

Sex-biased parasitism and expression of a sexual signal

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Given that sexual signals are often expressed more highly in one sex than the other, they can impose a sex-specific cost of reproduction through parasitism. The two primary paradigms regarding the relationship of parasites to sexual signals are the good genes hypothesis and the immunocompetence handicap hypothesis; however, there are other ecological, morphological and energetic factors that might 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 in 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 suggests 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 might structure life-history trade-offs.

ADDITIONAL KEYWORDS: anole – *Anolis* – ectoparasite – good genes hypothesis – immunocompetence handicap hypothesis – mite.

INTRODUCTION

Sexual signals, which are any traits that attract 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). Given that 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;

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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 & 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, the 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.*, 2013). The second paradigm is the immunocompetence handicap hypothesis, which proposes that pleiotropic hormones, such as testosterone in vertebrates, mediate a trade-off between immunocompetence and the 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, 2015a; Lindsay *et al.*, 2011) but is also immunosuppressive (Folstad & Karter, 1992; Muehlenbein & Bribiescas, 2005). The immunocompetence handicap hypothesis generally predicts that the parasite load should increase with the quality or size of a sexual signal (Folstad & Karter, 1992), because 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, b; Reedy *et al.*, 2016). Like the good genes hypothesis, there is also broad support for this hypothesis among diverse taxa (e.g. *Psammmodromus* 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 signalling of mate quality (good genes hypothesis) and trade-offs regulating the expression of sexual signals (immunocompetence handicap hypothesis). However, other ecological and morphological factors can impose a sex-specific ectoparasite cost of reproduction that is 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 the 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 the 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 at certain locations on the body, such as 'mite pockets' (Jovani & Serrano, 2001, 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 (e.g. size of fat storage organs) that can differ between the sexes (Gross & Fritz, 1982; Sikkil *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* Köhler & Sunyer, 2008; 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, 2017); expression of the dewlap can be condition dependent and is regulated by testosterone (Lovern *et al.*, 2004; Cox *et al.*, 2009b, 2015a; Curlis *et al.*, 2017), and males suffer from increased ectoparasitism (Zippel *et al.*, 1996; Reedy *et al.*, 2016). However, whether increased numbers of ectoparasites are attributable to immunosuppression or simply to larger 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 signalling or

fitness trade-offs 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 with females, and ectoparasitic infections were modulated further by both morphology and energetics in a sex-specific fashion.

MATERIAL 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 (Köhler & Sunyer, 2008). Slender anoles are short lived (annual population turnover) and extremely abundant (the most abundant vertebrate; Rand & Myers, 1990) in their forested habitat (Andrews & Nichols, 1990; Andrews, 1991). Although 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 (Köhler & Sunyer, 2008; Stapley *et al.*, 2011). In contrast, female slender anoles have either a very small dewlap (Fig. 1) or none at all (Köhler & 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 cf. 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 a tube, termed 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 behaviour (Cook *et al.*, 2013; Johnson *et al.*, 2019), can decrease growth and body condition (Curtis & Baird, 2008; Cook *et al.*, 2013) 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 to be a reaction of the immune system (Arnold, 1986; Goldberg & Bursey, 1991; Goldberg & Holshuh, 1992).

FIELD COLLECTION

We collected slender anoles in July 2017 and July 2018 (wet season) from Soberania National Park, Panama. 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 with a lizard catch-pole, which is composed of the top section of a 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 present only in males. We measured body temperature by inserting a type-K thermocouple (Omega, Stamford, CT, USA) into the cloaca. We included body temperatures in subsequent analyses only 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 centimetre, using an extendable tape measure. Perch diameter at the location where the lizard was initially detected was recorded using digital callipers (Neiko Tools, China) to the nearest millimetre.

QUANTIFICATION OF 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). Given that 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 (Supporting Information, Table S1).

We measured the size of the dewlap of both males and females. We used forceps to extend the dewlap of each



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) and the inguinal and axillary regions (female, bottom right).

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 square centimetres). Finally, we measured the snout–vent length (SVL; Watson *et al.*, 2019) using digital callipers (Neiko Tools, China) and the body mass of each individual (to the nearest 0.01 g) using a digital balance (ScoutPro; Ohaus Corporation, Parsippany, NJ, 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 (Supporting Information, 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, the mass of the testes represents investment in postcopulatory fitness

(Todd, 2008; Kahrl *et al.*, 2016), whereas in females the mass of the ovaries 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, Göttingen, 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 analysed 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 the rate of mite infection (mite prevalence). We analysed mite abundance, intensity and prevalence for the entire lizard and according to 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. Owing to 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. Given that 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 given 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_{10} -transformed as necessary before analysis to meet the assumptions of statistical models. All analyses were completed in JMP v.13.0 (SAS Institute, Cary, NC, USA).

RESULTS

ANATOMICAL DISTRIBUTION OF PARASITES

We found that males were more heavily parasitized than females, which was driven by a heavy ectoparasite load on male dewlaps. We captured 515 Panamanian slender anoles (232 females and 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

Table 1. Ecological correlates of ectoparasite infection

Parameter	Sex			Habitat variable		Sex-by-habitat variable	
	d.f.	χ^2	<i>P</i> -value	χ^2	<i>P</i> -value	χ^2	<i>P</i> -value
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

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 bold.

Table 2. Body size (mass and SVL) correlates of ectoparasite infection

Parameter	d.f.	Sex		Size		Size-by-sex	
		χ^2	<i>P</i> -value	χ^2	<i>P</i> -value	χ^2	<i>P</i> -value
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

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 bold. Abbreviation: SVL, snout-vent length.

abundance, $\chi^2 = 486.7$, $P < 0.0001$; Wilcoxon test, test statistic $Z = -6.04$, $P < 0.0001$) more mites than females (Fig. 2). Infected males (range of one to 54 mites, median of nine mites) also had significantly more mites than females (range of one to 24 mites, median of five 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, $Z = -4.35$, $P < 0.0001$). Females were significantly more likely to be parasitized in the axillary (contingency

Table 3. Dewlap size correlates of ectoparasite infection

Parameter	d.f.	Dewlap area		SVL		SVL-by-dewlap area	
		χ^2	<i>P</i> -value	χ^2	<i>P</i> -value	χ^2	<i>P</i> -value
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
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

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 analysed separately because of non-overlapping dewlap size. Both dewlap area and SVL were \log_{10} -transformed before analyses. Statistically significant results are in bold. Abbreviation: SVL, snout-vent length.

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, $\chi^2 = 1.7$, $P = 0.1898$; 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, $\chi^2 = 4.3$, $P = 0.0382$; Wilcoxon test, $Z = -2.12$, $P = 0.0343$).

Table 4. Energetic correlates of ectoparasite infection

Parameter	d.f.	Sex		Organ mass		Body mass		Sex-by-organ mass	
		χ^2	<i>P</i> -value	χ^2	<i>P</i> -value	χ^2	<i>P</i> -value	χ^2	<i>P</i> -value
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	–	–

Results are from generalized linear models with a log-link function to a Poisson distribution for mite abundance. Males and females were also analysed separately because of divergent organ masses. Statistically significant results are in bold.

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, $F_{1486} = 54.24$, $P < 0.0001$; Wilcoxon test, $Z = -7.10$, $P < 0.0001$) and had marginally warmer body temperatures (linear regression model,

$F_{1504} = 4.16$, $P = 0.0478$; Wilcoxon test, $Z = -1.61$, $P = 0.1069$) than females, but perch diameter did not differ between the sexes (linear regression model, $F_{1466} = 0.644$, $P = 0.4225$; 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,

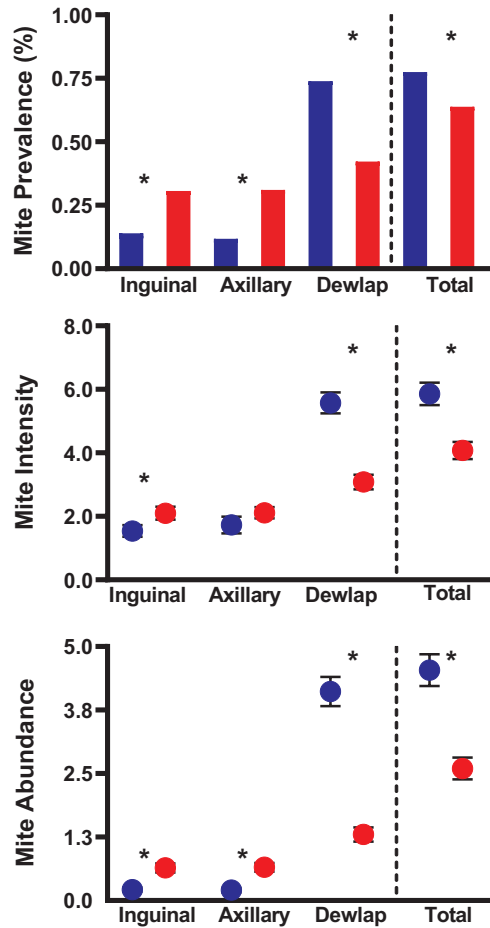


Figure 2. Mite prevalence (top), intensity (middle) and abundance (bottom) of male (blue) and female (red) Panamanian slender anoles across three different body regions. Symbols for mite abundance and intensity are means \pm SEM. Males had greater prevalence, intensity and abundance of mites than females 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.

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, $F_{1513} = 6.03$, $P = 0.0144$), but sexes did not differ in SVL (\log_{10} -transformed SVL, $F_{1513} = 3.43$, $P = 0.0647$). 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). Likewise, 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 analysed 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). 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 for 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). Given that 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 lower numbers of mites (generalized linear model of mite abundance: dewlap area, $\chi^2 = 7.77$, $P = 0.0053$; SVL, $\chi^2 = 63.10$, $P < 0.0001$; dewlap area-by-SVL interaction, $\chi^2 = 7.95$, $P = 0.0048$; dewlap area-by-dewlap area interaction, $\chi^2 = 27.21$, $P < 0.0001$), but not females (generalized linear model of mite abundance: dewlap area, $\chi^2 = 0.02$, $P = 0.8961$; SVL, $\chi^2 = 14.64$, $P = 0.0001$; dewlap area-by-SVL interaction, $\chi^2 = 0.02$, $P = 0.8948$; dewlap area-by-dewlap area interaction, $\chi^2 = 2.25$, $P = 0.1337$). Use of LOWESS (locally weighted scatterplot smoothing) curves confirmed the linear and non-linear 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 seemed to be driven by the fact that larger dewlaps had greater mite abundance in males but not females (significant main effect of dewlap area; Table 3), but infected lizards did not experience increased dewlap mite numbers with larger dewlaps (dewlap mite intensity; Table 3). We also found that the size of the dewlaps was positively

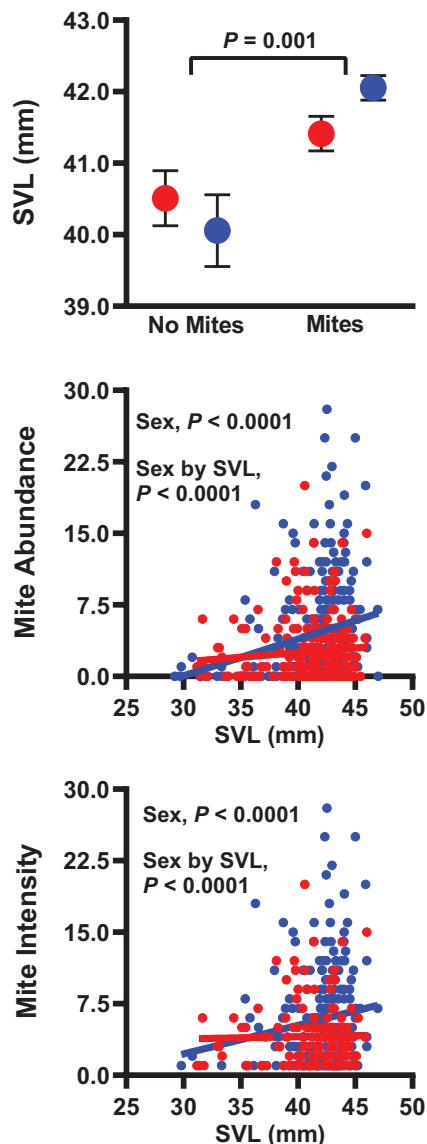


Figure 3. Relationship between body size [snout-vent length (SVL)] and mite prevalence (top), intensity (middle) and abundance (bottom) of male (blue) and female (red) Panamanian slender anoles. Symbols (top) are means \pm SEM. Lines (middle and bottom) are best fit-lines from ordinary least-square regression analyses. Both males and females with mites were larger than those without mites (top). Mite abundance (middle) and intensity (bottom) increased with body size for male but not female anoles.

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

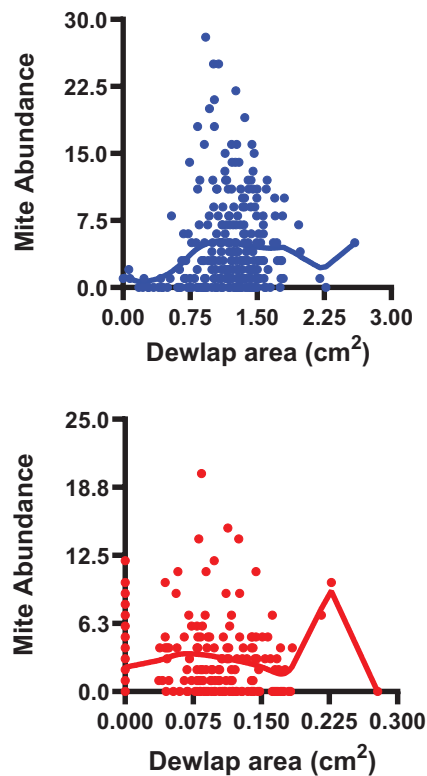


Figure 4. Relationship between mite abundance and area of the dewlap for males (top) and females (bottom). Lines are LOWESS (locally weighted scatterplot smoothing) to reflect linear and non-linear components to the relationship between dewlap size and mite infection.

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, whereas 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. Mite abundance and intensity increased with the size of the ovaries in females, whereas testicular size was negatively correlated with mite intensity (but not abundance) in males (Fig. 5; Table 4). There was no relationship between

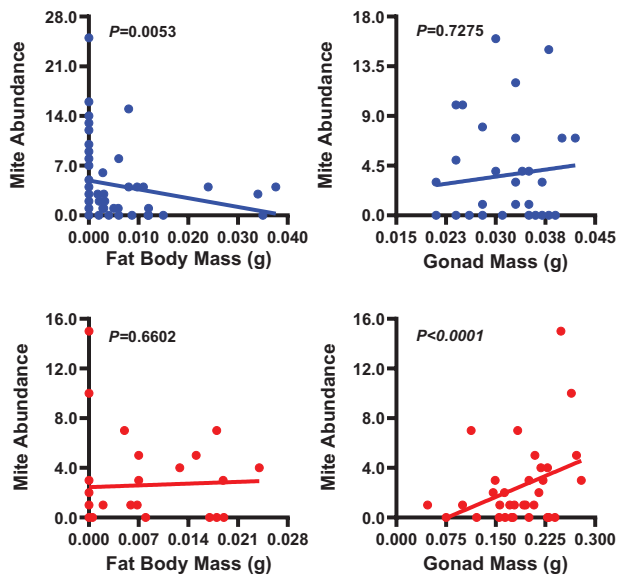


Figure 5. Relationship between fat body mass (left) and gonad mass (right) mass and mite abundance for males (top) and females (bottom). Lines (middle and bottom) are best-fitting lines from ordinary least-square regression analyses. Mite abundance decreases with fat body mass for males but not for females,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

mite prevalence and the mass of the 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 in females. Mite infection was modulated by energetics in a sex-specific fashion in 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.

Given that we found that the presence and expression of a sexual signal were 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 the good genes hypothesis in males with maximal expression of the dewlap. Regardless, although 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 the risk of parasitism. Note that we did not measure circulating testosterone levels directly in the 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 in many lizard species (*Psammmodromus algirus*, Salvador *et al.*, 1996; *Sceloporus undulatus*, Cox *et al.*, 2005; *Sceloporus jarrovi*, Cox *et al.*, 2008), and hormone implant studies have revealed that both dewlap size and male-typical colour are 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, 2015a). Although 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 attributable 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 with females. Spacing between the gorgetal scales might 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 and an ideal microclimatic environment for mites to limit water loss. If mites prefer dewlaps for structural reasons, this provides a new 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), whereas 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. Likewise, we found that mite parasitism increased with body size in males but not in females, even when 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. Likewise, 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 explained, in part, by differences in habitat use (Gross & Fritz, 1982; Sikkil *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. Although 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. Several 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 might also mediate ectoparasite infection in male slender anoles. Second, we found that the size of the gonads was positively associated with the number of mites in females, but not in males. Gonad mass is linked to investment in precopulatory sexual selection in males (Kahrl *et al.*, 2016), whereas 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). Given that most of the mass of the ovaries is the lipid-rich yolk of the developing follicles and the shelled egg (Andrews & Rand, 1974), the mass of the gonads 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 trade-off between reproduction and immunity. Given that 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

might arise from the increased affinity of mites for the dewlap itself, rather than solely from an indirect relationship of dewlap size to 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), maximal investment in the dewlap might ultimately limit energetic resources for growth and reproduction. It is thus plausible that the fitness trade-offs 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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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Sex, ecological and morphological variables and parasitism for the field study (see Material and Methods). Abbreviations: CTmin, critical thermal minimum (in degrees Celsius); mites, mite abundance; PH, perch height; PW, perch diameter; Tb, body temperature (in degrees Celsius); VTmax, voluntary thermal maximum (in degrees Celsius). Dewlap area is in square centimetres (cm² in the table). Mite abundance and intensity are count data, whereas prevalence is the presence (1) or absence (0) of mites on individual lizards.

Table S2. Sex, morphology, parasitism and organ mass for the laboratory energetics study (see Material and Methods). Abbreviation: Total mites, mite abundance. Mite abundance and intensity are count data, whereas prevalence is the presence (1) or absence (0) of mites on individual lizards.