure of angular dispersion. Although Rayleigh's test is very useful with unimodal data sets, it becomes problematic with multimodal samples. Certain distributions, such as distinct groupings of data that oppose each other in orientation relative to the center point, can be determined randomly dispersed by Rayleigh's test. For this reason the second test chosen was Rao's spacing test, which is equally powerful with unimodal and multimodal directional data. This test essentially evaluates spacing by measuring the length of the arcs between adjacent sample points. These lengths subsequently reveal to what extent the spacing between consecutive points of the parent data deviates from that of points distributed evenly around a circle. This test provides a measure of the degree to which the data points are clumped despite data groupings that oppose each other in orientation. This combination of the very powerful but susceptible Rayleigh's uniformity test with the more flexible Rao's spacing test formed the basis of the directional analysis of the corpse depositions. Using both tests in tandem allowed for other insights to be made given their inherent strengths and weaknesses; if data were shown to be significant by Rao's test but not by Rayleigh's this implies the data is somewhat multimodal as well as non random, if data were shown to be significant by Rayleigh's test but not by Rao's the data likely has a strong unimodal tendency. This means that much can be inferred about corpse deposition tendencies if significant and insignificant values generated by these tests show trends across colonies.

For the carry distances the data were arranged in the same fashion; each colony was evaluated in respect to nestmate carries separately from conspecific carries. For each of these 20 groupings of figures I calculated mean carry distance, standard deviation, and variance. An F-test for variance was also conducted to determine if nestmate and conspecific carry distance variances for each respective colony differed significantly from one another.

In an effort to ascertain to what degree the origin (nest mate or conspecific) predicted carry distance for an individual corpse I calculated the Akaike Information Criterion (AIC) of two models. The AIC is a measure of the relative quality of a statistical model for a given set of data. By creating two models with a select difference and evaluating their AICs, I could determine the importance of the singled out factor, which in this case was corpse origin. Both models shared colony designation as a random effect in order to account for the inherent variation between nests. One model had corpse origin as a fixed effect while a second model lacked this distinction. A  $\Delta AIC$  value, or difference between the calculated AICs, was determined following these tests, and allows for evidentiary statements about the relative effectiveness of the models to be made (Burnham et al., 2011). If this value is large I could accept corpse origin as a strong influence on transport distance. Conversely, a relatively small  $\Delta AIC$  would indicate that corpse origin does not consistently or reliably predict carry distance given the similar effectiveness of the models that would be suggested by a small  $\Delta AIC$ .

I estimated the size of the colony from the size of the nest cone taken as  $\text{Ln} [\text{Length} \times \text{Width} \times (\text{Height} + 1\text{cm})]$ . In 1995 Wiernasz and Cole found this resulting value to be highly correlated to estimates of worker population in this species.

# Results

## Directional analysis

Circular statistics were used to compare whether nestmate and non-nestmate corpses were oriented differently relative to the nest entrance. The circular data showed no consistent pattern

of direction in which corpses were taken. Depending on the colony, significant differences from random distributions were found for nest mates groups only, conspecific groups only, both nest mates and conspecific groups, or neither. All of the non-significant comparisons generated with Rayleigh's test and Rao's test indicate a uniformly distributed parent population, and these corpse depositions can consequently be accepted as randomly dispersed. Trends could not be ascertained between the random or non-random deposition of corpses between colonies.

Directional Data										
Colony #	Rayleigh Test						Rao's Spacing Test		Slope	
	z Statistic ( $z_{critical} = 2.91$ at $\alpha .05$ )		Mean Vector Length / Angular Dispersion (r)		Mean Angle / Azimuth		U Statistic ( $U_{critical} = 173.68$ )		Angle of Incline	Heading (declining)
	Nestmate	Conspecific	Nestmate	Conspecific	Nestmate	Conspecific	Nestmate	Conspecific		
1	0.979	1.161	0.33	0.359	187°	168°	<b>174</b>	155	NONE	
2	1.843	<b>3.53</b>	0.453	<b>0.626</b>	29°	<b>61°</b>	147	162	20°	108°
3	<b>3.911</b>	<b>7.159</b>	<b>0.659</b>	<b>0.892</b>	<b>238°</b>	<b>256°</b>	<b>197</b>	<b>232</b>	NONE	
4	<b>3.651</b>	0.673	<b>0.637</b>	0.273	<b>216°</b>	201°	<b>180</b>	<b>189</b>	NONE	
5	1.495	0.776	0.408	0.294	93°	283°	<b>189</b>	169	NONE	
6	0.343	0.658	0.195	0.27	31°	65°	112	125	18°	5°
7	<b>3.146</b>	1.781	<b>0.591</b>	0.445	<b>220°</b>	205°	165	<b>191</b>	9°	97°
8	1.653	0.767	0.429	0.292	121°	31°	<b>182</b>	133	NONE	
9	<b>3.975</b>	2.665	<b>0.665</b>	0.544	<b>85°</b>	57°	163	152	20°	9°
10	<b>3.209</b>	<b>4.266</b>	<b>0.597</b>	<b>0.689</b>	<b>112°</b>	<b>80°</b>	151	173	NONE	

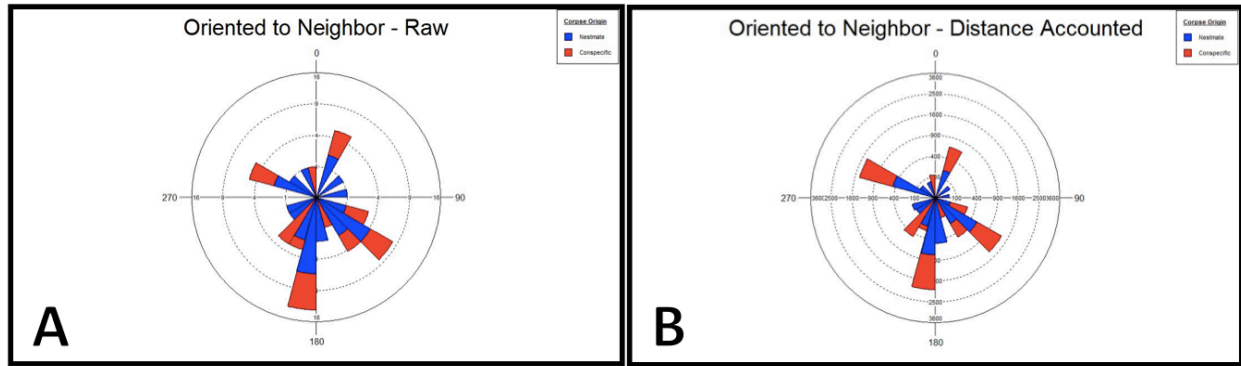
**Table 1**

Directional data is provided in this table. Values that proved to be significant are shown in bold. The Rayleigh test generates a z value, r value, and azimuth (mean sample direction). If the z value is larger than  $z_{critical}$  the corresponding r value and azimuth are considered significant. The value of r is also a measure of angular dispersion, with a value of 0 signifying a perfectly uniform distribution around the center point and a value of 1 signifying complete concentration in one direction. A significant U value generated by Rao's spacing test determines that the sample data is non-random, and some type of one-sidedness exists. Four of the ten colonies were situated on sloped terrain. The magnitudes and directions of these slopes are provided for comparison to other directional statistics.

Possible influences of slope and nearby neighbors on corpse deposition sites were also evaluated. When colonies were evaluated for orientation with respect to close proximity neighbors or slope, the results were as indiscriminate as the rest of the directional data. No

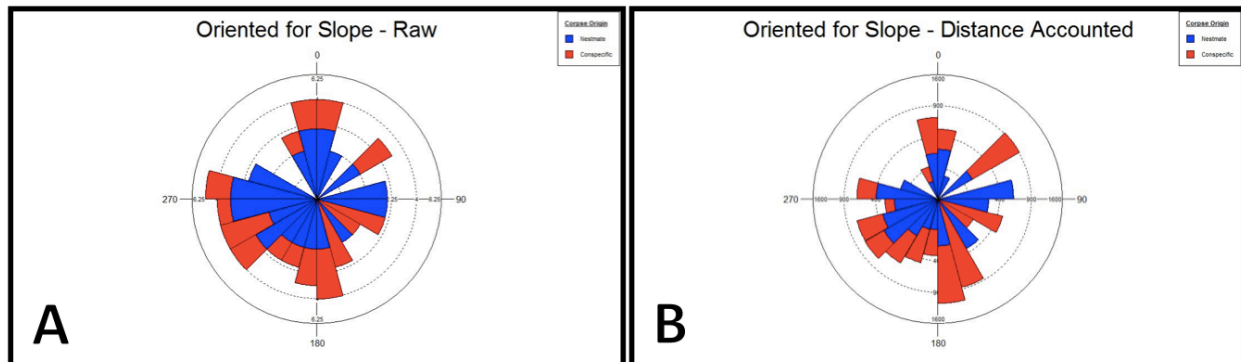
discernable difference was found between the depositional patterns of isolated colonies versus colonies with close neighbors. For colonies lacking neighbors but situated on sloping terrain I further analyzed necrophoric carry data and found that undertaker ants were not necessarily more likely to carry corpses downhill or to carry corpses further downhill than uphill. For colonies situated on sloped terrain corpses were essentially deposited with the same regularity and distance against the slope as with it.

Figures 3 and 4 show these potential influences on corpse placements. Figure 3 represents the depositions of nestmates and conspecifics for 3 colonies that had a single neighbor in close proximity (less than 9 meters) and were situated on terrain with no discernable slope. Figure 4 represents the depositions of nestmates and conspecifics for 3 colonies that had significant slopes ( $9^\circ$  angle of inclination or greater) and lacked a nearby neighbor. All of the headings of the depositions were adjusted such that 180 degrees on the graphs are representative of the direction of the closest neighbor for Figure 3 or the downhill direction for Figure 4. Statistical analysis of these colony composites reveal that with depositions adjusted in this manner, depositions must still be considered random by virtue of Rayleigh's and Rao's tests as no preferred direction was indicated at a significant level. This indicates that corpse depositions are likely not shaped by the presence of nearby rival colonies, nor do the necrophoric workers seem to be operating with a geotaxis. It is also apparent that there is no preferred direction based on corpse origin, as nestmate and conspecific corpses exhibit the same general dispersal patterns.



**Fig. 3**

**A.** Stacked rose diagram ( $n=54$ ) with all depositions oriented towards a neighbor located at  $180^\circ$ . “Raw” indicates that the size of the wedges is representative of the frequency of depositions which fall into the given degree range; only headings are taken into account. Blue represents nestmate corpse depositions and red represents non-nestmates **B.** Stacked rose diagram ( $n=54$ ) with all depositions oriented towards a neighbor located at  $180^\circ$ . “Distance Accounted” indicates that the size of the wedges is representative of the total distance that corpses were transported in the given degree range. In the “Distance Accounted” representation, longer corpse carries equate to larger wedges.



**Fig. 4**

**A.** Stacked rose diagram ( $n=54$ ) with the downhill direction located at  $180^\circ$ . “Raw” indicates that the size of the wedges is representative of the frequency of depositions which fall into the given degree range; only headings are taken into account. Blue represents nestmate corpse depositions and red represents non-nestmates **B.** Stacked rose diagram ( $n=54$ ) with the downhill direction located at  $180^\circ$ . “Distance Accounted” indicates that the size of the wedges is representative of the total distance that corpses were transported in the given degree range. In the “Distance Accounted” representation, longer corpse carries equate to larger wedges.

## Carry distance analysis

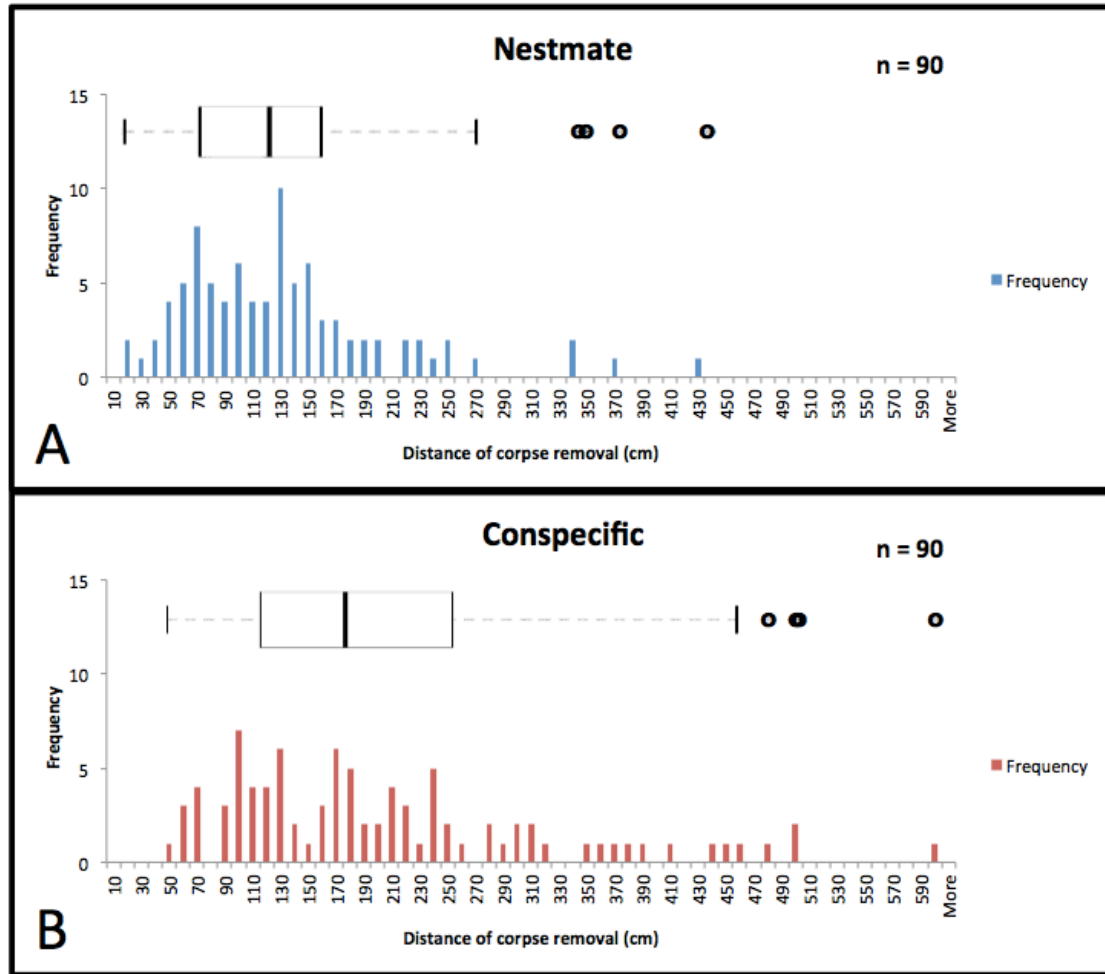
Analysis of carry distances yielded more cohesive results than that of circular data, and distinct trends became evident. As shown in table 2, every tested colony on average transported non-nestmate corpses a greater distance than nestmate corpses. Not only were non-nestmate corpses placed further away from the nest entrance, their transport distances were also more variable. All colonies displayed a higher variance in deposition distance with conspecifics, and an F-Test showed 6 of the 10 colonies to have significantly different variances between their mean nestmate and conspecific corpse depositions in terms of distance. Figure 5 clearly illustrates how conspecific corpses are deposited in a more diffuse manner, minimizing concentration to an even further extent than nestmates are. The conspecific corpses were dispersed over a higher range of distances, and dispersed more uniformly within those distances than nestmate corpses. Figure 6 is a circular scatterplot mapping of all corpse deposition locations of the study. This alternate graphical representation depicts the low probability that an undertaker ant carrying a deceased nestmate will travel extreme distances to abandon its cargo. The shortest necrophoric carries are those of nestmates, while the furthest corpse transports are consistently those of non-nestmates.

Non-directional Data								
Colony #	Mean Carry Distance ( $\bar{x}$ ) in cm		Standard Deviation (s) in cm		Variance (s <sup>2</sup> )		F-Test for Equality of Variances (F <sub>critical</sub> = 3.1789)	Nest Worker Force Estimation (Ln)
	Nestmate	Conspecific	Nestmate	Conspecific	Nestmate	Conspecific		
1	178	206	104	110	10891	11995	1.1	11.09
2	183	277	97	102	9496	10329	1.09	10.86
3	107	152	28	73	772	5368	<b>6.96</b>	10.5
4	88	156	33	78	1101	6127	<b>5.57</b>	9.51
5	114	183	34	107	1173	11442	<b>9.75</b>	10.2
6	66	124	32	80	1015	6474	<b>6.38</b>	8.37
7	60	113	40	41	1630	1689	1.04	10.27
8	110	220	50	111	2481	12296	<b>4.96</b>	11.87
9	204	273	87	131	7611	17088	2.25	12.99
10	166	314	35	161	1211	26078	<b>21.53</b>	12.84

**Table 2**

Non-directional data is provided in this table. Values that proved to be significant are shown in bold. The nest worker force estimation is based on calculations made from nest cone dimensions and is displayed as a natural log.

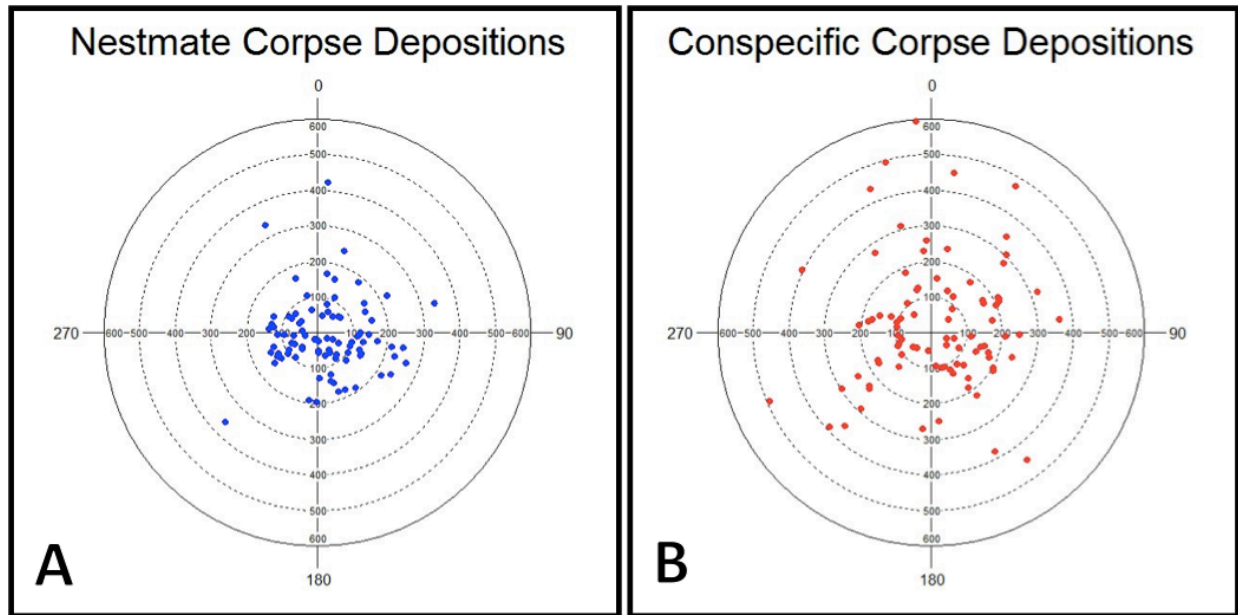




**Fig. 5**

**A.** Histogram displaying the frequency of nestmate corpses transported the distance indicated by the x-axis. This includes the depositions of all 10 colonies, and the distances are not normalized in any way. n=90. Boxplot superimposed to show inner quartile range (a robust measure of distribution) and outliers **B.** Same as for histogram “A” but for conspecific corpses.

The  $\Delta AIC$  of the two statistical models that I assessed was 36.484, a rather high value in this context. The greater this value, the more that information loss is minimized by one of the models relative to the other. This  $\Delta AIC$  translates to an evidence ratio of 83,637,287 between the two models. This specifies that the evidence of best fit is about 83 million times stronger for the model accounting for corpse origin than the model that does not. This indicates that corpse origin (nest mate or conspecific) has an exceptionally identifiable influence on carry distance.



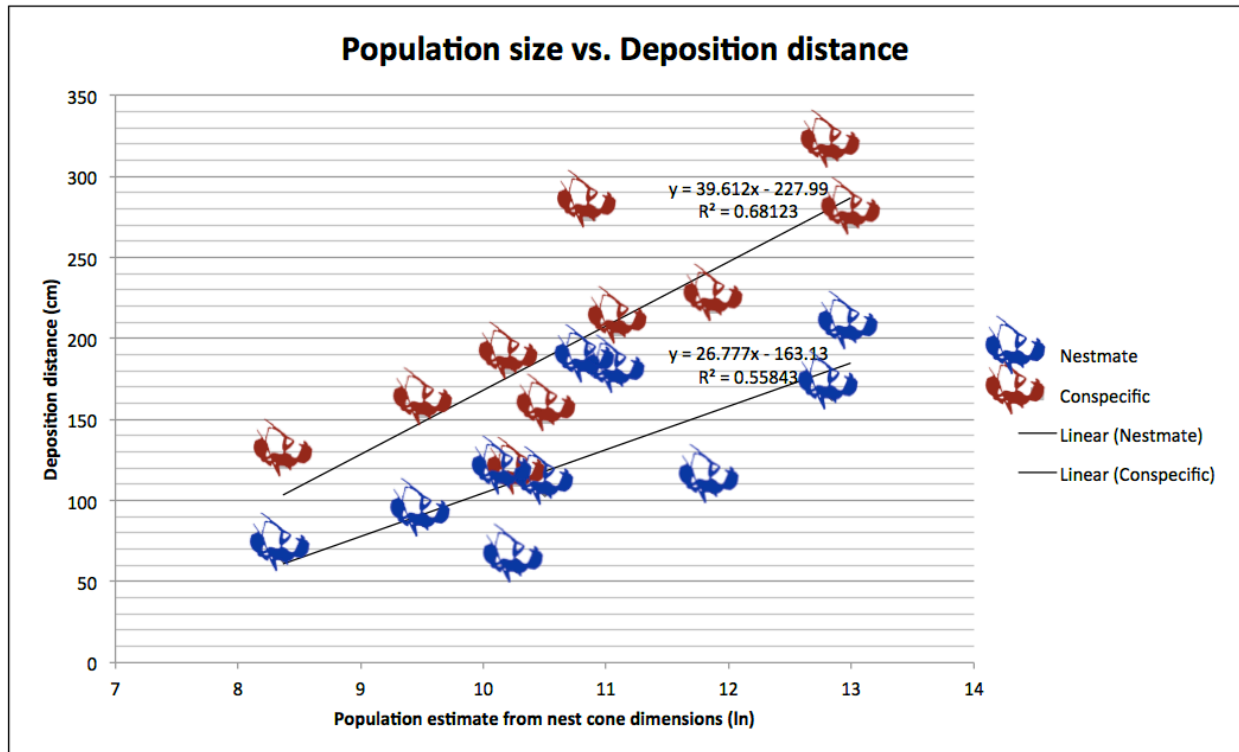
**Fig. 6**

**A.** Circular scatterplot of all nestmate corpse depositions. The center of each plot represents the nest entrance. This is a composite of all 10 colonies. The distances are not normalized in any way.  $n=90$ . **B.** Same as for circular scatterplot “A” but for conspecific corpse depositions.

## Colony size effects

Another well-defined trend was seen when colony worker population was plotted against the mean carry distances for the colony. This positive correlation can be seen in figure 7.

Essentially the larger colonies transported corpses further away from their nest's than did smaller, younger colonies. The trend lines signify a meaningful relationship, with an  $R^2$  for conspecific carry distance of .68123, compared to an  $R^2$  for nestmates of .55843. This suggests that nest worker population can somewhat predict the general magnitude of corpse carry distance of a colony.



**Fig. 7**

This scatterplot denotes the increasing mean carry distance with increasing colony worker population. Each colony has its mean carry distance for nestmate and conspecific corpses placed along the x-axis according to a calculated estimation of worker population. The x-axis represents exponential change in colony population (log natural). Trend lines are presented for all nestmate averages and all conspecific averages.

## Observations

Several intriguing observations were made while conducting these trials in the field. Most noticeable was the obvious difference in sinuosity, or straightness paths, of necrophoric workers compared to that of ants carrying out other nest duties such as foraging. Workers engaged in corpse removal regularly chose straighter paths than their nestmates that were performing other tasks; a direction was picked at the onset of necrophoresis and it was seldom deviated from.

Seemingly aggressive displays also sometimes took place during removal of conspecific corpses from the immediate nest area. Undertaker ants in possession of a conspecific corpse

would occasionally stop periodically during their transport to sting the corpse, sometimes for several minutes. This behavior was particularly odd considering that aggressive displays such as threats with open mandibles or hostile posturing were not witnessed at any time during the workers' interaction with the corpse. In contrast to the aggressive behavior that workers exhibited towards live or very recently killed conspecifics, these corpses were treated in a calm and methodical manner and seemed to be recognized to not be an immediate or active threat. This stinging behavior was witnessed only in regard to conspecific corpses and on an inconsistent basis. Another curious behavior observed during almost all of the corpse transports. Transporting ants were observed dropping their payload, antennating the ground of the proximate area for several seconds, and then continuing their transport or abandoning the corpse. This was a very common occurrence, and seemed to be some type of evaluation of the suitability of the location as a gravesite. A final observation of interest was a suite of interactions between *P. occidentalis* and *Dorymyrmex insanus* (Buckley, 1866), the latter also known as the crazy ant. *D. insanus* was frequently observed to be in very close proximity with the harvester ant nests at both sites, and was sometimes even seen to have their nests within the nest clearings of the harvester ants. Antagonistic behavior was not witnessed despite great intermingling between the two species. Some of the typical interactions witnessed between these two ants living among each other are displayed in figure 8.

## Discussion

My most important finding was the discrimination in treatment between nestmate and non-nestmate corpses in the context of carry distance. In comparison to ant carcasses that

originated from the colony of the undertaker ant, heterocolonial corpses were transported substantially further on average from the nest entrance. This greater transport distance necessarily comes at greater cost to the undertaker ant, suggesting that there are ecological benefits to this behavior. Benefits to colony fitness are presumably associated with this increased carry distance for heterocolonial corpses, as foreign colonies may host pathogens unknown to the colony, and greater carry distances increase the effective isolation of the corpse from living members of the colony.

Findings regarding the small or non-existent influence that slope had on necrophoresis in *P. occidentalis* were also quite noteworthy, mostly due to their unexpectedness. The trivial effect that inclines had on the deposition of ant carcasses was contradictory to previous findings in other species of ants. Howard and Tschinkel (1976) documented that *S. invicta* undertaker ants decisively prefer downhill vectors relative to the nest. This preference was evident at as little as 5° of incline and increased with greater slopes. Ants that began necrophoresis in an uphill direction routinely adjusted their orientation to a downhill direction. The leaf cutting ant *A. colombica* places dead nestmates in a fashion dependent on slope as well. Carcasses of these ants are amassed in heaps outside of the nest instead of scattered, and these heaps are placed downhill from the nest at a distance that decreases with increasing slope (Hart and Ratneiks, 2002). Interestingly, necrophoric workers of *P. occidentalis* do not display sensitivity to slope of the nest area as other species have demonstrated. It might be worth recognizing that the ant species identified to date to be influenced by slope in this regard live in regions where rain is relatively frequent. The general understanding is that dangerous refuse is deposited downhill of nest entrances in ants to prevent rain events from flushing debris such as corpses back into the nest. This hypothesis holds true for *P. occidentalis* as rain is not a frequent occurrence throughout its range. A harvester ant that lives in areas more frequently exposed to rain, such

as *P. badius*, would be an interesting comparison in this respect; ants reacting to slope would support this hypothesis, but ignoring it would mirror closely related taxa.

## Implications of directional and linear data

The directional data suggest that the centrifugal path chosen by necrophoric workers is largely random. Despite the presence of some significant comparisons in the directional analysis, the overall lack of trends in the data set suggests that these significant values could very well be the product of stochastic events coupled with rather small groupings of data. Larger sample sizes may negate the significance found within these limited corpse depositions. Even with the acceptance of some of these data groups as significantly one-sided, most of data comparisons are decidedly random in their orientations.

*P. occidentalis* workers dropped their nestmate cargo at highly variable distances ranging between 19 and 422 cm from the nest entrance. This variability is reflective of corpse transport distances observed in other ant species that scatter this type of refuse around their nest mound. Distances between 7 and 315 cm were recorded for *S. invicta* (Howard and Tschinkel, 1976) and of 7 to 289 cm for *M. rubra* workers conveying dead nestmates (Diez et al., 2012) are very comparable to my findings. Similar to other studies focusing on necrophoresis, corpse-carrying ants exhibited a sinuosity distinct from that of workers involved in other activities such as foraging. Necrophoric workers consistently followed the straightest paths while executing their duties. This serves to reduce contact time between the undertaker ant and their potentially hazardous cargo.

Although corpses were dropped within distances that allow for the possibility of contact with healthy foragers, several factors may contribute to the removal distances I observed. Costs related to corpse transport increase with distance from the nest. These costs theoretically limit

the degree to which necrophoresis is profitable to the colony. Longer corpse carries result in a higher energetic or metabolic cost to the ant (Franks et al., 2004), higher exposure to possible predation, and a higher chance of disorientation and becoming lost. Since necrophoric workers do not lay a scent trail while leaving the nest, extremely long trips come with a reasonable expectation of not finding the way back to the nest. These reasons imply that forays into territory so remote that they are never explored by nestmates are simply not worth the costs or risks they entail.

Larger harvester ant colonies had greater mean removal distances than smaller colonies. Similarly, larger colonies of *Atta colombica*, have been documented as having their waste heaps located at greater distances from the nest (Hart and Ratnieks, 2002). Interestingly, necrophoric workers of species that tend to accumulate dead nestmates in one location as well as species that disperse corpses are somehow able to account for colony size in the execution of their corpse removals. This novel tactic assures that colonies producing more waste are able to achieve comparable levels of corpse densities around their nest. Unfortunately, with an emphasis in research of initiating factors of necrophoresis as opposed to terminating factors, the selective forces for this colony scaling effect is unknown. Researchers have yet to identify the information that ants use in determining when to cease corpse removal behavior.

This raises the question of how a worker ant knows that it must deposit a corpse a shorter or further distance away. What are the cues involved? In 2011, nest mounds of *Pogonomyrmex barbatus* were shown to have a detectable gradient of colony-specific hydrocarbons that was strongest near the nest entrance and declined towards the periphery of the nest area (Sturgis et al., 2011). These hydrocarbons apparently make their way onto the nest mound by years of contact with worker ants. Hydrocarbons may be actively secreted by ants onto the nest mound from exocrine glands (Soroker et al., 2003), or passively transferred to the surface by various

types of contact with an insect's cuticle (Soroker et al., 1995; Vienne et al., 1995). Something as simple and commonplace as footfall from daily ant traffic would contribute to the transfer of cuticular hydrocarbons to the nest mound. Other activities such as the rearranging of pebbles on the nest mound using the mandibles, a common pastime of *Pogonomyrmex* midden workers that specialize in waste management, would also serve to strengthen the nest odor. It can be expected that these compounds would accumulate over time given the long-term stability of these hydrocarbons (Martin et al., 2009). The antennation during the pit stops of necrophoresis are evaluating something by contact chemoreception, and colony-specific hydrocarbons are a reasonable candidate. The fact that these hydrocarbons are expressed as a gradient on and around the nest means that they can be used as an accurate gauge of the relative use of a particular area by nestmates. The periodic antennation witnessed could possibly be a surveying of a gradient such as this, and if it is determined that the concentration of the targeted compound is weak enough the corpse could be safely abandoned. This may give an alternate reasoning as to why conspecific corpses are carried further, as this would help to preserve the colony-specific hydrocarbon profile that is used to regulate important tasks such as foraging (Sturgis et al., 2011).

It cannot be said with certainty that undertaker ants do not exhibit more pronounced and variable rejection of conspecific corpses from the nest area due to some type of aggressive response to a non-nestmate, but the lack of aggressive displays or posturing before the onset of necrophoresis of conspecifics seems to suggest that this is not the case. Conspecifics of foreign colonies may represent competition, or perhaps imminent attack to the undertaker ant, but the behaviors seen in response to conspecific corpses did not resemble encounters with live non-nestmates. Corpses of both origins were approached in a restrained manner lacking frenzied or erratic movements characteristic of alarm and aggression in ants. At this point it seems unlikely



that a combative condition is driving conspecifics corpses to be dispersed differently than nestmate corpses.

## Stinging of non-nestmate corpses (observation)

The occasions of undertaker ants periodically stopping to ostensibly sting their cargo may warrant investigation. It is possible that this behavior is simply aggression elicited by the encountering of an unfamiliar ant, but toxins produced by some other ant species are used for sanitizing tasks. Venoms and poisons of various apocrita are proving to be beneficial to colonies in more ways than traditional offensive and defensive applications against prey and predators. Social paper wasps are known to coat the walls of hibernation sites with antiseptic substances effective against both gram-negative and gram-positive bacteria produced by their venom glands (Turillazzi et al., 2006). *Tetramorium bicarinatum*, a common ant tramp species often found within manmade structure, has venom that shows antimicrobial activity on par with modern antibiotics (Rifflet et al., 2012). Formicine ants utilize their formic acid poison to disinfect fungus-exposed brood; ants uptake and store poison in their mouth during acidopore grooming and indirectly transfer this to the brood via pupae grooming (Tragust et al., 2013). This behavior was shown effective in fungus suppression. In scarcer events these ants were witnessed directly spraying poison on the brood (Tragust et al., 2013). This direct application is not very unlike fire ant queens coating eggs with poison sac contents during oviposition (Vander Meer and Morel, 1995).

The chemical analysis of the venom of select ponerine ants has revealed vigorous and unique capabilities. The peptides found in these venoms have since been aptly named ponericins and have been noted as having insecticidal, hemolytic, and antimicrobial properties. So effective are these compounds that researchers have hypothesized that the venom serves to preserve colony

health by effectively sterilizing prey items that will eventually be brought back to the nest and shared (Orivel et al., 2001).

Members of the genus *Myrmecia*, also known as bulldog ants, have also been acknowledged as having strong antimicrobial peptides in their venom that would aid in the sterilization of prey (Inagaki et al., 2004). While this makes sense for ant species that rely exclusively on hunting live prey for nourishment, the connection is lost for largely granivorous species such as harvester ants. Previous studies of toxicity potentials point towards the evolution of *Pogonomyrmex* venom as a stout deterrent to vertebrates (Schmidt and Blum, 1978), contrary to a hunting tool. Although it is possible that this behavior could be somehow sterilizing the corpse given the absence of any antimicrobial evaluations of *Pogonomyrmex* venom (Vander Meer, 2012), without this information it is reasonable to assume that there has simply been selective pressure for workers to ensure the death of foreign ants in their vicinity. Even if *P. occidentalis* venom were shown in the future to have antimicrobial capabilities, this would only be the first step in linking it to necrophoric behavior in this manner, as these properties in hymenopteran venoms are seen as increasingly common.

## Interactions with other ants (observation)

Complex interactions exist between *Pogonomyrmex* and *Dorymyrmex* genera, at least some of which revolve around waste management. *Dorymyrmex* species, commonly known as “pyramid ants,” are widespread in the Americas. These small ants have a wide dietary breadth and can usually be identified on a preliminary basis by their characteristic open conical nest mounds. Pyramid ants have been cited several times in the scientific literature as being established in extremely close vicinity to harvester ant nests (Gregg, 1963; Allred, 1982), and on occasion even taking residence within *P. occidentalis* nest mounds. Despite numerous

acknowledgements of a relationship between these two species this has yet to be expounded upon by researchers. *D. insanus* was frequently seen cohabitating with *P. occidentalis* during this study at both sites. This seems probable given the preference of both species to nest in open, xeric habitats at these elevations. Pyramid ants also have the preference of nesting in areas of open soil without vegetative cover, which is increased in areas with harvester ant habitation. During the hottest parts of the day harvester ants abandon foraging efforts, while the pyramid ants are notorious for braving high midday temperatures for food collection. On many occasions harvester ant corpses were witnessed being scavenged by *D. insanus* workers, suggesting that they play a substantial part in the prevention of the buildup of biological waste around harvester ant nests. The harvester ants receive the benefit of the complete removal of a number of their discarded corpses while the pyramid ants have access to a regular supply of substantial meals. This interaction perhaps contributes to the toleration of *D. insanus* by the much larger harvester ants.

Wilson (1958) described almost identical interactions between *Pogonomyrmex badius* and another *Dorymyrmex* species in Florida and Alabama. Wilson suspected that this quick interception of harvester ant corpses by the much smaller *Dorymyrmex* ants patrolling the area prevents accumulation of corpses around *P. badius* nests. Similarly, Gordon (1984) observed several instances of pyramid ants claiming dead ants from *P. badius* nest yards and hauling them back to their own nests. Instances of live and apparently injured *P. badius* workers being seized and hauled away were also witnessed. This prompted Gordon (1984) to speculate that middens, or colony refuse aggregations, may deter the active predation of injured ants within their own nest clearings by somewhat satiating local predatory ants.

Small ants other than those of genus *Dorymyrmex* were also observed interacting with harvester ant refuse. Unidentified Myrmicine ants were found cohabitating with some harvester ant

colonies at site #2. These particular ants seemed to prefer to pillage harvester ant refuse to foraging far into surrounding areas. *P. occidentalis* seemed oblivious to these scavengers. It is possible that these scavenging ants nest preferentially near mature harvester ant colonies and obtain much of their nutrition from dead and dying *P. occidentalis* workers, as Cole et al. have hypothesized about the honeypot ant, *Myrmecocystus mexicanus*.



**Fig. 8**

**A.** Unidentified Myrmicines were frequently seen scavenging the refuse of *Pogonomyrmex* at some nests. Items of interest were often various isopod remains as well as *Pogonomyrmex* corpses. These ants were met with zero opposition **B.** An event witnessed on several occasions. *D. insanus* finding a recently abandoned harvester ant corpse and initiating the arduous task of dragging the much larger ant to it's nest.

## Overview

Corpse removal in social insects is a critical duty that directly impacts the overall fitness and success of groups (Diez, 2014). Cleanliness is paramount in high density living, and waste poses a very real threat. I describe the spatial patterns of nestmate and non-nestmate corpses that are removed from the nest mounds of *P. occidentalis*. Corpse-carrying ants did not

transport their payload to areas that could be called “graveyards” or “cemeteries” but instead scattered corpses into their surroundings.

Slope did not have a detectable impact on the directions chosen or the distances traveled for necrophoric workers in this species, indicating that it is not a relevant factor for workers engaged in this activity. Similarly, nearby neighbors had no discernable effect on corpse placement. Nest mound size was found to be a predictor of the general magnitude of corpse transport, with larger colonies exhibiting substantially further carry distances.

The combination of largely random orientations, highly variable carry distances, and a general distance scaling with colony size apparently facilitates uniform scattering of corpses into the environment regardless of colony size, thereby preventing areas of high sanitary risk.

Aggregations of corpses that could extend the risk associated with certain pathogens by providing them strongholds to persist within are avoided by this set of behaviors.

More energy was invested into removing non-nestmate versus nestmate corpses to more remote areas. Conspecific ant carcasses were rejected further into the nest surroundings on average, decreasing the risk of reencounter by foraging workers relative to other types of waste to include the corpses of nestmates. Although mechanisms for this are unclear at this point, this differential treatment of homocolonial and heterocolonial corpses illustrates plasticity of the behavior of corpse removal.

## Future directions

I would like to conduct an experiment to categorize the necrophoric behavior for *P. occidentalis* as either a strictly preventative disease mitigation tactic (a hard wired response) or a possibly remedial behavior. My research has shown that there is plasticity in necrophoresis for western harvester ants. Even so, this plasticity so far can only be attributed to decisions made by a

single ant based on characteristics of the corpse, and could be nothing more than a static response with multiple levels of execution. Perhaps necrophoric workers are able to integrate even more information, specifically that of colony status, and act appropriately. Honeybees will increase antimicrobial resin collection in response to fungal infection (Simone-Finstrom and Spivak, 2012), indicating that the action is therapeutic as well as prophylactic. Contrastingly, wood ants do not increase foraging efforts for antimicrobial conifer resin after colony infection with entomopathogenic fungus (Castella et al., 2008). I would explore this notion with *P. occidentalis* by observing a single colony in a manner similar to this study, except that this new study would involve only nestmate corpses. After gathering data on corpse deposition sites I would simulate a health threat to the colony. Capturing a portion of the population, inoculating the ants with a suspension of a common entomopathogenic fungus, and reintroducing them to the colony could achieve this. After this point I would also offer corpses that have been inoculated by the very same fungus that was previously introduced to the colony *en masse*. I would then continue to monitor corpse depositions to determine if necrophoresis is augmented after the colony has been exposed to a substantial pathogen load. Results from an experiment such as this could yield insight to how static or adaptive the act of corpse removal is in this species. This would also be a productive experiment because of the similar methodology to my 2014 experiment coupled with a much larger sample size could allow me to confirm findings and resolve speculations of trends in the circular data previously gathered.

I would also be interested in determining the variability of this behavior set within individual undertaker ants. Straightforward laboratory experiments on the same ants presented with corpses of different origin or treatment could provide insight into this.

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