



Microclimate and host body condition influence mite population growth in a wild bird-ectoparasite system



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ARTICLE INFO

Keywords:

Ectoparasites
Microclimate
Host condition
Population growth
Bird nest

ABSTRACT

Parasite populations are never evenly distributed among the hosts they infect. Avian nest ectoparasites, such as mites, are no exception, as their distribution across the landscape is highly aggregated. It remains unclear if this pattern is driven by differences in transmission events alone, or if the environment that parasites inhabit after transmission also plays a role. Here, we experimentally examined the influence of the post-transmission microclimate, nest characteristics, and host condition on ectoparasite population growth in a bird-ectoparasite system. We infested barn swallow (*Hirundo rustica erythrogaster*) nests with a standardized number of Northern Fowl Mites (*Ornithonyssus sylvarium*) and analyzed both biotic (nestling mass, wing length, number of other arthropods present in the nest, and brood size) and abiotic (temperature, humidity, nest lining, nest dimensions, and substrate upon which the nest was built) predictors of mite population growth. Our results suggest that mite populations were most successful, in terms of growth, in nests with higher temperatures, lower humidity, few other arthropods, and hosts in good condition. We also found that nests built on wooden substrates support larger populations of mites than those constructed on metal or concrete. These findings lend insight into the factors that drive large-scale patterns of ectoparasite distributions.

1. Introduction

The majority of parasites in nature are characterized by an aggregated distribution, where most of the parasite burden is accounted for by a small proportion of possible hosts (Atkinson et al., 2009). Despite the widespread nature of this pattern, there remain important gaps in our understanding of why parasite populations thrive in certain hosts and locations, but not others (Atkinson et al., 2009). In particular, it is unclear whether these aggregated distribution patterns arise solely from differences in exposure to and transmission of parasites, or if more localized effects of variation in host quality and the environment in which the parasites live is also important (Whiteman and Parker, 2004; Balakrishnan and Sorenson, 2007; Gómez-Díaz et al., 2008; Thamm et al., 2009; Dallas and Presley, 2014). The ecology and population dynamics of parasites that commonly infect humans and domesticated animals has been fairly well described; however, there is still much to learn about the ecology of parasites in wild host populations and the mechanisms that shape the distributions of these parasites across the landscape.

Bird-ectoparasite systems have been widely used to study the impact of ectoparasites on host immunity, physiology, and survival (Møller, 1990; Christe et al., 1998; Møller et al., 2009; Eisner Pryor and Casto, 2015). Many of these studies focus on ectoparasite infestations that occur exclusively at nest sites of avian species with altricial nestlings (which require extensive parental care) (e.g. Brinkhof et al., 1999; Roulin et al., 2003; Dubiec et al., 2006; Drobnik et al., 2013). These systems are convenient to study, as parasites and hosts (nestlings) are confined to a discrete physical location (the nest) for up to several weeks during development. Additionally, it is relatively easy to control, manipulate, and accurately assess ectoparasite infestations in the nest environment throughout the nestling development period (Møller, 1990; Owen et al., 2010; Hund et al., 2015b). Ectoparasites have been shown to impose important fitness costs on their avian hosts, including increased nestling mortality (Proctor and Owens, 2000; Møller et al., 2009), reduced body size (Eisner Pryor and Casto, 2015), diminished secondary sexual trait expression (Lehmann, 1993; Proctor and Owens, 2000), and changes in parental resource provisioning (Bouslama et al., 2002; Buechler et al., 2002; Hund et al., 2015a). Moreover, the effect of

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<https://doi.org/10.1016/j.ijppaw.2018.07.007>

Received 30 May 2018; Received in revised form 29 July 2018; Accepted 31 July 2018

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Table 1
Question and hypotheses tested in this manuscript, along with description of data we collected to evaluate each hypothesis.

Question	Hypothesis	Data Collected
What are the relative contributions of abiotic and biotic factors to nest mite population growth?	Mite population size is influenced by abiotic factors alone. (H ₁)	Nest substrate, nest dimensions, amount of lining, nest temp and humidity.
	Mite population size is influenced by biotic factors alone. (H ₂)	# of nestlings, body condition of nestlings.
	Mite population size is influenced by a combination of abiotic and biotic factors. (H ₃)	Test for correlations and interactions between abiotic and biotic factors
	Mite population size is not influenced by any of the abiotic or biotic factors we measured. (H ₀)	Mite populations are variable, but not correlated with abiotic and biotic measures.

ectoparasites on the expression of hormones and immune function in the host has been an area of interest in ecological immunology (Casto et al., 2001; Klein, 2004; Owen et al., 2010; Eisner Pryor and Casto, 2015). To date, most of the research on host-parasite relationships in birds has focused on the effects of parasites on the hosts, and not what drives the fitness and reproduction of the parasites themselves. Here, we set out to investigate the abiotic and biotic factors which influence ectoparasite populations after they are transmitted to nests, and thus address an important knowledge gap related to host-parasite relationships in altricial birds.

Competing hypotheses in the literature currently make it unclear how the condition and number of hosts may influence the success of nest ectoparasites. For example, higher nestling body condition has been shown to increase immunocompetence and skin inflammation (making it harder for parasites to obtain a blood meal) (Owen et al., 2009), and it is suggested that nestlings in good condition can contribute more resources towards ectoparasite defense (Norris and Evans, 2000; Brommer et al., 2011). However, other studies show that nestlings with more food resources support greater parasite populations (Bouslama et al., 2002; Tschirren et al., 2007). Similar mixed predictions exist for variation in host number, or brood size. Nestlings that must compete with more siblings often have fewer resources and are in worse condition compared to nestlings with fewer siblings (Neuenschwander et al., 2003) and the presence of ectoparasites has been shown to increase variance in offspring quality (Christe et al., 1998; Szép and Møller, 2000). This can result in similar ectoparasite levels across different brood size manipulations (Hörak et al., 1998). However, it has also been suggested that larger brood sizes may mean more available hosts and a larger carrying capacity for ectoparasites (Møller, 1991; Richner, 1995).

Beyond host condition and number, the influence of the abiotic environment remains a relatively unexplored, but potentially important predictor of ectoparasite population growth in wild systems. Work assessing temperature and humidity conditions in domestic poultry suggests that ectoparasites do best within a specific environmental range (DeVaney and Beerwinkle, 1980; Nordenfors et al., 1999). However, it is largely unknown how other environmental factors, such as nest area and substrate, may influence ectoparasite success. Further, the relative contributions of and interactions between abiotic and biotic factors that may influence ectoparasites are largely unknown as abiotic and biotic factors are rarely assessed in the same study.

The hematophagous Northern Fowl Mite, *Ornithonyssus sylviarum*, hereafter 'NFM', is a common ectoparasite in the North American barn swallow (*Hirundo rustica erythrogaster*). While several studies have investigated the population dynamics of similar mites in commercial poultry systems (Chen and Mullens, 2008; Mullens et al., 2009; Owen et al., 2009; Halbritter and Mullens, 2011; De La Riva et al., 2015), very few studies have examined how abiotic and biotic factors influence the population size and distribution of mites in wild systems, such as the nests of barn swallows. Here, we examine how abiotic factors, such as temperature, humidity, amount of nest lining, nest dimensions, and nest

substrate influence NFM population growth in the microenvironment of barn swallow nests. We also compare the influence of these abiotic factors to biotic factors, including body condition (nestling mass divided by wing length) and number of nestlings (brood size). Using these measures, we seek to evaluate the following questions: 1) Is the aggregated distribution of NFM that we observe in nature driven only by differences in transmission, or do nest level differences in microclimate and host quality play a role? 2) What specific abiotic and biotic factors explain variation in mite population size in barn swallow nests? In this study, we experimentally control for transmission (using equal initial starting populations), which allows us to test if mite population size is influenced by transmission alone, in which case we would predict very similar final population sizes, or if local nest and host characteristics influence the success of mite infections. This 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 associated with these hypotheses are shown in Table 2.

2. Study organisms

2.1. Northern Fowl Mites (*Ornithonyssus sylviarum*)

The NFM is a common pest in industrial poultry, but is known to infect over 70 species of wild birds (Murillo and Mullens, 2013). In North American barn swallows, the NFM completes its entire life cycle in the nest, moving between the nest material and the birds. Its life cycle consists of five stages: egg, larva, protonymph, deutonymph, and adult. Protonymphs and adults require blood meals (Murillo and Mullens, 2013). The life cycle from egg to adult is completed in 5–12 days (Murillo and Mullens, 2013). Virgin females can lay unfertilized eggs, which result in predominantly male offspring, which they can then mate with to begin an infestation (Murillo and Mullens, 2013).

Research investigating the biotic and abiotic factors driving population dynamics in NFMs has primarily been conducted with commercial poultry (Chen and Mullens, 2008; Mullens et al., 2009; Owen et al., 2009; De La Riva et al., 2015). In general, the poultry literature shows that mites survive longer without a blood meal in humid environments (~85%), and in relatively lower temperatures (Chen and Mullens, 2008). Additionally, NFM populations are negatively impacted by strong host inflammatory responses in these systems (Owen et al., 2009).

However, the host specificity of NFM is unknown and wild populations may differ from those studied in commercial poultry in important ways. For example, NFM populations studied in commercial poultry settings cannot survive long without a blood meal (Chen and Mullens, 2008), whereas mite populations in wild systems are known to survive between breeding seasons (Barclay, 1988; Hund et al., 2015a, 2015b). This may indicate that mites infecting barn swallows are

Table 2

Data collected for each nest, whether that variable is abiotic or biotic in nature, and prediction and rationale for how that variable may influence mite population growth.

Variable	Type	Prediction	Rationale
Nest Area	Abiotic	Unsure.	Exploratory, could have an effect on density dependent factors limiting mite growth
Substrate	Abiotic	Unsure.	Exploratory, but likely has an effect on temperature and other microclimate factors.
Amount of feather 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 et al., 2015)
Nest 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 and Mullens, 2011; Chen and Mullens, 2008)
Nest Cup humidity	Abiotic	Positively correlated with population size.	Halbritter and Mullens, 2011 found that higher humidity positively impacted mite survivability when off host.
Body condition	Biotic	Could positively or negatively impact population growth.	Inflamed skin blocks mite access to blood meal and compromises survival and development of mites (Owen et al., 2009). Tschirren found that nestling mass and PHA response were positively correlated in great tit nestlings (2007). However, Tschirren et al., 2007 also found a positive correlation between supplementary feeding of nestlings, and mite population size.
Number of nestlings (Brood Size)	Biotic	Positively correlated with mite population size.	More nestlings likely means more hosts and surface area, allowing more access to uncompromised tissues (and tissue recovery as they move on to new sites).

adapted to a broader range of environmental contexts, including prolonged periods without food such as those encountered during the winter.

2.2. North American barn swallows (*Hirundo rustica erythrogaster*)

Barn swallows are one of the most common birds in the world in terms of both abundance and geographic distribution (Brown and Brown, 1999). Barn swallows nest in loose social colonies; in this study, group size ranged from one to 35 breeding pairs. Barn swallows nest almost exclusively in human buildings and other structures, including barns, sheds, and infrastructure such as bridges and culverts. Their close association with humans (and relatively large colony sizes) have led researchers to leverage the system to answer a variety of questions within ecology and evolutionary biology (Møller, 2000; Safran et al., 2005; Scordato and Safran, 2014; Hund et al., 2015a; Romano et al., 2017).

Barn swallows build mud cup nests in which small mud pellets are sewn together with straw and horsehair and 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 breeding delays (Safran, 2006). Pairs lay 3–5 eggs per brood and incubate for 12 days. Nestlings typically fledge around 20 days after hatching, but the range is 15–24 days (Brown and Brown, 1999). Before fledging, they are restricted to their nest site, where they are cared for by both parents.

Previous work on NFM in a Colorado population of barn swallows found that NFM are the most common nest parasite and that they feed primarily on nestlings. Out of 172 monitored nests, 58.1% were infected with NFM, with a mean infection intensity of 101.09 mites. Infections ranged from 0 to up to 1500 mites (Hund, 2017). Mites in this study were counted in the field as described below, and are an indication of, but not an exact count of, the total NFM population size within the nest.

Barn swallow nests are not only home to NFM, but a variety of other arthropods. Although we do not formally classify the other arthropods encountered in this study, previous work has identified these to include beetles, flies, pseudo-scorpions, spiders, and lice (Hund et al., 2015b). Although rarely encountered, we placed these other arthropods into a single group for statistical analysis.

3. Methods

3.1. Mite treatment

Research took place at 24 barn swallow breeding colonies located in Boulder County Colorado from May–August 2016. Adult barn swallows were captured using mist nets and each bird was given a USGS metal identification band and a unique combination of color bands. Breeding pairs were identified and assigned to nests through behavioral observations. All nests at each of the study colonies were monitored every other day to track lining activity, egg laying, and the hatching and growth of nestlings. This study was part of a larger experiment examining the role of genes and environment in color development in nestling barn swallows. For all experimental nests ($n = 58$), existing NFM were removed from nests using a heat disinfection method (Hund et al., 2015b) three days after clutch completion. Briefly, eggs were removed and a heat gun was used to heat all parts of the nests to 125 °C at least two times. The heating process took approximately 5 min. 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 NFMs.

3.2. iButtons

iButton DS1923 data loggers (Maxim Integrated) are miniature sensors (17.4 mm in diameter, and 6.4 mm in depth) that were used to track temperature and humidity throughout nestling development within a subset of our experimental nests ($n = 38$). iButtons were installed in the nest at the same time that NFMs were experimentally added to the nest (3 days after clutch completion). iButtons were placed in the nest cup between the feather lining and mud structure to capture the microclimate in which NFMs live. Nest temperature and humidity measurements were collected every 10 min for 14 days for a total of around 2024 measurements for each nest.

3.3. Mite counts

Nest parasites were counted in the field when nestlings were 12 days old. We counted 1) how many mites were on the field assistant's hand after being placed in the nest for 30 s, 2) the number of mites on each nestling, and 3) the number of mites in the container used to hold

the nestlings. These three counts were added together and used as a proxy for mite population size in each nest. Similar methods have been used in previous studies and correlate with mite population counts from nests placed in Berlese funnels (Møller, 1990; Hund et al., 2015b).

Whole nests containing iButtons were collected 10 days (range: 7–12) after nestlings fledged (unless a new clutch had already been started) and placed into Berlese funnels for 24 h to get a more precise measure of final mite population size ($n = 20$, seven nests failed and were not included, five had new clutches before collection and were not included). Arthropods that were collected from the Berlese funnels were then sorted and counted using a dissecting microscope, according to procedures used previously (Hund et al., 2015b). The samples were separated into two categories: NFM 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.

3.4. Nestling measurements

Nestling mass, right wing length, and the number of nestlings were measured for each nest on day 12. Nestling mass was measured in grams using an electronic balance (± 0.01 g, AWS-100). Flattened wing length was measured for the right wing in millimeters (± 0.5 mm) using a wing rule (AFO Banding Supplies). Nestling mass was divided by wing length to calculate individual body condition. Individual nestling body condition scores were used to calculate an average nestling body condition for each nest. Brood size consisted of the number of live nestlings on day 12.

3.5. Nest characteristics

On day 12 after hatching, nest lining (amount of feathers in the nest cup) 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 widest point of the nest cup by the height of the nest. The substrate on which the nest was built was categorized as either wood, metal, concrete, or other.

3.6. Treatment of animals

The handling and measuring of adult and nestling barn swallows, as well as the manipulation of nest parasite infections, was done in accordance with the guidelines set by the University of Colorado Institutional Animal Care and Use Committee (IACUC). All procedures in this study were approved by the IACUC (protocol number 1303). Parasite manipulations were within the natural range of infections that we observe for these birds.

3.7. 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., 2015). Before model selection, we investigated the extent of collinearity between fixed effects (correlation matrices and pair plots in supplementary material). Temperature and humidity data from iButtons were highly correlated, so we collapsed these variables using a principle components analysis with the R function “prcomp.” 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 (for additional details see supplement).

Given that iButtons were placed in a subset of our experimental nests ($n = 20$), we did not have the power with this small sample size to include all the variables we measured for each nest in one model. We were particularly interested in understanding the relative contribution of abiotic and biotic factors, and interactions between these factors, to mite population growth. Thus, for this dataset, we chose to focus on the iButton temperature and humidity data (nest microclimate, PC1) and host number and body condition. We analyzed the rest of the abiotic nest characteristics with the larger dataset (see below). We also did not have the power to include data on other arthropods in this model, so we analyzed these data separately to test for an association between mite population success and other arthropods in the nest. The response variable for these models was Berlese funnel mite counts. These Berlese funnel counts were raw final population counts, not adjusted for the original starting populations of 100 mites per nest. Given that all experimental nests had an identical starting point (100 mites), the end population size is a good proxy for population growth or decline: populations with fewer than 100 mites declined in size whereas populations with greater than 100 mites grew in size.

For our full data set, which had a larger sample size ($n = 42$, 16 of the original 58 nests failed and were not included in the analysis), we were again interested in understanding the relative contribution of, and interaction between, biotic and abiotic factors to mite success. In this dataset, we focused on the different nest characteristics that we measured along with host number and body condition. We built a model with nest lining, nest area, nest substrate, nestling number, and body condition; the response variable in this model was the number of mites found within nests and on nestlings when they were 12 days of age. We were not able to include all pair-wise interactions in this model due to constraints of statistical power. As we were still interested in exploring these interactions, we analyzed their significance using separate models. None of these interactions were statistically significant, so our full model contained all fixed effects without interactions before model selection. Given our results, we performed additional analyses with our iButton data subset to determine whether and how temperature and humidity PCs correlated with our other nest measures (lining, nest area, and substrate).

All models were generalized linear mixed models with a Poisson distribution for the response variables and site as a random effect. Abundance data for nest mites were over dispersed; to improve the performance of our models, we therefore included an observation level random effect (a random effect that models extra Poisson variation of count data) (Harrison, 2014). Numerical fixed effects were z-transformed to improve model stability, as well as to allow for comparison of the parameter estimates between fixed effects. Model selection was conducted using the R package MuMIn and was based on AICc (for small sample sizes). This approach penalizes model complexity while ranking models to find the best fit, and thus helps to prevent overfitting of models with multiple fixed effects (Burnham et al., 2011; Aho et al., 2014). Results for all final models with a delta AICc smaller than two are reported in the main text (Johnson and Omland, 2004).

4. Results

4.1. Data from field sampling and Berlese funnel sampling

To test if field counts on day 12 were a good proxy for total mite populations, we compared field counts to the counts we extracted using the Berlese funnel 7–12 days after the nestlings fledged ($n = 20$). We found that the field mite counts on day 12 were significantly correlated with our Berlese funnel counts ($F = 15.26$, $p < 0.001$, $b = 0.01$, $n = 20$; Fig. 1). This indicates that field counts are representative of the total

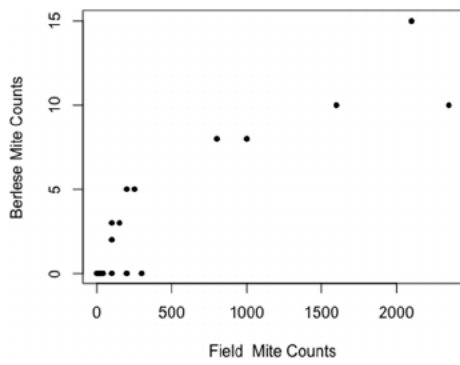


Fig. 1. Relationship between nest mite counts when nests were placed in a Berlese funnel 30–35 days after initial infections of 100 live mites (x-axis) and counts taken for the same nest ~10 days earlier in the field when nestlings were 12 days old and still in the nest (y-axis). There was a significant correlation between these two different counts.

mite population in a nest.

4.2. Subset of nests with iButtons and Berlese funnel counts

As expected, the mite counts in the Berlese funnel samples were aggregated (Fig. 2). Many populations declined to zero, while some populations grew to over 2000 mites.

For the Berlese funnel data set, the best-supported model contained PC1, nestling body condition, and the interaction between these variables. PC1 and body condition were both positively associated with mite counts, as was the interaction (*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*: $F = 16.91$, $p < 0.001$, $b = 2.07$, $n = 20$). The raw data associated with this model are presented in the supplement. Many populations fell below the starting population size of 100 individuals by the end of the experiment, indicating that some populations declined or went extinct. Although many populations did not increase, a few populations within certain ranges of both microclimate (PC1) and average nestling body condition were fairly large in size. Mite populations were larger in nests that were relatively warm, had low humidity, and contained nestlings in good body condition. We also found a significant negative association between mites and the presence of other arthropods ($F = 6.51$, $p = 0.013$, $b = -0.008$, $n = 20$; Fig. 3), where nests with a higher abundance of other arthropods had fewer mites.

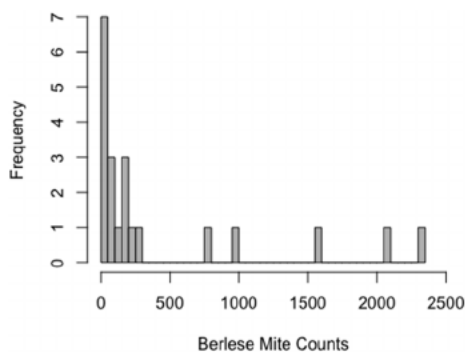


Fig. 2. Distribution of nest mite population sizes estimated when nests were placed in a Berlese funnel after nestlings had fledged. All nests began the experiment with the same population size (100 live mites), mimicking identical transmission, but ending population sizes 30–35 days later were highly variable. This suggests that factors of the nest environment or hosts may be playing an important role in mite population growth.

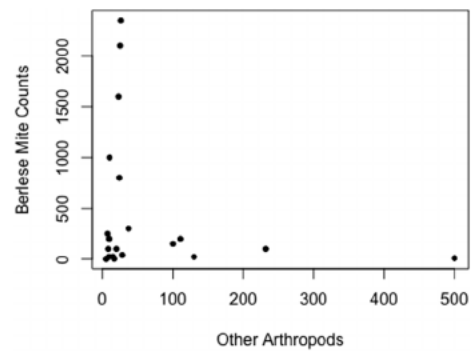


Fig. 3. Relationship between the number of non-mite arthropods (x-axis) and nest mites (y-axis) that were recovered when experimental nests were removed from the field after nestlings fledged and placed in a Berlese funnel. Nests with more arthropods had significantly fewer nest mites. This graph was made using raw data, but models reported in text had a Poisson distribution and included site as a random effect.

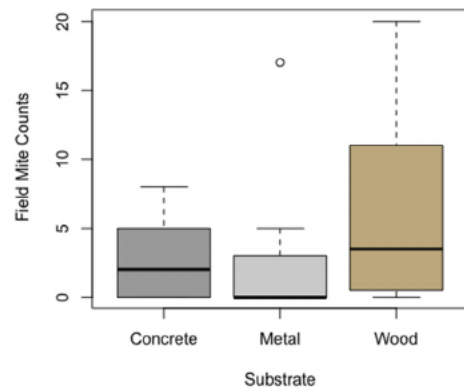


Fig. 4. The relationship between the substrate the nest was built on: concrete, metal, or wood (y-axis) and the number of mites estimated in the field when chicks were 12 days old. Nests built on wooden substrates had significantly more mites compared to nests built on concrete or metal substrates. This graph was made using raw data, but models reported in the text included site as a random effect.

4.3. Larger data set (all day 12 parasite nests)

The final model for the larger data set included both substrate (Fig. 4) 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 in our data. Together, these results indicate larger populations of mites are present in nests constructed on wooden substrates compared to concrete or metal substrates, and in nests that contain nestlings with high body condition.

When we compared characteristics of the nest (substrate, lining, area) and our temperature/humidity PC, only substrate was kept in the final model (Fig. 4) (*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 PC1 values between those of nests on metal or concrete. These intermediate values of PC1 indicate that the wooden substrate may have a stabilizing influence on nest microclimate.

5. Discussion

We found that nest mite populations are affected by both abiotic and biotic factors: final population size was associated with microclimate in the nest (PC1, temperature and humidity), host body condition, the interaction between microclimate and body condition, the

substrate upon which the nest is built, and the number of other arthropods in the nest. Mite populations experienced the greatest growth in warm, dry nests built on wood that contained nestlings with high body conditions and few other arthropods. Given that we kept transmission (initial population size) constant in our experiment, these findings suggest that the aggregated distribution of ectoparasites seen naturally in this system may not be driven by variation in transmission alone, and that variation in post-transmission environmental conditions and host quality are also important factors in mite population success.

Little is known about the optimal environmental conditions for ectoparasites in wild systems and how host quality contributes to population growth or decline. In barn swallows, as is the case for most avian populations that host nest ectoparasites, most nests are uninfected, or have very few mites, while others have very large populations (Hund, 2017). In this study, we experimentally removed existing parasites from nests and then infected them with 100 live mites. We then analyzed predictors of mite population size 12 days after nestlings hatched, as well as after the nestlings fledged and left the nest. Equalizing the starting point for these populations allowed us to look at the effects of environment and host quality alone on mite population size, removing the influence of variable transmission of ectoparasites into nests. In this system, NFM transmission between nests does occur, but at relatively low levels during the period of time encompassed by our experiment. In 2016, the larger experiment associated with this study included 49 disinfected nests. Of those nests, we found that 10.20% of them did show evidence of mite transmission, but at low levels relative to our starting infection (average infection intensity of 7.71 mites by day 12) (Hund unpublished data). We still do not have a good understanding of how mites move between nests and more work is needed to better understand the mechanisms of mite transmission in the North American barn swallow system.

In the Berlese funnel data subset, we found that mite populations had the greatest growth in warm, dry nests. We also found that higher average nestling body condition and fewer arthropods were associated with greater final NFM population size. Further, there was an interaction between microclimate and nestling body condition. If both microclimate and nestling body condition were favorable, the effect was amplified. The larger data set similarly showed a positive relationship between body condition and NFM population growth. Additionally, we found that nests built on wood supported greater NFM populations compared to nests built on other substrates. These results suggest that both abiotic and biotic factors are influencing ectoparasite success.

Based on previous studies and theory (Table 2), we predicted that mite populations might be smaller on nestlings in good body condition. For example, the tasty chick hypothesis predicts that nestlings in lower body condition are preferred by ectoparasites because they are not able to mount a strong immune system response and invest resources in parasite defense (Christe et al., 1998; Norris and Evans, 2000). Additionally, it has been shown that inflammation in the skin blocks mite access to blood vessels, limiting feeding and therefore negatively impacts their survival and fecundity (Owen et al., 2009). These results, coupled with findings in other altricial birds which indicate mass is positively correlated with general immunocompetence (PHA response; Tschirren et al., 2007), led to the prediction that body condition and mite population size would be negatively correlated. However, our findings indicate that mite densities were greater in nests that contained nestlings in higher average body condition. This is consistent with findings of other studies that 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 chickens studied in Owen et al. 2009, given that barn swallows are much smaller birds. Thus, the benefit of greater resource availability may overwhelm the negative effects of greater immune response in higher condition nestlings.

Previous work on NFM populations on chickens suggests that cool

and humid environments are optimal conditions for mite growth (Chen and Mullens, 2008; Halbritter and Mullens, 2011). However, this pattern of larger ectoparasite populations in cool, humid conditions was not supported in our study population. Instead, we found greater mite populations in warmer, less humid nest environments. These findings may be due to differences in life history. While NFMs infecting poultry spend most of their time on the hosts (Owen and Mullens, 2004), mites in the barn swallow system live primarily in the nest and only climb on the host to feed. This difference in life history may lead to different optima in terms of temperature and humidity. Similar results have been found in other avian-ectoparasite systems. Heeb et al. (2000) examined the effects of humidity on hen flea populations in great tit nests, and found that flea immigration into uninfected nests was more likely in dry nests than in wet nests. However, they also found that there was no difference in adult flea population numbers between their wet and dry treatments. In another study where counts of three ectoparasites of pied flycatchers were evaluated against local weather data, the parasites fared best in warmer, drier years (Merino and Potti, 1996). It is also possible that differences between the results of this experiment and previous studies with NFMs in poultry are related to the environmental context of the study itself. Whereas this 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, these studies indicated an optimal temperature for mites of 30 °C (Chen and Mullens, 2008), a temperature that was rarely observed in our study.

Additionally, we found that when both the microclimate and the body condition parameters were favorable, mite populations grew. While most populations were less than, or similar to the starting population, some were quite large (Fig. 2). Greater population size was associated with certain ranges of microclimate and nestling body condition, with a general trend towards warm, dry nests with higher average nestling body condition. 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 positively correlated with mite population size. Future work may consider experimental manipulations of the biotic and abiotic factors we found to be important in order to elucidate those mechanisms and determine the exact nature of these relationships.

In the full day 12 data set, we found that substrate influenced mite population size. Nests built upon wooden structures (e.g., beams or rafters) were associated with higher mite population counts than those constructed on metal or concrete; additionally, nest substrate significantly correlated with variation in temperature and humidity. Given that wood is a better thermal insulator than concrete or metal (Ankersmit and Stappers, 2016), the nests built on wood were likely not subjected to the same temperature fluctuations as those constructed on materials that provide less insulation. The thermal properties of the wood substrate may thus 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 mite eggs). Differences in the thermal properties of nest substrates likely affected the nest microclimate, and therefore mite populations.

In summary, we found that NFM population growth was determined by both host quality and characteristics of the nests in which the ectoparasites live. This suggests that the distribution of mites across the landscape is influenced by characteristics of both hosts and the environment following transmission events. These findings shed light on the mechanisms that generate the observed distribution of NFM within barn swallow populations. By exploring the abiotic and biotic factors that predict parasite success and population growth, we gain new insight into environmental features that structure host-parasite interactions in nature, and generate the distributions of parasites that we observe.

Conflicts of interest

The authors do not have any conflicts of interest to declare.

Acknowledgements

We would like to thank Pieter Johnson for his advice on statistical modeling and Ryan Terrien for his help with data processing. We would also like to thank the Safran Lab for their helpful feedback throughout this process. Thank you to Eric Burger for his assistance in editing the manuscript. Thank you to Nancy Emery for her advice on iButton use and data processing, as well as lending us some iButtons. Thank you to our undergraduate field assistants: Mara Hernandez, Claire Mastrangelo, and Kelley McCahill; as well as middle school teachers Kimberly Greene, Heidi Reeg, Daniel Tomlin (who were funded through a grant to RJS: The National Science Foundation (USA) DEB-CAREER 1149942, Research Experience for Teachers (RET)), for their help with fieldwork. Also, we thank the site owners, without whom, none of this work would be possible. This project was funded by The National Science Foundation (USA) Doctoral Dissertation Improvement Grant (DDIG) IOS-1601400 (AKH) with additional support from the Undergraduate Research Opportunities Program (UROP) at the University of Colorado. Additionally, WCD was supported by a National Science Foundation (USA) Research Experience for Undergraduates (REU) grant to RJS:DEB-CAREER 1149942). AKH and SPT were funded by the National Science Foundation (USA) Graduate Research Fellowship Program (GRFP). Publication of the manuscript was funded by the University of Colorado, Boulder libraries open access fund.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ijppaw.2018.07.007>.

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