Sex-biased parasitism and expression of a sexual signal

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Running title: Parasitism and sexual signals

Because sexual signals are often expressed more highly in one sex than the other, they can impose a sex-specific cost of reproduction through parasitism. While the two primary paradigms regarding the relationship of parasites to sexual signals are the good-genes hypothesis and the immunocompetence handicap hypothesis, there are other ecological, morphological, and energetic factors that may influence parasite infections in a sex-specific fashion. We tested the relationship between expression of a sexual signal (the dewlap) and ecological, morphological, and energetic factors mediating ectoparasite (mite) load between male and female Panamanian slender anoles (*Anolis apletophallus*). We found that males were more highly parasitized than females because of the preponderance of ectoparasites on the larger dewlap of males. Indeed, ectoparasite infection increased with both body size and dewlap size in males but not females, and parasite infection was related to energy storage in a sex-specific fashion for the fat bodies, liver, and gonads. Our work and previous work on testosterone in anoles suggest that this pattern did not arise solely from immunosuppression by testosterone, but that mites prefer the dewlap as an attachment site. Thus, the expression of this sexual signal could incur a fitness cost that may structure life-history tradeoffs.

Keywords: Immunocompetence handicap hypothesis, good-genes hypothesis, ectoparasite, mite, anole, *Anolis*

INTRODUCTION

Sexual signals, which are any trait that attracts mates and increases fitness through reproduction, are a ubiquitous form of phenotypic diversity in nature (Symonds & Elgar, 2007; Seddon et al., 2013; Schaefer & Ruxton, 2015). Because they are linked strongly to reproduction and thus fitness, many organisms invest heavily in sexual signals, even to the extent of incurring survivorship costs (Fisher, 1930; Ryan et al., 1982; Hoefler et al., 2007). Sexual signals are often (but not always) present or highly expressed in only one of the sexes (e.g., breeding turbercles in fish; Taskinen & Kortet, 2002; dewlaps of anoles, Harrison & Poe, 2012; bird coloration, Dale et al., 2015), highlighting the potential of sexual signals to impose sex-specific fitness costs. One mechanism whereby sexual signal expression can modulate fitness is through their relationship to parasitism. Parasites can be a powerful selective force that can reduce performance, survival, and reproduction (Schall, 1983; Richner et al., 1993; Brown et al., 1995; Lamarre et al., 2018; Edworthy et al., 2019; Jones et al., 2019) in a sex-specific fashion (Smith, 1996; Perez-Orella & Schulte-Hostedde, 2005; Reedy et al., 2016).

There are many paradigms that have been used to understand the relationship between parasites and sexual signals. First, Hamilton and Zuk (1982) proposed that sexual ornamentation can be used as an honest signal of the ability of an individual to resist parasitic infection. This so-called “good genes” hypothesis generally predicts that as the quality of the sexual signal increases, parasite load should decrease (Hamilton & Zuk, 1982), assuming that good genes are associated with parasite resistance and not merely increased tolerance. This hypothesis has been tested widely and has found support among diverse taxa (e.g., turkeys, Buchholz, 1995; roach fishes, Taskinen & Kortet, 2002; bowerbirds, Doucet & Montgomerie, 2003; anole lizards, Cook et al., 2013b). The second paradigm is the immunocompetence handicap hypothesis, which proposes that pleiotropic hormones, such as testosterone in vertebrates, mediate a tradeoff between immunocompetence and quality of a sexual signal (Folstad & Karter, 1992). Mechanistically, this is because testosterone can stimulate expression of sexual signals (Fernald, 1976; Cox et al., 2005; Lindsay et al., 2011; Cox et al., 2015a), but is also immunosuppressive (Folstad & Karter, 1992; Muehlenbein & Bribiescas, 2005). The immunocompetence handicap hypothesis generally predicts that parasite load should increase with the quality or size of a sexual signal (Folstad & Karter, 1992) as hormones such as testosterone can mediate a shift in energetics from investment in the immune system to reproduction (Boughton et al., 2007; Cox et al., 2015a; Cox et al., 2015b; Reedy et al., 2016). Like the good genes hypothesis, there is also broad support for this hypothesis among diverse taxa (e.g., Psammodromus lizards, Salvador et al., 1996; house sparrows, Poiani et al., 2000; damselflies, Siva-Jothy, 2000; humans, Rantala et al., 2012). Rather than representing strict alternatives, the good genes hypothesis and immunocompetence handicap hypothesis represent ends of a continuum between honest signaling of mate quality (good-genes hypothesis) and tradeoffs regulating expression of sexual signals (immunocompetence handicap hypothesis). However, other ecological and morphological factors can impose a sex-specific ectoparasite cost of reproduction that are not directly linked to immunosuppression by testosterone.

Parasite infection can also be modulated by ecological and morphological factors that alter the susceptibility of hosts or encounter rate between hosts and parasites in a sex-specific fashion. Microhabitat or dietary differences between the sexes can alter the likelihood of infection if certain microhabitats contain more parasites or allow more efficient infection of hosts (Wiles et al., 2000; Durden et al., 2004; Biaggini et al., 2009; Leung & Koprivnikar, 2016; Jones et al., 2019). Morphological differences between host sexes can also impact parasite infection through differences in attachment sites available to ectoparasites (e.g., body size, organ size, or ideal attachment sites for ectoparasites). For example, ectoparasites such as mites and ticks are often more abundant on larger individuals (Bauwens et al., 1983; Rocha et al., 2008), which has clear ramifications for sex-specific parasitism in species where the sexes differ in body size (Dudek et al., 2016). Alternatively, parasites can cluster in preferred locations for feeding and defense (Spears et al., 1999; Wiles et al., 2000; Jovani et al., 2006; Dudek et al., 2016), and some morphological features can concentrate mite infections on certain locations on the body such as "mite pockets" (Jovani & Serrano, 2001; Jovani & Serrano, 2004; Jovani et al., 2006; Rocha et al., 2008). Therefore, morphological features such as sexual signals that are dimorphic in expression could increase sex-biased infection by parasites. Finally, parasite infection can be influenced by the energetic state of the host, which in turn is influenced by ecological (e.g., differences in food availability between sex-specific habitats) and morphological factors (i.e., size of fat storage organs) that can differ between the sexes (Gross & Fritz, 1982; Sikkel et al., 2000; Lajeunesse et al., 2004; Reedy et al., 2016) and can explain sex-differences in parasitism in some species (Reedy et al., 2016).

We sought to understand how ecology, morphology, and energetics can impact ectoparasitic infection in a sex-specific fashion in the Panamanian slender anole (*Anolis apletophallus*; hereafter, slender anole). The slender anole possesses a dramatic sexual signal (the dewlap), can be heavily parasitized by trombiculid mites (Cox et al., 2020), and is sexually monomorphic in body size (Andrews & Stamps, 1994). In other anole species with male-biased sexual size dimorphism, testosterone circulates at higher levels in males (Lovern et al., 2001; Cox et al., 2009a; Cox et al., 2017), expression of the dewlap can be condition-dependent and is regulated by testosterone (Lovern et al., 2004; Cox et al., 2009b; Cox et al., 2015a; Curlis et al., 2017), and males suffer from increased ectoparasitism (Zippel et al., 1996; Reedy et al., 2016). However, whether increased ectoparasites is due to immunosuppression or simply an increase in surface area and body mass in males is difficult to disentangle in species with sexual size dimorphism. Hence, the highly sexually dimorphic expression of the dewlap in a generally sexually monomorphic lizard renders this species an ideal system for studying the relationship between ectoparasitism and sexual signals. We combined field surveys and laboratory dissections to address the following questions: 1) Is the relationship between sexual signals and ectoparasites consistent with honest signaling or fitness tradeoffs between signal expression and ectoparasite infection? 2) Do male and female slender anoles differ in ectoparasite load and are ectoparasites distributed differently on their bodies? 3) Is variation in habitat use, field-active body temperature, and energetics among individuals correlated with ectoparasite infection? We found evidence of sex-biased parasitism that was driven by the larger dewlap of male slender anoles compared to females, and ectoparasitic infections were further modulated by both morphology and energetics in a sex-specific fashion.

MATERIALS AND METHODS

STUDY SYSTEM

The slender anole is a small (~1.5 g), insectivorous lizard that is found in lowland, closed-canopy forests in eastern and central Panama (Kohler & Sunyer, 2008). Slender anoles are short-lived (~10 month generation time) and are extremely abundant (the most abundant vertebrate; Rand & Myers, 1990) in their forested habitat (Andrews & Nichols, 1990; Andrews, 1991). While this species is sexually monomorphic in most traits, including body size and coloration (Andrews & Stamps, 1994), males have a large dewlap (Fig. 1; Stapley et al., 2011), which is a colorful and extendable flap of skin in the gular region that functions as a social and sexual signal (Kohler & Sunyer, 2008; Stapley et al., 2011). In contrast, female slender anoles have either a very small dewlap (Figure 1) or none at all (Kohler & Sunyer, 2008). Males also have larger home ranges, disperse farther, and tend to perch higher in the vegetation than females (Talbot, 1979; Andrews & Rand, 1983), which could alter their risk of parasite infections. Both sexes can be heavily parasitized (Fig. 1) by both endo- and ectoparasites (Telford, 1974; Cox et al., 2020), including the trombiculid mite (*Eutrombiculus* c.f. *alfreddugesi*., Lance Durden pers. comm). Anoles do not have mite pockets (areas of scale-less skin that tend to concentrate ectoparasite infections), unlike many other lizard genera (Arnold, 1986). Mite infestations vary seasonally in some temperate species of lizards (Werman, 1983; Klukowski, 2004; Huyghe et al., 2010; Lumbad et al., 2011). However it is unknown whether mite infestations vary between wet and dry seasons for the tropical slender anole, although they do not vary seasonally for a different species of tropical lizard (Heredia et al., 2014). Trombiculid mites pierce the integument of the lizard with at tube called a stylostome to inject saliva and digestive enzymes (Arnold, 1986) and consume the resulting mixture of blood, lymph, and dissolved cells (Arnold, 1986). These ectoparasite infections in lizards cause integumental lesions (Goldberg & Bursey, 1991; Goldberg & Holshuh, 1992), alter social and thermoregulatory behavior (Cook et al., 2013b; Johnson et al., 2019), can decrease growth and body condition (Curtis & Baird, 2008; Cook et al., 2013a), and act as vectors for diseases (Arnold, 1986). Trombiculid mite infections can also instigate an immune response in vertebrates (Wright et al., 1988), and induce lesions in lizards that are likely a reaction of the immune system (Arnold, 1986; Goldberg & Bursey, 1991; Goldberg & Holshuh, 1992).

FIELD COLLECTION

We collected most slender anoles during surveys in Soberanía National Park, Panama, during the wet season in July 2017, with an additional collection period in July 2018. This period of time is during the primary reproductive period in slender anoles (Andrews & Rand, 1974; Andrews, 1979). Adult lizards were captured either by hand (Moseley et al., 2015) or by a lizard catch-pole, which is comprised of the top section of two-piece fishing rod and a braided fishing line with a loop created by a slipknot. Upon capture, we determined sex and measured body temperature, perch height, and perch diameter. We determined sex by the presence of a large dewlap, which is only present in males. We measured body temperature by inserting a type-K thermocouple (Omega, Stamford, CT, USA) into the cloaca. We only included body temperatures in subsequent analyses if we were able to record them within 30 s (Dzialowski, 2005) of capture (98.2% of lizards). We measured perch height as the distance above the ground where the lizard was initially located to the nearest cm using an extendable tape measure. Perch diameter was recorded at the location where the lizard was initially detected using digital calipers (Neiko Tools, China) to the nearest mm.

QUANTIFYING ECTOPARASITES, DEWLAPS, AND ENERGY STORAGE

We quantified ectoparasite loads by visually inspecting lizards for the presence of trombiculid mites (Cox et al., 2020). In slender anoles, these small mites are visible to the naked eye and are located on the dewlap, the axillary region, or the inguinal region (Fig. 1). Because anoles have very small scales with minimal overlap, even smaller mites are unlikely to be able to conceal themselves under scales, although it is possible that we did not detect very small larvae. For each individual lizard, we recorded the total number of mites on each of the three regions by visual inspection (Table S1).

We measured the size of the dewlap of both males and females. We used forceps to extend the dewlap of each animal over graph paper with a scale and photographed the dewlap. The dewlap was then digitally traced to the base of the throat using the freehand line tool in the program ImageJ (Schneider et al., 2012) to measure area in cm^2. Finally, we measured snout-vent length (Watson et al., 2019) using digital calipers (Neiko Tools, China) and the body mass of each individual to the nearest 0.01 g using a digital balance (ScoutPro, Ohaus Corporation, Parsippany, New Jersey, USA).

In both 2017 and 2018, we dissected a subset of lizards in our study (*n*=107) and quantified the size of several internal organs that are related to energetics and metabolism (Table S2). Specifically, we measured the mass of the discrete visceral fat bodies, liver, and gonads. In squamate reptiles, fat bodies and the liver are both associated with energy storage (Derickson, 1976; Cox et al., 2015a). In males, testis mass represents investment in post-copulatory fitness (Todd, 2008; Kahrl et al., 2016), whereas in females ovary mass represents energetic investment into offspring (Andrews & Rand, 1974; Cox et al., 2010). Each organ was dissected, lightly blotted once on laboratory tissue (Kimwipe™, Kimberly-Clark, Irving, TX, USA), and weighed to the nearest gram on tared weighing paper using an analytical balance (Sartorius Handy Analytical Balance, Sartorius AG, Grottingen, Germany).

STATISTICAL METHODS

We conducted analyses on mite abundance, mite prevalence, and mite intensity. Mite abundance is the total number of mites on each lizard (Klukowski, 2004). Mite intensity is the number of mites on lizards that have mites, excluding animals with zero mites (Diaz-Real et al., 2014; Reedy et al., 2016; Cox et al., 2020). Mite prevalence is the proportion of lizards that have mites at a population level, which is analyzed at an individual level as a binary variable that represents presence or absence of mites on an individual (Diaz-Real et al., 2014; Reedy et al., 2016; Cox et al., 2020). These three measures of mite infection are useful for characterizing overall mite load (mite abundance), the relative severity of mite infections on infected lizards (mite intensity), and rate of mite infection (mite prevalence). We analyzed mite abundance, intensity and prevalence for the entire lizard as well as separated by body region (dewlap, axillary, and inguinal regions). We used linear regression models (ANOVA or regression) on response variables that were normally or log-normally distributed, such as body size, perch height and width, and body temperature. Because of the high frequency of lizards without mites, continuous ectoparasite data were not normally distributed and possessed a strong right skew. Therefore, we conducted generalized linear model analyses when the response variable was mite abundance or intensity, which allowed the specification of the Poisson distribution through a log-link function. Because presence/absence (i.e. prevalence) of mites is a binary variable, we used either nominal logistic regression or contingency analyses when mite presence/absence was a response variable. Independent variables included sex, ecology, morphology, and organ mass data, with body size and other covariates as appropriate. Details of statistical models are in Tables 1-4. Finally, we confirmed the major results of our analyses using bivariate non-parametric analyses (e.g. Kruskal Wallis, Spearman’s Rank Correlation). Variables were log-transformed as necessary prior to analysis to meet the assumptions of statistical models. All analyses were completed in JMP v13.0 (SAS Institute, Cary, North Carolina, USA).

RESULTS

ANATOMICAL DISTRIBUTION OF PARASITES

We found that males were more heavily parasitized than females, which was driven by heavy ectoparasite load on male dewlaps. We captured 515 Panamanian slender anoles (232 females, 279 males) of which 366 (71%) were infected with ectoparasitic trombiculid mites. Males were significantly (contingency analysis of mite prevalence; $\chi^2=12.335$, $P=0.0004$) more likely (prevalence of 77.5%) to be infected than females (prevalence of 63.4%, Fig. 2), and males had significantly (generalized linear model of mite abundance; $\chi^2=486.7$, $P<0.0001$, Wilcoxon test; Z=-6.04, $P<0.0001$) more mites than females (Fig. 2). Infected males (range of 1-54 mites, median of 9 mites) also had significantly more mites than females (range of 1-24 mites, median of 5 mites) that were infected (generalized linear model with mite intensity; $\chi^2=262.6$, $P<0.0001$, Wilcoxon test; Z=-5.44, $P<0.0001$). This pattern was driven by the fact that dewlaps of males were significantly more likely to have mites than those of females (contingency analysis of mite prevalence; $\chi^2=12.335$, $P=0.0004$), and male dewlaps were significantly more parasitized than female dewlaps (generalized linear model of mite abundance; $\chi^2=385.2$, $P<0.0001$, Wilcoxon test; Z=-8.37, $P<0.0001$). Considering only dewlaps with mite infestations, dewlaps of males also had significantly more mites than the infected dewlaps of females (generalized linear model of mite intensity; $\chi^2=92.7$, $P<0.0001$, Wilcoxon test; Z=-4.35, $P<0.0001$). Interestingly, females were significantly more likely to be parasitized in the axillary (contingency analysis of mite prevalence; $\chi^2=28.2$, $P<0.0001$) and inguinal regions than males (contingency analysis of mite prevalence; $\chi^2=20.2$, $P<0.0001$) and had significantly more mites in the axillary (generalized linear model of mite abundance; $\chi^2=63.1$, $P<0.0001$, Wilcoxon test; Z=5.44, $P<0.0001$) and inguinal regions (generalized linear model of mite abundance; $\chi^2=56.3$, $P<0.0001$, Wilcoxon test; Z=4.7, $P<0.0001$). For animals with mites in the axillary region, the intensity of infection in the axillary region did not differ between the sexes (generalized linear model of mite intensity;

χ^2=1.7, P=0.1898, Wilcoxon test; Z=-1.75, P=0.0804), although females had slightly greater intensity than males (Fig. 2). However, females had greater intensity of infection in the inguinal region than males (generalized linear model of mite intensity; χ^2=4.3, P=0.0382, Wilcoxon test; Z=-2.12, P=0.0343).

ECOLOGICAL CORRELATES OF ECTOPARASITE INFECTION

We found that ecological factors did not impact mite abundance, mite prevalence, or mite intensity. Males were found on significantly higher perches (linear regression model; $F_{1,486}$=54.24, P<0.0001, Wilcoxon test; Z=--7.10, P<0.0001) and had marginally warmer body temperatures (linear regression model; $F_{1,504}$=4.16, P=0.0478, Wilcoxon test; Z=-1.61, P=0.1069) than females, but perch diameter did not differ between the sexes (linear regression model; $F_{1,466}$=0.644, P=0.4225, Wilcoxon test; Z=-0.23, P=0.8150). We found that there was no main effect of body temperature, perch height, or perch diameter on the mite prevalence, abundance, or intensity, although the sexes still differed in mite prevalence, abundance and intensity when controlling for habitat by including perch diameter in the statistical models (Table 1). We did detect an interaction between sex and body temperature on mite abundance, and between sex and both perch diameter and body temperature on mite intensity (Table 1), suggesting that habitat and thermal physiology could have a minor role in mite infections.

MORPHOLOGICAL CORRELATES OF ECTOPARASITES

We found that mite infection increased with size and that the relationship between size and mite infection differed between the sexes (Fig. 3). Females (1.55±0.02 g) were slightly heavier than males (1.48±0.02 g, $F_{1,513}$=6.03, P=0.0144), but sexes did not differ in snout-vent length (log-transformed SVL; $F_{1,513}$=3.43, P=0.0647). However, mite prevalence increased significantly with body size in both sexes (Fig. 3, Table 2). Mite abundance increased with SVL and body mass (Fig. 3, Table 2), but the strength of this relationship differed between males and females (Fig. 3, Table 2, significant sex by size interaction). Similarly, the number of mites on infected lizards also increased with SVL and body mass (mite intensity, Table 2) for males but not for females (Fig. 3, Table 2, significant sex by size interactions, confirmed with separate analyses for the sexes). When mite infections on body regions were analyzed separately, mite infection increased with body size for the dewlap (mite abundance, intensity, and prevalence) and axillary regions (abundance and intensity), but not the inguinal region (Table 2). Interestingly, we found a sex-specific relationship between body size and mite infection (abundance, intensity, and prevalence) for the dewlap (significant sex by body size interaction, Table 2), but not other body regions.

We found that the mite abundance and intensity of mite infection increased with the size of the dewlap for males but not for females (significant main effect of dewlap area, Fig. 4, Table 3). The size of the dewlap was not related to the prevalence of mite infection in males or females (Table 3). Because the relationship between dewlap size and mite infection appeared to be non-linear (Fig. 4), we constructed a statistical model *a posteriori* that included a quadratic dewlap term. This analysis indicated that males with the largest dewlaps tended to have decreasing numbers of mites (generalized linear model of mite abundance; dewlap area, χ^2=7.77, P=0.0053; SVL, χ^2=63.10, P<0.0001; dewlap area by SVL, χ^2=7.95, P=0.0048; dewlap area by dewlap area, χ^2=27.21, P<0.0001), but not females (generalized linear model of mite abundance; dewlap area, χ^2=0.02, P=0.8961; SVL, χ^2=14.64, P=0.0001; dewlap area by SVL, χ^2=0.02, P=0.8948; dewlap area by dewlap area, χ^2=2.25, P=0.1337). Use of LOWESS (locally weighted scatterplot smoothing) curves confirmed the linear and nonlinear components to the relationship between mite abundance and dewlap size for males, but not females (Fig. 4). Most of the effect of dewlap area on mite abundance seems to be driven by the fact that larger dewlaps have greater mite abundance in males but not females (significant main effect of dewlap area, Table 3), but infected lizards do not experience increased dewlap mite number with larger dewlaps (dewlap mite intensity, Table 3). Interestingly, we also found that the size of the dewlaps was positively associated with mite prevalence in the axillary region (significant main effect of dewlap area, Table 3), perhaps because of the physical proximity of the axillary region to the dewlap. Besides axillary mite prevalence, dewlap area was not associated with mite abundance, intensity, or prevalence in the axillary or inguinal regions (Table 3).

ENERGY STORAGE, REPRODUCTIVE INVESTMENT, AND ECTOPARASITES

We found evidence that ectoparasite infection was associated with the mass of organs of energy storage. We found that male anole lizards with larger fat bodies tended to have lower mite abundance and intensity than those with small fat bodies, while fat body mass was not associated with mite abundance or intensity in females (Fig. 5, Table 4). There was no relationship between mite abundance and liver mass for males or females (Table 4). However, liver mass was positively associated with mite intensity for both males and females. While mite abundance and intensity increased with the size of the ovaries in females, testis size was negatively correlated with mite intensity (but not abundance) in males (Fig. 5, Table 4). There was no relationship between mite prevalence and mass of fat bodies, liver, or gonads in either sex (Table 4).

DISCUSSION

We found evidence of a sex-specific relationship between parasitism and a sexual signal in a species that is sexually monomorphic in most traits. Importantly, we found that larger sexual signals increased ectoparasite infection in males, but not females. Mite infection was modulated by energetics in a sex-specific fashion on slender anoles, but we found little evidence that ecological differences between the sexes impacted ectoparasitism. Broadly, our results are consistent with the notion that male anoles could pay an ectoparasite-driven fitness cost for large sexual display organs.

Because we found that the presence and expression of a sexual signal was associated with increased rather than decreased parasitism, our results are generally congruent with the immunocompetence handicap hypothesis (Folstad & Karter, 1992) rather than the good genes hypothesis (Hamilton & Zuk, 1982). However, we did find that the largest dewlaps were associated with a diminished parasite load, which could be consistent with good-genes hypothesis in males with maximal expression of the dewlap. Regardless, while the immunocompetence handicap hypothesis suggests that immunosuppression by testosterone is responsible for the higher mite load in males (Folstad & Karter, 1992), our results might suggest an indirect mechanism whereby testosterone can increase risk of parasitism. Note that we did not directly measure circulating testosterone levels in slender anoles, and therefore our conclusions with respect to the relationship between ectoparasitism and testosterone-based immunosuppression must be considered preliminary. Nevertheless, there is a strong positive correlation between sexual signal size and circulating testosterone species in many lizard species including anoles (Psammodromus algirus, Salvador et al., 1996; Sceloporus undulatus, Cox et al., 2005; Sceloporus jarrovii, Cox et al., 2008), and hormone implant studies have revealed that both dewlap size and male-typical color is stimulated in many of the *Anolis* congeners of the

Panamanian slender anole (*Anolis carolinensis*, Lovern et al., 2004; *Anolis sagrei*, Cox et al., 2015a). In addition, females of many lizard species (including anoles) have lower circulating testosterone than males (Cox & John-Alder, 2005; Cox et al., 2009b; Cox et al., 2015a). While female slender anoles are similar in body size to males, they have more mites than males on all body regions (axillary and inguinal) except for the dewlap (Fig. 1). If larger dewlaps of male slender anoles are regulated by testosterone and females have lower circulating testosterone than males (as is the case in other anole species), our work suggests that the increased levels of parasitism experienced by the sex with the larger dewlaps is unlikely to be due solely to immunosuppression.

In the absence of apparent immunosuppression, the increased parasitism of the dewlap in male slender anoles could be caused by the physical characteristics of the dewlap itself. Although the dewlap remains unobtrusively folded most of the time, there is wide spacing of dewlap scales (gorgetal scales) to facilitate dewlap extension, and this spacing would be more pronounced in the larger dewlap of males compared to females. Spacing between the gorgetal scales may allow mites a more propitious attachment location, analogous to how mites cluster in scale-less anatomical regions such as “mite pockets” of other lizard species (Rocha et al., 2008; Dudek et al., 2016), which are hypothesized to concentrate mites and limit damage to the lizard integument (Arnold, 1986; Rocha et al., 2008). Additionally, the folded dewlap might provide additional surface area as well as an ideal microclimatic environment for mites to limit water loss. If mites prefer dewlaps for structural reasons, this provides a novel mechanism inducing sex-specific costs of reproduction.

Our work also documents a sex-specific cost of a sexual signal in a species that is sexually monomorphic for many traits, including body size. Males with larger dewlaps suffered from increased parasitism (while including a covariate for body size), while dewlap size was not related to parasitism in females. The lack of a relationship between dewlap size and infections in females could be explained by the small size of the dewlap in females, with limited scope for attachment sites for mites. Similarly, we found that mite parasitism increased with body size in males, but not females, even while controlling for dewlap size. Together, this suggests that the cost of parasitism increases with both body size and the size of a sexual signal. Previous research in other lizard species has found that larger body size in males can be associated with increased parasitism (Bauwens et al., 1983; Rocha et al., 2008), but this has primarily been investigated in species that are sexually dimorphic in body size and other traits (Cox & John-Alder, 2007; Dudek et al., 2016). A positive correlation between parasitism and body size could be caused by parasite accumulation during development and age-specific immune responses (Fichet-Calvet et al., 2003; Hawlena et al., 2006), but the rapid growth and short lifespan of slender anoles limits this possibility. Similarly, other research has found that the size of a sexual signal increases parasitism risk, but this has been associated with underlying correlations between testosterone, expression of a sexual signal and immunosuppression (e.g., *Psammodromus* lizards, Salvador et al., 1996; Olsson et al., 2000; house sparrows, Poiani et al., 2000; damselflies, Siva-Jothy, 2000; humans, Rantala et al., 2012). Our work highlights how the single dimorphic trait of dewlap size can enhance parasitism risk in a sex-specific fashion for a species where the sexes are otherwise (e.g., length, mass) very similar.

We did not find that parasite load was linked to the three habitat variables in this study, despite differences in habitat use and body temperature between males and females. Unlike other species of anoles, where larger males perch higher in the vegetation than females (Andrews, 1971; Delaney & Warner, 2016; Kolbe et al., 2016), perch height seems decoupled from body size in the slender anole, which is sexually monomorphic in body size. Previous research has found that ecological factors can mediate parasite infection (Wiles et al., 2000; Durden et al., 2004; Biaggini et al., 2009; Leung & Koprivnikar, 2016; Jones et al., 2019). Hence, sex-differences in parasite load can often be partially explained by differences in habitat use (Gross & Fritz, 1982; Sikkel et al., 2000; Lajeunesse et al., 2004), and diet (Reimchin & Nosil, 2001; Friesen et al., 2015). Our study only identified interactions (but no main effects) between sex, body temperature, and perch width on mite abundance and intensity, suggesting that habitat and thermal physiology could play a sex-specific (albeit minor) role in mite infections. While our study found limited evidence that sex differences in ecology impacted parasitism, we interpret these results cautiously given the limited timespan and scope of the ecological component of our study, and the lack of repeated ecological measures for individuals. Future research should explore how sexual dimorphism in habitat use and physiological ecology could influence parasitism in a sex-specific fashion in slender anoles.

Similar to previous work, we found evidence of a sex-specific relationship between parasitic infections and energy storage (Reedy et al., 2016). First, we found that male slender anoles with decreased fat stores had more mites, but there was no relationship between mite infection and fat body mass in females. Multiple mechanisms could explain this relationship, including 1) males with ample fat stores can invest energetically into the immune system and thus have fewer mites, 2) ectoparasite infection could be energetically costly and deplete fat stores or 3) an indirect correlate of lower fat body mass facilitates mite infection. Immune responses incur energetic costs in a number of species (Svensson et al., 1998), including congeners of the slender anole (Cox et al., 2015b), which supports the idea that energetics may mediate ectoparasite infection in male slender anoles as well. Second, we found that the size of the gonads was positively associated with the number of mites in females, but not in males. While gonad mass is linked to investment in precopulatory sexual selection in males (Kahrl et al., 2016), gonad mass in females reflects direct energetic investment into offspring. For anoles, the bilateral ovaries are a series of follicles that mature in a stepwise fashion, with alternate energetic investment to the left and right ovaries (Andrews & Rand, 1974; Cox & Calsbeek, 2010). As most of the mass of the ovaries is the lipid-rich yolk of the developing follicles as well as the shelled egg (Andrews & Rand, 1974), the gonad mass of females is a direct measurement of their energetic investment into offspring (Cox et al., 2010). We found that females that invest heavily into reproduction can experience increased parasitism, which implies a potential energetic tradeoff between reproduction and immunity. Because reproduction can be associated with increased stress and circulating corticosterone in lizards (Wilson & Wingfield, 1992; French et al., 2007), our results are also consistent with reproduction-associated immunosuppression in female slender anoles. Broadly, our results suggest that the role of energetics in mediating parasite infection could involve sex-specific relationships among energy storage, immune function, and reproduction.

We found that a social and sexual signal, the dewlap of anole lizards, imparts a sex-specific cost of increased parasitism. Intriguingly, this cost may arise from the increased affinity of mites to the dewlap itself, rather than solely from an indirect relationship of dewlap size with testosterone-mediated immunosuppression. Sexual selection resulting from female choice for larger male dewlaps could potentially increase parasitism and incur a survival cost to males. Given that parasitism is often associated with an energetic decrement (Booth et al., 1993; Scantlebury et al., 2007), investing maximally in the dewlap might ultimately limit energetic resources for growth and reproduction. It is thus plausible that the fitness tradeoffs associated with expression of the dewlap could be a factor limiting both body and dewlap size in anoles, even in species that are sexually monomorphic in body size.

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AUTHOR CONTRIBUTIONS

C.L.C, M.L.L., and W. O. M. conceived the study. C.L.C, M.L.L., D. J. N., J.D.C., and A.K.C. designed the study. C.L.C, M.L.L., D. J. N., J.D.C., A.K.C. and Z. D. collected data for the study. A. A. R., D. J. N., and C.L.C wrote the first draft of the study. All authors contributed to statistical analyses, figure construction, manuscript revision, and gave final approval to the manuscript.

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SHARED DATA

All data will be archived upon acceptance.

Table 1. Ecological correlates of mite infection. Results are from generalized linear models with a log link function to a Poisson distribution (mite abundance and intensity) and nominal logistic regression (mite prevalence). Statistically significant results are in bolded italics.

		Sex		Habitat Variable		Sex by Habitat Variable	
	d.f.	χ^2	*P*	χ^2	*P*	χ^2	*P*
Mite Abundance							
Perch Height	484	461.83	***<0.0001***	0.06	0.8099	0.01	0.9315
Perch Diameter	464	498.36	***<0.0001***	2.49	0.1148	2.13	0.1448
Body temperature	502	496.71	***<0.0001***	1.51	0.2197	20.83	***<0.0001***
Mite Intensity							
Perch Height	342	240.17	***<0.0001***	1.86	0.1728	0.57	0.4496
Perch Diameter	328	252.61	***<0.0001***	1	0.3168	4.314	***0.0378***
Body temperature	358	271.62	***<0.0001***	0.02	0.8808	12.62	***0.0004***
Mite Prevalence							
Perch Height	488	12.65	***0.0004***	0.46	0.5002	0.23	0.6354
Perch Diameter	468	14.89	***0.0001***	0.3	0.5846	0.26	0.6097
Body temperature	506	13.15	***0.0003***	1.1	0.2965	0.71	0.3984

Table 2. Body size (mass and SVL) correlates of ectoparasite infection. Results are from generalized linear models with a log link function to a Poisson distribution (mite abundance and intensity) and nominal logistic regression (mite prevalence). SVL=snout-vent length. Statistically significant results are in bolded italics.

	Sex			Size		Size by Sex	
	d.f.	χ^2	*P*	χ^2	*P*	χ^2	*P*
Mite Abundance							
SVL	511	363.01	***<0.0001***	148.17	***<0.0001***	20.88	***<0.0001***
Mass	511	481.42	***<0.0001***	196.44	***<0.0001***	26.63	***<0.0001***
Mite Intensity							
SVL	362	228.35	***<0.0001***	26.19	***<0.0001***	11.84	***0.0006***
Mass	362	268.12	***<0.0001***	29.52	***<0.0001***	17.27	***<0.0001***
Mite Prevalence							
SVL	515	11.34	***0.0008***	21.89	***<0.0001***	2.79	0.0946
Mass	515	18.41	***<0.0001***	30.7	***<0.0001***	3.21	0.0733
Dewlap Mite Abundance							
SVL	511	301.70	***<0.0001***	61.42	***<0.0001***	6.10	***0.0136***
Mass	511	373.03	***<0.0001***	80.01	***<0.0001***	9.46	***0.0021***
Dewlap Mite Intensity							
SVL	302	83.85	***<0.0001***	4.94	***0.0263***	1.49	0.2228
Mass	302	93.87	***<0.0001***	7.88	***0.0050***	2.77	0.0733
Dewlap Mite Prevalence							
SVL	515	51.18	***<0.0001***	28.17	***<0.0001***	7.45	***0.0063***
Mass	515	67.69	***<0.0001***	34.43	***<0.0001***	10.30	***0.0013***
Axillary Mite Abundance							
SVL	511	66.55	***<0.0001***	7.55	***0.0063***	0.82	0.3645
Mass	511	55.64	***<0.0001***	5.01	***0.0252***	0.05	0.8193
Axillary Mite Intensity							
SVL	101	2.51	***0.1132***	3.87	***0.0492***	1.52	0.2181
Mass	101	1.35	0.2455	0.70	0.4033	0.26	0.6134
Axillary Mite Prevalence							
SVL	515	28.51	***<0.0001***	0.32	0.5701	0.38	0.5369
Mass	515	25.37	***<0.0001***	1.38	0.2406	0.28	0.5989
Inguinal Mite Abundance							
SVL	511	56.04	***<0.0001***	0.01	0.9053	0.11	0.7356
Mass	511	53.40	***<0.0001***	0.13	0.7173	0.54	0.4639
Inguinal Mite Intensity							
SVL	106	4.34	***0.0373***	0.23	0.6299	0.12	0.7324
Mass	106	4.31	***0.0379***	0.01	0.9408	0.63	0.4280

Inguinal Mite Prevalence							
SVL	515	19.61	<***0.0001***	0.17	0.5783	0.01	0.9342
Mass	515	19.61	<***0.0001***	0.17	0.6791	0.01	0.9332

Table 3. Dewlap size correlates of ectoparasite infection. Results are from generalized linear models with a log link function to a Poisson distribution (mite abundance and intensity) and nominal logistic regression (mite prevalence) for three different body regions. Males and females were analyzed separately because of non-overlapping dewlap size. SVL=snout-vent length. Both dewlap area and SVL were log-transformed prior to analyses. Statistically significant results are in bolded italics.

		Dewlap Area		SVL		SVL by Dewlap Area	
	d.f.	χ^2	*P*	χ^2	*P*	χ^2	*P*
Mite Abundance							
Males	276	44.74	***<0.0001***	67.57	***<0.0001***	44.99	***<0.0001***
Females	230	0.14	0.7101	14.68	***<0.0001***	0.14	0.7094
Total Intensity							
Males	212	29.87	***<0.0001***	28.89	***<0.0001***	29.53	***<0.0001***
Females	144	0.21	0.6475	0.01	0.9349	0.17	0.6789
Total Prevalence							
Males	276	0.20	0.6542	7.53	***0.0061***	0.20	0.6537
Females	227	0.07	0.7873	4.83	***0.0280***	0.09	0.7603
Dewlap Mite Abundance							
Males	276	25.38	***<0.0001***	32.69	***<0.0001***	25.43	***<0.0001***
Females	230	0.70	0.4041	4.95	***0.0260***	0.67	0.4132
Dewlap Mite Intensity							
Males	203	1.40	0.2380	6.03	***0.0140***	1.43	0.2312
Females	94	1.12	0.2895	0.05	0.8293	1.08	0.2973
Dewlap Mite Prevalence							
Males	276	3.44	0.0637	4.69	***0.0304***	3.38	0.0660
Females	227	0.01	0.9925	2.46	0.1168	0.01	0.9895
Axillary Mite Abundance							
Males	276	1.91	0.1666	10.75	***0.0010***	1.68	0.1951
Females	229	0.06	0.8138	3.30	0.0694	0.06	0.8001
Axillary Mite Intensity							
Males	29	0.07	0.7955	5.49	***0.0191***	0.17	***0.6844***
Females	50	0.15	0.7019	0.36	0.5482	0.16	0.6904
Axillary Mite Prevalence							
Males	276	3.76	***0.0482***	0.70	0.4045	3.84	***0.0460***
Females	227	0.02	0.8867	0.90	0.3422	0.02	0.8889

		Dewlap Area		SVL		SVL by Dewlap Area	
	d.f.	χ^2	*P*	χ^2	*P*	χ^2	*P*
Inguinal Mite Abundance							
Males	276	0.34	0.5580	0.35	0.5546	0.33	0.5642
Females	229	2.00	0.1573	1.50	0.2208	1.91	0.1660
Inguinal Mite Intensity							
Males	35	2.21	0.1369	0.05	0.8337	2.32	0.1281
Females	67	0.54	0.4633	1.11	0.2922	0.54	0.4636
Inguinal Mite Prevalence							
Males	275	0.60	0.4398	0.01	0.9318	0.64	0.4240
Females	227	0.45	0.5040	0.01	0.9069	0.41	0.5204

Table 4. Energetic correlates of ectoparasite infection. Results are from generalized linear models with a log link function to a Poisson distribution for mite abundance. Males and females were also analyzed separately because of divergent organ masses. SVL=snout-vent length. Statistically significant results are in bolded italics.

		Sex		Organ Mass		Body Mass		Sex by Organ Mass	
	d.f.	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Mite abundance									
Fat Body									
Both Sexes	94	31.64	***<0.0001***	7.78	***0.0053***	53.49	***<0.0001***	10.56	***0.0011***
Males	64	-	-	33.99	***<0.0001***	52.34	***<0.0001***	-	-
Females	29	-	-	0.19	0.6602	4.45	***0.0349***	-	-
Liver									
Both sexes	97	42.54	***<0.0001***	0.02	0.8829	31.25	***<0.0001***	1.1	0.22994
Males	64	-	-	1.38	0.2398	35.63	***<0.0001***	-	-
Females	31	-	-	2.37	0.1236	0.79	0.3732	-	-
Gonads									
Both Sexes	-	-	-	-	-	-	-	-	-
Males	33	-	-	0.121	0.7275	1.86	0.1726	-	-
Females	31	-	-	18.51	***<0.0001***	0.02	0.9	-	-
Mite Intensity									
Fat Body									
Both Sexes	65	2.18	0.1396	5.50	***0.0190***	37.58	***<0.0001***	3.95	***0.0468***
Males	45	-	-	13.91	***0.0002***	19.58	***<0.0001***	-	-
Females	19	-	-	0.14	0.7109	19.23	***<0.0001***	-	-
Liver									
Both sexes	67	8.64	***0.0033***	0.44	0.5075	18.06	***<0.0001***	11.33	***0.0008***
Males	46	-	-	4.40	***0.0360***	19.07	***<0.0001***	-	-
Females	20	-	-	8.43	***0.0037***	1.11	0.2923	-	-
Gonads									
Both Sexes	-	-	-	-	-	-	-	-	-
Males	20	-	-	8.65	**0.0033**	1.39	0.2387	-	-
Females	20	-	-	14.54	***0.0001***	0.94	0.3329	-	-
Mite Prevalence									
Fat Body									
Both Sexes	84	0.14	0.7079	0.48	0.4893	0.26	0.6134	0.08	0.7835
Males	56	-	-	1.37	0.2426	2.13	0.1441	-	-
Females	27	-	-	0.04	0.8462	0.98	0.3213	-	-
Liver									

Both sexes	97	0.45	0.5005	0.66	0.4162	0.03	0.8689	2.38	0.1231
Males	65	-	-	0.75	0.3881	0.21	0.3881	-	-
Females	31	-	-	0.05	0.8306	0.54	0.4620	-	-
Gonads									
Both Sexes	-	-	-	-	-	-	-	-	-
Males	34	-	-	1.84	0.1749	0.01	0.9835	-	-
Females	31	-	-	1.30	0.2543	1.67	0.1961	-	-

Figures

Figure 1. Male (left) and female (right) Panamanian slender anoles with mite infections. Male anoles (top left) have larger dewlaps than females (top right). Mites can infect the dewlap (male, bottom left) as well as the inguinal and axillary regions (female, bottom right).

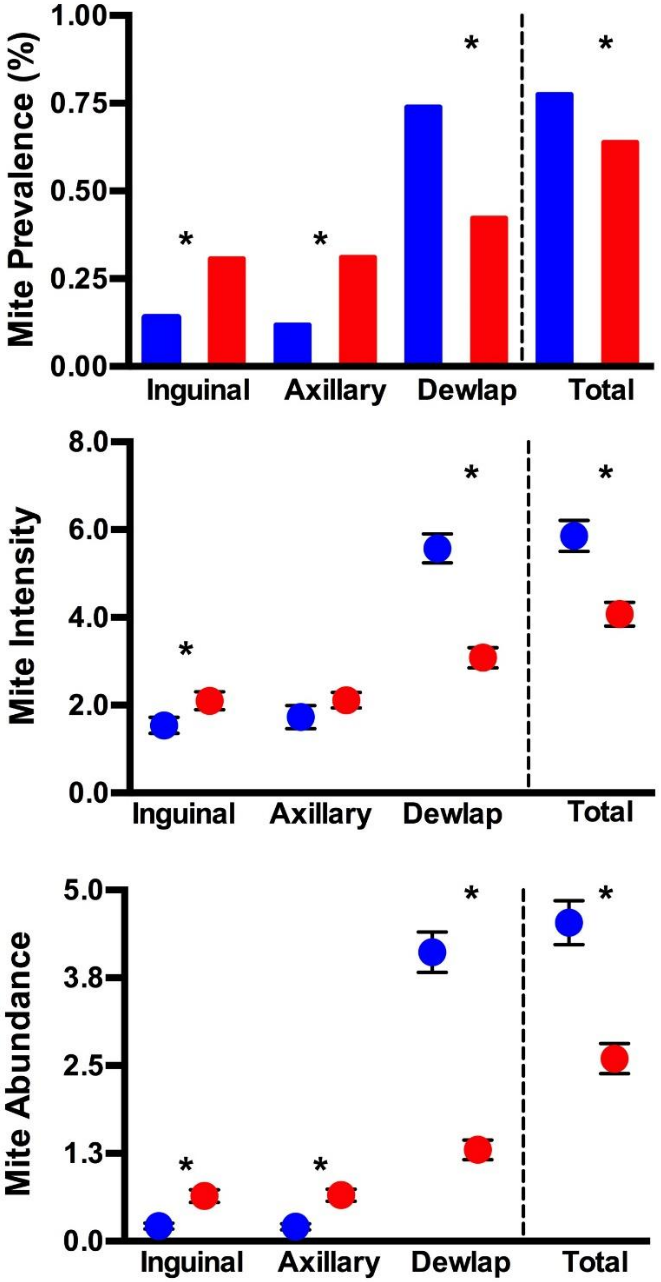

Figure 2. Mite prevalence (top), intensity (middle) and abundance (bottom) of male (blue) and female (red) Panamanian slender anoles across three different body regions. Symbols for mite abundance and intensity are means ± S.E.M. Males had greater prevalence, intensity, and abundance of mites than females when summed across body regions. Females had greater prevalence, intensity, and abundance of mites than males in the inguinal and axillary regions.

However, males had far greater prevalence, intensity, and abundance of mites on their dewlaps than females.

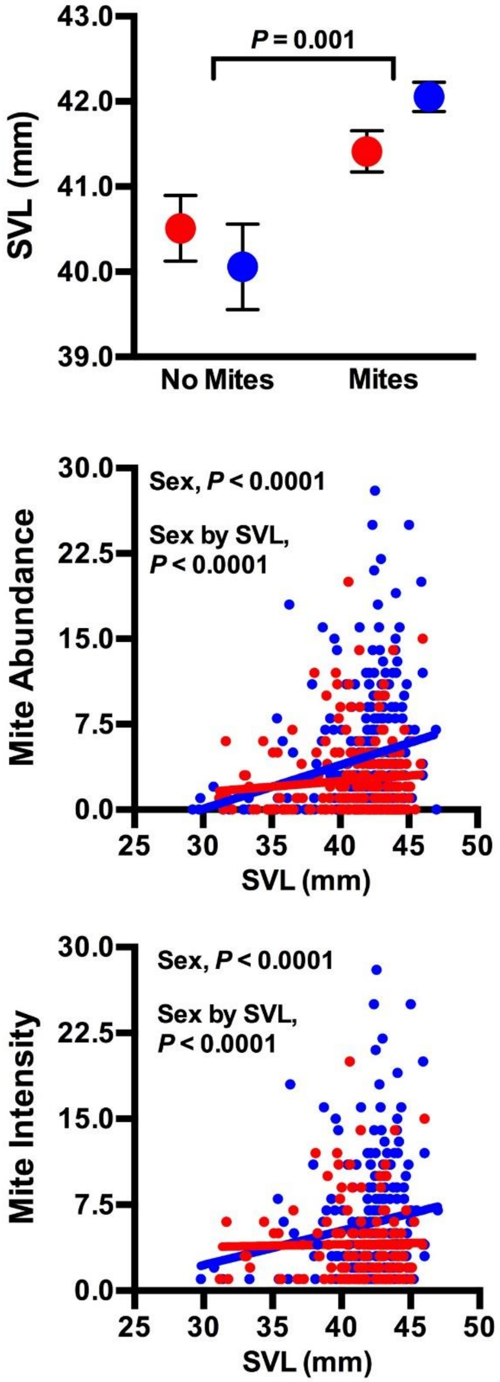

Figure 3. Relationship between body size and mite prevalence (top), intensity (middle) and abundance (bottom) of male (blue) and female (red) Panamanian slender anoles. Symbols (top)

are means ± S.E.M. Lines (middle and bottom) are best fit-lines from ordinary least-square regression analyses. Both males and females with mites were larger than those without mites (top). Mite abundance (middle) and intensity of (bottom) increased with body size for male but not female anoles.

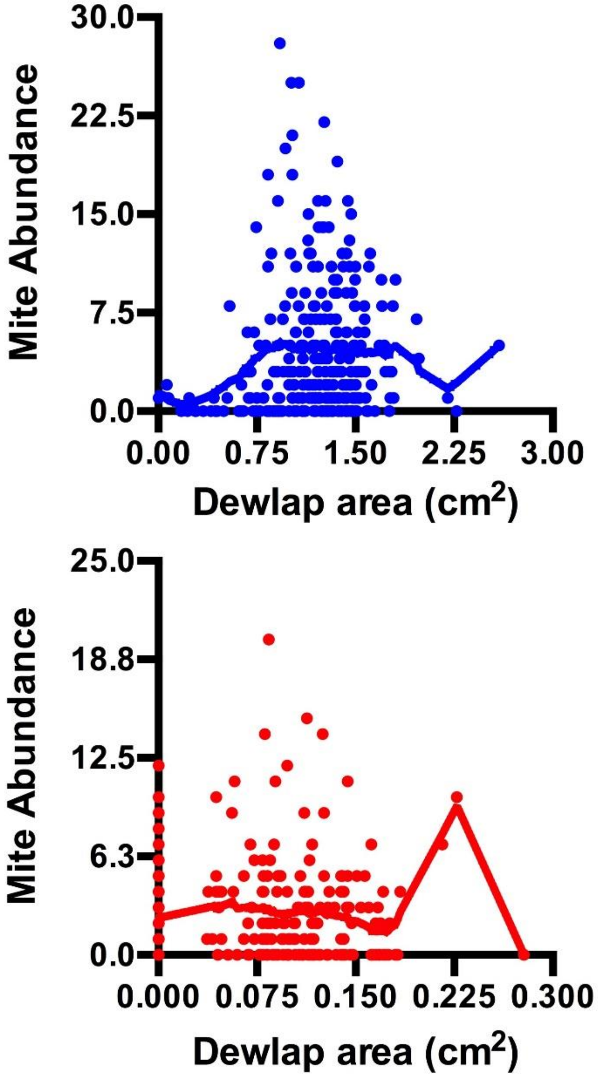

Figure 4. Relationship between mite abundance and area of the dewlap for males (top) and females (bottom). Lines are LOWESS (locally weighted scatterplot smoothing) to reflect linear and non-linear components to the relationship between dewlap size and mite infection.

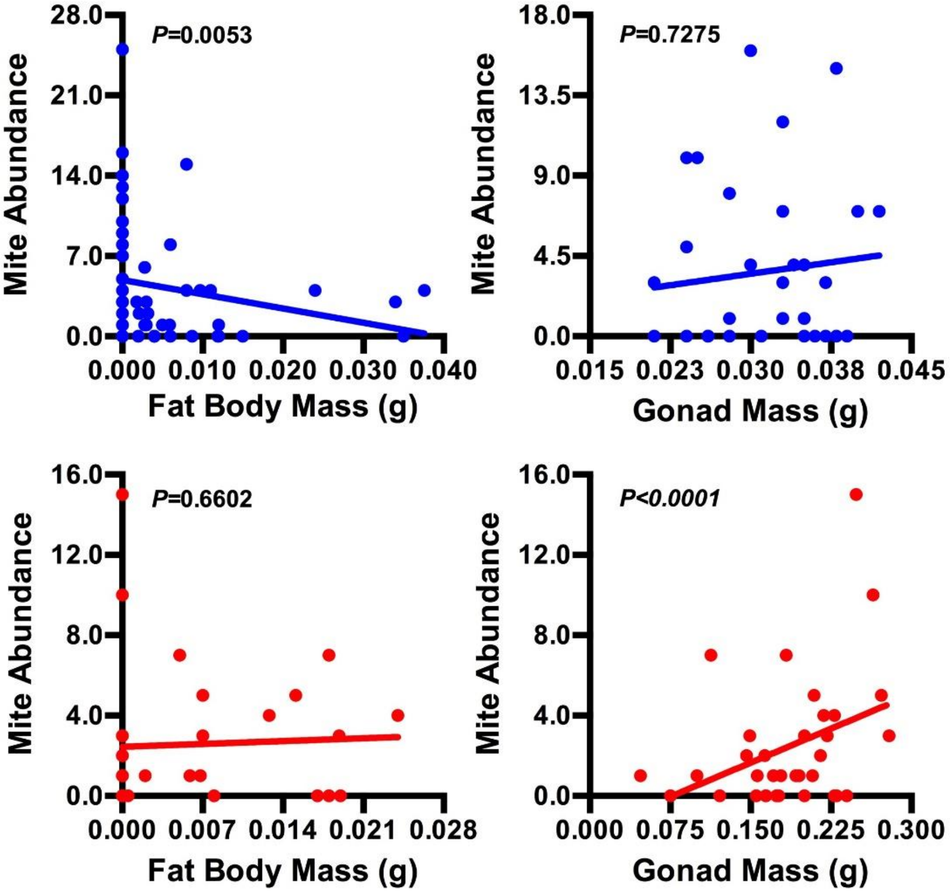

Figure 5. Relationship between fat body mass (left) and gonad (right) mass and mite abundance for males (top) and females (bottom). Lines (middle and bottom) are best-fit lines from ordinary least-square regression analyses. Mite abundance decreases with fat body mass for males but not females, while gonad mass increases with mite abundance for females but not males.