Microclimate and Host Body Condition Influence Mite Population Size in a Bird-Ectoparasite System

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Abstract

Parasite populations are not evenly distributed among the hosts they infect. Nest ectoparasites, such as mites, are no exception as their distribution is highly aggregated with considerable variability between and within sites. Here, I examine the influence of microclimate, nest characteristics, and host condition on ectoparasite population size in a bird-ectoparasite system. I experimentally infested Barn Swallow (*Hirundo rusitca erythrogaster*) nests with Northern Fowl Mites (*Ornithonyssus sylvarium*) and analyzed both biotic (nestling mass, wing length, and brood size) and abiotic (temperature, humidity, nest lining, nest dimensions, and substrate upon which the nest was built) predictors of mite population size. Temperature and humidity measurements were collected every ten minutes for 14 days using iButtons (Maxim Integrated), which are small data loggers that collect and store temperature and humidity measurements until retrieved from the field. My results suggest that mite populations are largest in nests that have hosts in good condition, with higher temperatures, lower humidity, and low prevalence of other arthropods. I also found that nests built on wood substrates support larger populations of mites than those constructed on metal or concrete. These findings lend insight into where one may expect to find higher prevalence of mites, when considering microclimate and host condition.

Introduction

Parasites, by definition, inflict a cost on their hosts (Lehmann, 1993). Parasites can be divided into two main categories: endoparasites include parasites that live inside their host (tape-worms, malaria); and ectoparasites are parasites that live outside the host (mites, fleas, lice). Most parasites have an aggregated distribution, where most of the parasite burden is accounted for by a small proportion of the possible hosts (Atkinson, Thomas, & Hunter, 2009). However, there remain gaps in knowledge in terms of what drives this uneven distribution pattern of parasites on a small subgroup of hosts (Atkinson, Thomas, & Hunter, 2009). Further, the ecology and population dynamics of parasites are fairly well described in cases of human or livestock infections; however, there is still a lot to learn about the ecology of parasites in wild host populations.

One commonly studied type of wild parasite-host interactions is the bird-ectoparasite system, with a particular focus on ectoparasite infections that occur in nest sites of altricial birds (in which offspring are completely dependent upon parental care and remain in the nest up to several weeks after hatching from their eggs) (Owen, Nelson, & Clayton, 2010). These systems are convenient to study, as parasites and hosts (nestlings) are confined to a discrete physical location (the nest). Additionally, it is relatively easy to control, manipulate, and accurately observe parasites in the nest environment across the nestling development period (Owen, Nelson, & Clayton, 2010). Ectoparasites have been shown to impose important fitness costs on their avian hosts, some of these include increased nestling mortality (A. P. Møller, Arriero, Lobato, & Merino, 2009; Proctor & Owens, 2000), diminished secondary sexual trait expression (Lehmann, 1993; Proctor & Owens, 2000), and changes in parental resource provisioning (Hund, Aberle, & Safran, 2015). Additionally, the interaction of parasite load and expression of hormones in the host have been an area of interest in ecological immunology (Owen, Nelson, & Clayton, 2010). Despite the interest that exists for host-parasite relationships in altricial birds,

research generally focuses on the effects of parasites on the hosts, and not what drives the fitness and reproduction of the parasites themselves.

Given the impact of ectoparasites on their avian hosts, it is important to understand ectoparasite ecology in these systems. In the North American Barn Swallow (*Hirundo rustica erythrogaster),* a common ectoparasite is the hematophagous Northern Fowl Mite (*Ornithonyssus sylviarum,* hereafter 'NFM'; Hund, Blair, & Hund, 2015). While there have been some studies on similar mites in commercial poultry systems (Chen & Mullens, 2008; De La Riva, Soto, & Mullens, 2015; Halbritter & Chen, 2011; Mullens et al., 2009; Owen et al., 2009), very few studies have examined how abiotic and biotic factors influence the population size and distribution of mites in wild systems, such as within the nests of barn swallows. While the effects of the host on mites and other ectoparasites in wild systems have been examined (Tschirren et al., 2007; Møller, 2000), these studies have not been exhaustive. For example, higher body condition increases immunocomptence (making it harder to obtain a blood meal), but nestlings with more resources support greater mite populations (Tschirren et al., 2007). Also, how the abiotic environment in which these parasites are living affects their population dynamics is relatively understudied.

Here, I examine how abiotic factors such as temperature, humidity, amount of nest lining, nest dimensions, and nest substrate influence NFM population size in the microenvironment of barn swallow nests. I also compare the influence of those abiotic factors relative to biotic ones, including body condition (nestling mass divided by wing length) and number of nestlings. Using these measures, I addressed the following question: what abiotic and biotic factors explain variation in mite population size in barn swallow nests? This question leads to four hypotheses: i) mite population size is influenced by abiotic factors alone, ii) mite population size is influenced by biotic factors alone, iii) mite population size is influenced by a combination of abiotic and biotic factors, iv) mite population size is not influenced by the abiotic or biotic factors that we

measured (table 1). The predictions for the results are included for both the abiotic factors and the biotic factors (table 2).

Question	Hypothesis	Data Collected
What are the relative contributions of abiotic and biotic factors to mite population size?	Mite population size is influenced by abiotic factors alone. (H_1)	Substrate, dimensions, amount of lining, nest temp and humidity.
	Mite population size is influenced by biotic factors alone. (H_2)	# of nestlings, mass of nestlings, nestling wing length.
	Mite population size is influenced by a combination of abiotic and biotic factors. (H_3)	Are biotic and abiotic measures (described above for H1 and H2) correlated? Which of these predict population size?
	Mite population size is stochastic, and is not influenced by any of the abiotic or biotic factors we measured. (H_0)	Use data above.

Table 1. Question and hypotheses table, along with description of data necessary to evaluate each hypothesis.

Variable	Type	Prediction	Rationale
Nest Area	Abiotic	Unsure.	Exploratory, could have an effect on density dependent factors.
Substrate	Abiotic	Unsure.	Exploratory, but likely has an effect on temperature and other factors in microclimate.
Amount of lining	Abiotic	Positively correlated with population size.	More lining would give the mites the ability to get further away from the hosts, allowing them to live and lay their eggs in areas where the temperatures are closer to their preferences. (De La Riva 2015)
Cup temp	Abiotic	Negatively correlated with population size. With an ideal around 28-30*C	Mites moved within an experimental temperature gradient, arresting at ~30°C. Additionally, mite eggs will not hatch if exposed to high temperatures (~39°C). (Halbritter 2011, Chen 2008)
Cup humidity	Abiotic	Positively correlated with population size.	Halbritter 2011 found that higher humidity positively impacted mite survivability when off host.
Body condition	Biotic	Negatively correlated with body mass.	Inflamed skin blocks mite access to blood meal and compromises survival and development of mites (Owen 2009). Tschirren found that nestling mass and PHA response were positively correlated in great tit nestlings (2007). However, Tschirren 2007 also found a positive correlation between supplementary feeding of nestlings, and mite population size.
Number of nestlings	Biotic	Positively correlated with mite population size.	More nestlings likely means more surface area to spread over, allowing more access to uncompromised tissues (and tissue recovery as they move on to new sites).

Table 2. Variables, predictions, and rationales.

Study Organisms

Northern Fowl Mites *(Ornithonyssus sylviarum)*

Much research has been done to investigate the biotic and abiotic factors driving population dynamics in NFMs infecting commercial poultry (Chen & Mullens, 2008; De La Riva, Soto, & Mullens, 2015; Mullens et al., 2009; Owen et al., 2009). In general, the poultry literature shows that mites survive longer without a blood meal in humid environments (~85%), and in lower temperatures, with an ideal around 30°C (Chen & Mullens, 2008). NFM populations have been shown to be negatively impacted by strong host inflammatory responses in these systems (Owen et al., 2009).

The host specificity of these mites is unknown (Hund, personal communication). The populations in Colorado may differ from those studied in commercial poultry in important ways. For example, the NFM populations studied in commercial poultry settings cannot survive long without a blood meal (Chen & Mullens, 2008), whereas mite populations in Colorado overwinter in nests (Hund, Blair, & Hund, 2015), ready to welcome the swallows back in the spring. This may indicate that mites living in the wild are adapted to a broader range of environmental contexts, including those required to survive during the winter and for long periods without feeding.

Barn Swallows *(Hirundo rustica)*

Barn Swallows are one of the most common birds in the world, in terms of both abundance and range (Brown & Brown, 1999). Although it is thought that they used to nest in caves and other natural settings (Brown & Brown, 1999), they now nest almost exclusively in buildings and other structures, including barns, sheds and road infrastructure such as bridges and culverts. This

close association with humans (and their relatively large colony sizes) has led to extensive study of the species to answer a variety of questions within ecology and evolutionary biology.

Barn Swallow nests are mud cups, lined with straw and horsehair, then typically lined further with feathers. New nests are constructed every breeding season, but Barn Swallows prefer to use nests from previous seasons in order to minimize delays in breeding (Safran, 2006). The nestlings are altricial, meaning they are unable to care for themselves upon hatching, and they require extensive parental care. Nestlings typically fledge (leave the nest) around 20 days after hatching, but the range is 15-24 days (Brown & Brown, 1999). Before they fledge, they are restricted to their nest site where they are cared for by both parents.

Methods

Mite Treatment

Existing mites in experimental nests were removed using a heat disinfection method (Hund, Blair, & Hund, 2015) three days after clutch completion (n=60). Briefly, eggs were removed and a heat gun was used to heat the nests to 125°C. The heating process took approximately five minutes. After the nests had cooled to <29°C, the eggs were returned to the nest. After nests were disinfected, they were re-infected with 100 live field-collected mites.

iButtons

Nest temperature and humidity measurements were collected every ten minutes for 14 days using iButton data loggers (Maxim Integrated) (n=30). The remaining parasite-treated nests did not have nest iButtons (n=30). iButtons were placed in the nest on the same day that nests were disinfected and then re-infected with 100 mites. The logger was placed just below the feather lining of the nest to capture the microclimate in which both mites and nestlings live.

After the iButtons were collected, the data was processed in R using the “Plyr” package (R Core Team, 2014; Wickham, 2011). This enabled us to manage the data in a single database from which a variety of variables could be exported, including the daily means, maxima, minima; as well as overall maximum and minimum measurements for temperature and humidity.

Mite counts

Field parasite counts were conducted when nestlings were 12 days of age. These measurements included counting 1) how many mites were on the field assistant’s hand after being placed in the nest for 30 seconds, 2) the number of mites on each nestling, and 3) the number of mites on the paper towel that the nestlings were on while removed from the nest. This method has been used in previous studies, and has been shown to correlate with mite population counts using Berlese funnels (Hund, Blair, & Hund, 2015; Møller, 1990). Nests that had iButtons were collected ten (range: 7-12) days after nestlings fledged (unless a new clutch had already been started) and were then put into Berlese funnels for 24 hours to get a more precise measure of final mite population (n=20; Hund, Blair, & Hund, 2015). A Berlese funnel is a metal funnel with a screen to put the nests on, with a beaker of ethanol underneath to catch anything that falls out. A bright lamp is placed over the funnel as a source of heat and light, causing any live arthropods within the nest to emerge. The arthropods then fall down the funnel into the beaker. Arthropods that were collected from the Berlese funnels were then sorted and counted using a dissecting microscope, according to procedures used in our lab previously (Hund, Blair, & Hund, 2015). The samples were separated into two categories: mites and other arthropods. Other arthropod numbers were small enough that they were counted individually. The mite populations were variable, but some were large enough that individual counting would have been unmanageable; for this reason, the number of mites in each sample was estimated by volume. To do this, 100 mites were counted and put in a micro-centrifuge tube as a

reference. Then, mites were added into a new tube until its volume was the same as that of the reference tube. Once all the mites in a nest were accounted for, the number of complete tubes was counted, and multiplied by 100 to get an estimate of mite population size for a given nest.

Nestling measurements

Nestling mass, right wing length, and number of nestlings were measured on day 12. Nestling mass was measured in grams using an electronic balance (+/- 0.01g, *AWS-100*). Wing length was measured on the right wing in millimeters (+/- 0.5 mm) using a wing rule (AFO Banding Supplies). Nestling mass was divided by wing length to calculate body condition. These body conditions were used to calculate average nestling body condition for each nest.

Nest Characteristics

On day 12 after hatching, nest lining was evaluated on a qualitative scale from zero to three (zero being no feather lining, three being so many feathers that they could barely fit in the nest cup). Nest dimensions were measured using a measuring tape and nest area was calculated by multiplying the width and height.

Statistical Analysis

All statistical analyses were performed with the statistical package R version 3.3.2 (R core Team 2016) and the lme4 package: linear mixed-effects models using 'Eigen' and S4 (Bates et al. 2016). Temperature and humidity data from iButtons were highly correlated (table 3), so we collapsed these variables using a principle components analysis with the R function "prcompand." We kept the first PC for further analysis. PC1 explained 45% of the variance; nests with high PC1 scores have high temperature and low humidity, and nests with low PC1 scores have low temperature and high humidity (table 4 and figure 1).

Variable	by Variable	Spearman ρ	Prob>| ρ |
avg_max_nt	mean_nt	0.6315	0.0005*
avg_min_nt	mean_nt	0.6684	0.0002*
avg_min_nt	avg_max_nt	-0.0783	0.7038
max_nt	mean_nt	0.2561	0.2067
max_nt	avg_max_nt	0.7299	<.0001*
max_nt	avg_min_nt	-0.2034	0.3189
min_nt	mean_nt	0.5050	0.0085*
min_nt	avg_max_nt	0.0168	0.9353
min_nt	avg_min_nt	0.7764	<.0001*
min_nt	max_nt	-0.1125	0.5843
mean_nh	mean_nt	-0.7306	<.0001*
mean_nh	avg_max_nt	-0.3491	0.0805
mean_nh	avg_min_nt	-0.5932	0.0014*
mean_nh	max_nt	-0.2000	0.3273
mean_nh	min_nt	-0.5193	0.0066*
avg_max_nh	mean_nt	-0.5432	0.0041*
avg_max_nh	avg_max_nt	-0.0434	0.8332
avg_max_nh	avg_min_nt	-0.6438	0.0004*
avg_max_nh	max_nt	0.0509	0.8048
avg_max_nh	min_nt	-0.5610	0.0029*
avg_max_nh	mean_nh	0.9029	<.0001*
avg_min_nh	mean_nt	-0.7162	<.0001*
avg_min_nh	avg_max_nt	-0.7730	<.0001*
avg_min_nh	avg_min_nt	-0.1863	0.3621
avg_min_nh	max_nt	-0.5897	0.0015*
avg_min_nh	min_nt	-0.0920	0.6550
avg_min_nh	mean_nh	0.5631	0.0027*
avg_min_nh	avg_max_nh	0.2684	0.1850
max_nh	mean_nt	-0.2540	0.2105
max_nh	avg_max_nt	-0.0291	0.8879
max_nh	avg_min_nt	-0.3272	0.1028
max_nh	max_nt	0.0010	0.9960
max_nh	min_nt	-0.5104	0.0077*
max_nh	mean_nh	0.5303	0.0053*
max_nh	avg_max_nh	0.6349	0.0005*
max_nh	avg_min_nh	-0.0605	0.7690
min_nh	mean_nt	-0.3463	0.0831
min_nh	avg_max_nt	-0.4790	0.0133*
min_nh	avg_min_nt	-0.0250	0.9037
min_nh	max_nt	-0.5084	0.0080*
min_nh	min_nt	0.0058	0.9775
min_nh	mean_nh	0.2909	0.1493
min_nh	avg_max_nh	0.0810	0.6940
min_nh	avg_min_nh	0.7436	<.0001*
min_nh	max_nh	-0.0557	0.7869

Table 3. Spearman's rank correlation matrix with temperature and humidity variables. "nt" means nest temperature, and "nh" means nest humidity.

Variable	PC1
Mean nest temperature	0.3942056
Average maximum nest temperature	0.1003406
Average minimum nest temperature	0.3621817
Maximum nest temperature	0.1036807
Minimum nest temperature	0.3354633
Mean nest humidity	-0.4520570
Average maximum nest humidity	-0.4036779
Average minimum nest humidity	-0.3032444
Maximum nest humidity	-0.3011739
Minimum nest humidity	-0.1734888

Table 4. Temperature and humidity variable loading onto PC1.

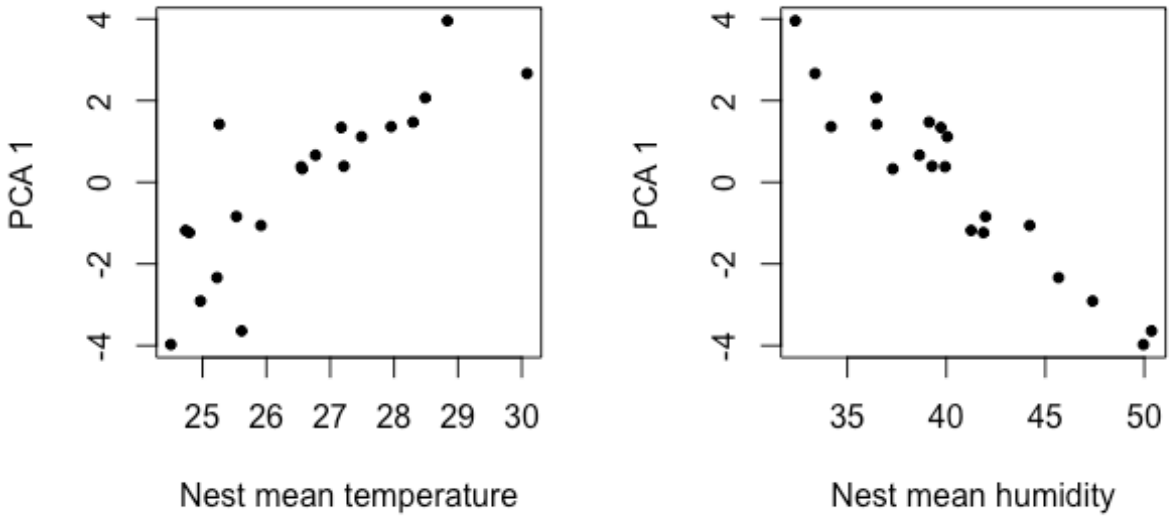

Figure 1. PC1 as a function of nest mean temperature (left) and nest mean humidity (right). Mean nest temperature is positively correlated with PC1, while nest mean humidity is negatively correlated with PC1. Note: This figure is constructed with raw data.

Given that iButtons were placed in a subset of our experimental nests, we analyzed the interactions between PC1, nestling number, and body condition for these nests (n = 20), with Berlese funnel mite counts as our response variable. These counts were raw end populations, not adjusted for the original starting populations of 100 mites per nest.

For our larger data set, we built a model with nest lining, nest area, nest substrate, nestling number, and body condition with day 12 mite counts as our response variable. Due to power constraints, we were not able to include all pair-wise interactions in this model. We therefore tested the importance of interactions separately. None of these interactions were significant, so our full model contained all fixed effects without interactions before model selection.

To test if counts in the field on day 12 were a good proxy for total mite populations, we compared counts in nests that also had Berlese funnel counts. Analyses were conducted to determine if mite counts were correlated with the presence of other arthropods, and whether and how temperature and humidity PCs correlated with other nest measures (lining, area, substrate).

All models were generalized linear mixed models with an over-dispersed Poisson distribution with site and nest as random effects. Numerical fixed effects were z-transformed (subtract the mean and divide by the standard deviation) to help with scale differences between our variables. Model selection was done using the R package MuMin and was based on AICc (because of small sample sizes). Results for all final models with a delta AICc smaller than two are reported.

Results

Berlese funnel count data subset

For the Berlese funnel data set, the final model contained PC1, nestling body condition and the interaction between these variables. PC1 and body condition were both positively associated with mite counts (*PC1: $F = 0.02$, $p = 0.01$, $b = 0.86$, Body Condition: $F=1.71$, $p<0.001$, $b = 1.90$, Body Condition x PC1: $F = 16.91$, $p<0.001$, $b = 2.07$, $n = 20$*). Figure 2 shows the raw data associated with the models. Many populations were below the starting population size of 100

individuals at the end of the experiment, indicating that some populations went extinct. Although many populations did not get larger in size, a few populations within certain ranges of both microclimate (PC1) and average nestling body condition were fairly large in size (figure 2). These results indicate that mite populations are larger in nests that are relatively warm, have low humidity, and have nestlings in good body condition. When comparing mite numbers to other arthropods, we found that there was a significant negative association (*other arthropods:* $F = 6.51$, $p = 0.013$, $b = -0.008$, $n = 20$; figure 3). Nests with more of the other arthropods had fewer mites.

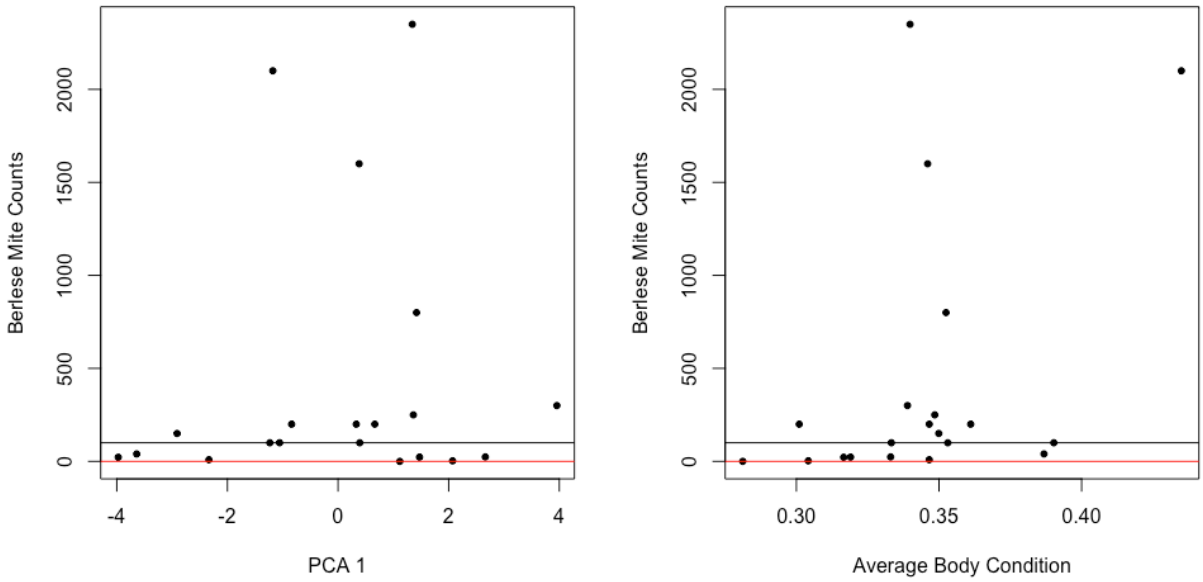

Figure 2. Berlese funnel mite counts as a function of PCA1 (Left), and the same counts as a function of average nestling body condition (Right). The black line indicates 100, which was the starting point of all populations, and the red line indicates zero. Points above the black line were larger than the starting population, points below the black line decreased in size and points on the red line went exinct. Note: This figure is constructed with raw data, and is not adjusted for random effects included in the model.

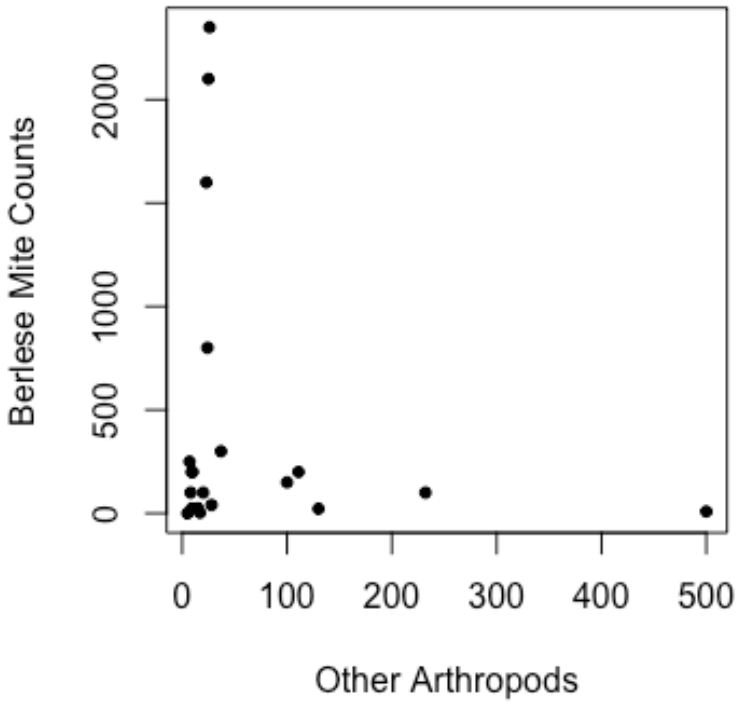

Figure 3. Berlese funnel mite counts as a function of other arthropods in the nest (F = 6.51, p= 0.013, b= -0.01, n= 20). Note: This figure is constructed with raw data, and is not adjusted for random effects included in the model.

Larger data set (all day 12 parasite nests)

We found that the field mite counts on day 12 were significantly correlated with our Berlese funnel counts (*F = 15.26, p= <0.001, b= 0.01, n= 20*; figure 4). This indicates that field counts are a good proxy for the total mite population in a nest.

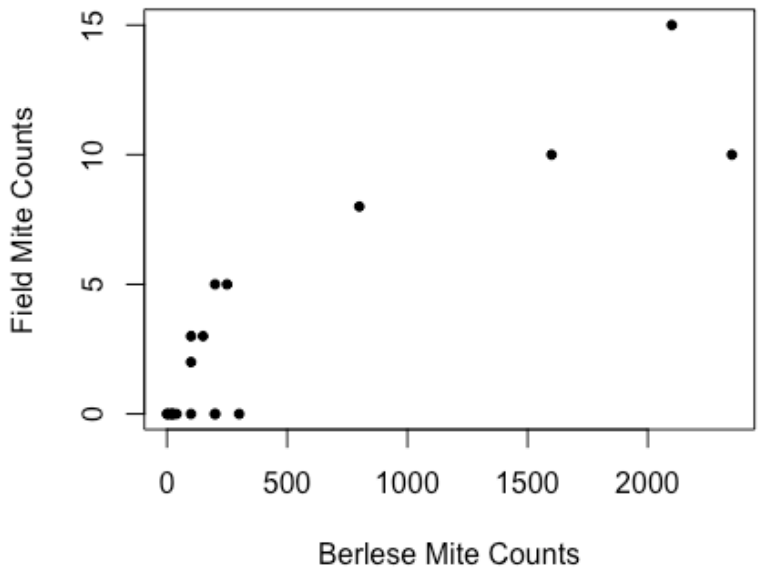

Figure 4. Field mite counts as a function of Berlese funnel counts (F = 15.26, p= <0.001, b= 0.01, n= 20). Note: This figure is constructed with raw data, and is not adjusted for random effects included in the model.

The final model for this larger data set kept both substrate (figure 5) and body condition as fixed effects (*Substrate (wood): F = 3.732, p= 0.013, b= 2.22; Body condition: F =6.37, p=0.017, b=0.57, n= 42*). Nestling number, nest lining, and nest area were not significant predictors of mite population sizes. Together, these results indicate larger populations of mites are present in nests constructed on wooden substrates than on concrete or metal substrates, and in nests that contain nestlings with high body condition.

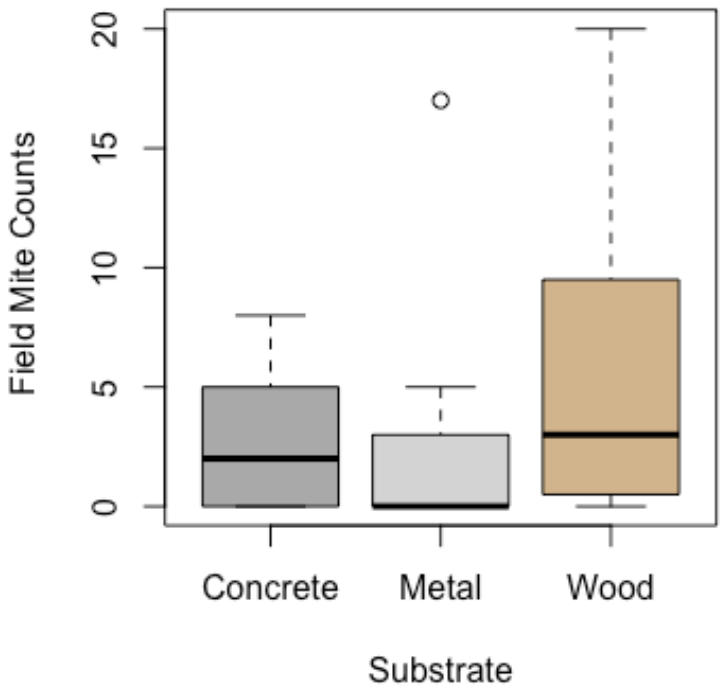

Figure 5. Field mite counts according to substrate on which the nest was built. Wood has the highest mean, while metal has the lowest (wood: F = 3.834, p= 0.012, b= 2.40, wood n = 16, metal n = 2, concrete n = 2). Note: This figure is constructed with raw data, and is not adjusted for random effects included in the model.

When we compared characteristics of the nest (substrate, lining, area) and our temperature/humidity PC, only substrate was kept in the final model (figure 6) (*substrate F* = 4.35, *p*= 0.05*, b*= 1.29, n= 20). Thus, the substrate upon which nests are constructed has an important influence on nest temperature and humidity, with nests on wood having temperature and humidity values between those of nests on metal or concrete.

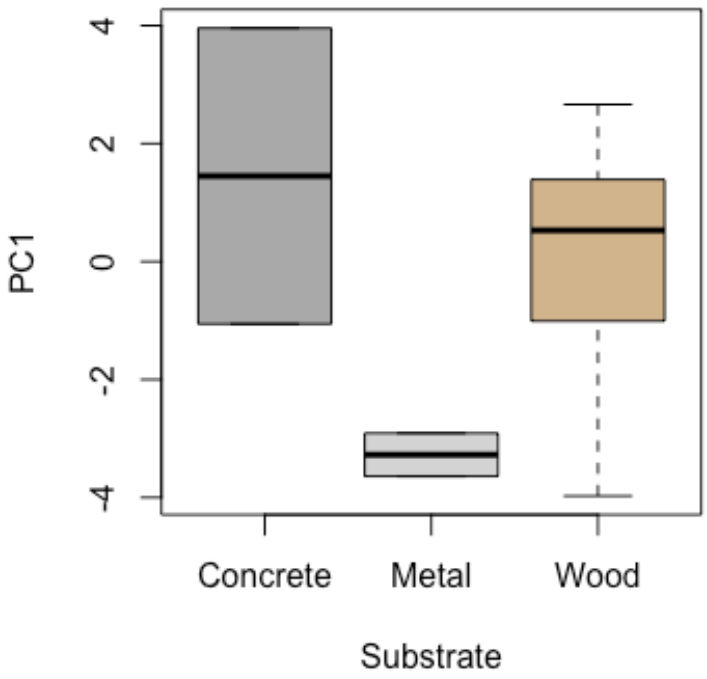

Figure 6. PC1 according to substrate on which the nest was built. Concrete has the highest mean, metal the lowest, and wood has an intermediate *(substrate F = 4.35, p= 0.05, b= 1.29, n = 20; wood n = 16, metal n = 2, concrete n = 2*). Note: This figure is constructed with raw data, and is not adjusted for random effects included in the model.

Discussion

Little is known about the optimal environmental conditions for ectoparasites in wild systems, and how abiotic and biotic factors contribute to population sizes. With barn swallows, most nests do not have any mites, while others have very large populations. While some studies in other systems have looked at how features of the host influence population size, the microclimate and other abiotic factors of the environment where ectoparasites are living and reproducing are not well studied. In this study, I experimentally added 100 mites to disinfected nests, and analyzed predictors of how many mites were present in each nest on day 12 after nestling hatching, as well as after the nestlings had fledged. To evaluate the influence of abiotic and biotic factors on mite populations, I constructed generalized linear mixed effect models to elucidate the effects of host body condition, host number, presence of other arthropods, nest microclimate (temperature and humidity), and features of nest construction (substrate on which the nest was constructed, amount of lining in the nest, and nest dimensions).

Results from the smaller (Berlese data set) and larger data set indicate that NFM population size is influenced by a combination of abiotic and biotic factors. iButton data from the Berlese funnel nest subset showed mite populations were larger in relatively warm, dry nests. While body condition explains population variation in both the Berlese funnel subset, as well as in the larger day 12 data set.

Body condition

Based on previous studies and theory (table 2), I predicted that populations of mites might be smaller on nestlings in good body condition. For example, the tasty chick hypothesis predicts that lower body condition nestlings are preferred by ectoparasites because they are not able to mount a strong immune system response in reaction to being parasitized (Christe, Møller, & Lope, 1998). Additionally, it has been shown that inflammation blocks mite access to blood

meals, and therefore negatively impacts their survival and development (Owen et al., 2009). This, coupled with findings in other altricial birds which indicate mass is positively correlated with general immunocompetence (PHA response; Tschirren et al., 2007), leads to an inference that body condition and mite population size would be negatively correlated. However, my findings indicate that mite densities are higher in nests in which nestlings are, on average, in higher average body condition. This is consistent with findings of other studies which suggest that the increased resource represented by hosts in better condition will support larger ectoparasite populations (e.g. Tschirren et al., 2007). It is possible that the inflammatory response of Barn Swallow nestlings is not as strong as that of the birds in the 2009 Owen study, given that Barn Swallows are much smaller than chickens. Thus, the benefit of greater resource availability may overwhelm the negative effects of greater immune response in higher condition nestlings.

Temperature and humidity

Previous work on NFM populations on chickens suggests that cool and humid environments are optimal conditions for mites (table 2). However, this prediction was not supported in my study population. Instead, I found that mite populations were found in warmer, less humid nest environments (figure 2). It is possible that this difference may be due to a difference in life history. While NFM in the chicken system spend most of their time on the hosts (Owen & Mullens, 2004), the mites in Barn Swallow nests primarily live in the nest and are only on the host to feed (Hund, personal communication). This difference in life history may be the cause of the different optima in terms of temperature and humidity. It is also possible that differences between the results of this study and ones conducted previously are related to the environmental context of the study itself. Whereas my study was conducted on wild populations subjected to variation in ambient climate conditions, the poultry studies were conducted in a highly controlled environment associated with the commercial poultry industry. Further, the

chicken study indicated an optimal temperature for mites as 30°C (Chen & Mullens, 2008), a temperature that was rarely observed in our study.

Additionally, we found that when both the abiotic conditions and the body condition parameters were favorable, this led to larger mite populations. While most populations were less than, or similar to the starting population, some were quite large (figure 2). We found that these larger populations were also associated with certain ranges of microclimate and nestling body condition, with a general trend towards warm, dry nests with higher average nestling body condition (figure 2). Taken together, the optimal conditions for NFM population size appear to be a combination of abiotic factors (high temperature and low humidity) and the biotic factor of nestling body condition, with higher average body condition being positively correlated with mite population size.

Substrate

In the day 12 data set, we found that substrate had influence on mite population size. Nests built upon wooden structures (e.g., beams) were associated with higher mite population counts than those constructed on metal or concrete; additionally, nest substrate was shown to explain variation in temperature and humidity. Given that wood is a better thermal insulator than concrete or metal (Ankersmit & Stappers, 2016), the nests built on wood are likely not subject to the same extremes of hot or cold. This stability may allow for quicker reproduction by keeping the temperature within a more tolerable range (preventing the temperature from getting too low and slowing mite egg development, or too high and killing the eggs). This nest characteristic is changing the nest microclimate, and therefore influencing mite population size.

Conclusion

NFM are patchily distributed across barn swallow nests both within and among nest sites (e.g., barns). As their potential effects on their hosts are highly costly in terms of nestling mortality (Hund *unpubl*), it is important to understand what influences their population size. The findings of this study help predict where mite populations will have the greatest success. These results indicate that NFM populations are not just influenced by their host, but also by the abiotic environment. In future work, it would be interesting to examine other possible features of nest construction and location that may influence the microclimate of the nest, and therefore mite success. Additionally, it would be prudent to examine populations in other locations to see how they are influenced by microclimate, and how they compare to the populations in this study.

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