The Effect of Exogenous Testosterone on Ectoparasite Loads in Free-Ranging Western Fence Lizards

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ABSTRACT
Numerous factors impact the dynamics of host-parasite relationships, such as host sex, hormonal state, reproductive condition, host health, and behavior. In particular, males from a variety of taxa frequently carry heavier parasite burdens than females, particularly during breeding season when testosterone concentrations are elevated. Using western fence lizards (*Sceloporus occidentalis*), we tested the hypothesis that high circulating testosterone concentrations in male lizards induce high tick and mite loads. We implanted male lizards with either testosterone or control implants in the field during the spring, when tick and mite loads are highest. One month later, testosterone-implanted males had significantly higher tick loads, but lower mite loads, than control males. These results suggest that testosterone differentially impacts ectoparasitic acarine burdens. Testosterone may modulate aspects of lizard physiology and behavior that enhance or diminish parasitism by certain acarines during periods of peak reproductive effort. *J. Exp. Zool. 317A:447-454, 2012.* © 2012 Wiley Periodicals, Inc.

Parasites may have significant impacts on host behavior, morphology, reproduction, and physiology (Dence, '58; Hindsbo, 72; Holmes and Bethel, '72; Baudoin, '75; Oetinger and Nickol, '81; Hamilton and Zuk, '82; Schall and Dearing, '87; Hurd, 90; Bakker, '97; Møller, '97; Lopé et al., '98; Møller et al., '99; Kristan and Hammond, 2000; Moore, 2002; Schwanz, 2006). The host attempts to mitigate parasitism via an immune response (Ribeiro, '89; Wheeler et al., '89; Wikel, '96; Wiel et al., 2006), grooming (Cotgreave and Clayton, '94; Giorgi et al., 2001; Mooring et al., 2004), or avoiding areas of high parasite abundance (Hart, '92; Karvonen et al., 2004). Parasites respond by such means as modulation of the host immune response (Ramachandra and Wikel, '92; reviewed in Wikel, '99; Brossard and Wikel, 2004). As a result of these contrasting aims, an evolutionary arms race occurs in which parasites attempt to manipulate the hosts to maximize their survival and the hosts attempt to prevent successful parasitism (Brooks, '79; Proctor and Owens, 2000; Soler et al., 2001; Zang and Maizels, 2001; Brossard and Wikel, 2004).

Parasite loads can be influenced by season, host age, health, genetic background, sex, and hormonal state. Seasonal shifts in parasite load have been observed across animal taxa, including fish (Mitchell, '89), crickets (Zuk, '87), sheep (Theodoropoulos et al., '98), birds (Teel et al., '98), and lizards (Eisen and Eisen, '99; Schall et al., 2000; Eisen et al., 2001; Klukowski, 2004; Lumbad et al., 2011). Lizard-parasite interactions have been particularly

well studied, largely because of their ease of manipulation in the field and laboratory to investigate physiological, immunological, and ecological consequences of parasitism. Several patterns emerge in studies of lizard parasitism: older and larger lizards have more parasites (Christian and Bedford, '95; Schall and Marghoob, '95; Sorci, '96), healthier lizards have fewer parasites (Dunlap and Mathies, '93; Clobert et al., 2000; Martín et al., 2007), specific genotypes of lizard species have increased resistance to parasites (Brown et al., '95; Olsson et al., 2005), and parasites are more abundant in males (Schall and Marghoob, '95; Schall et al., 2000; Klukowski and Nelson, 2001; Salkeld and Schwarzkopf, 2005). Higher parasite loads in males have also been observed in many other animal species (Poulin, '96; Zuk and McKean, '96), ranging from ball pythons (Aubret et al., 2005) and salamanders (Anthony et al., '94) to reindeer (Folstad et al., '89) and red jungle fowl (Zuk, '90).

The combination of seasonal, age, and sex effects on parasite loads suggests a potentially important relationship between the endocrine system and parasitism. The androgenic hormone testosterone (T) plays a role in numerous morphological, physiological, and behavioral processes. It has been shown to increase reproductive success of male lizards and stimulate increased energy expenditure during the mating season as males increase territorial behaviors (Moore, '86; Marler and Moore, '88,'89; Wingfield and Hahn, '94; Klukowski and Nelson, '98; Sinervo et al., 2000), home-range size (DeNardo and Sinervo, '94; Sinervo et al., 2000; Cox et al., 2005; John-Alder et al., 2009), endurance (Sinervo et al., 2000; John-Alder et al., 2009), movement (Olsson et al., 2000; Sinervo et al., 2000; Cox et al., 2005a; John-Alder et al., 2009), and expression of morphological secondary sex characteristics (Cooper et al., '87; Zuk et al., '95; Evans et al., 2000; Cox et al., 2005a,2005b). As activity increases and males patrol larger territories, they may become exposed to more parasites, leading to increased burdens. Testosterone may also affect parasite loads via inhibition of immune function directly, or indirectly by increasing reproductive energy expenditure at the cost of immune maintenance. Experimentally elevated T decreases leukocyte counts (Zuk et al., '95; Veiga et al., '98; Uller and Olsson, 2003) and suppresses the cell-mediated immune response (Duffy et al., 2000; Belliure et al., 2004; Oppliger et al., 2004) and the humoral immune response (Saad et al., '90; Duffy et al., 2000). Studies across multiple lizard taxa have demonstrated that males with higher concentrations of circulating T have higher parasite loads (Saino et al., '95; Salvador et al., '96; Olsson et al., 2000; Hughes and Randolph, 2001; Klukowski and Nelson, 2001; Roberts et al., 2004; Cox and John-Alder, 2007). However, some studies showed no significant relationship between circulating T concentrations and parasite loads in lizards (Lefcort and Blaustein, '91; Salvador et al., '97; Oppliger et al., 2004).

Western fence lizards (*Sceloporus occidentalis*) are major hosts for subadult western black-legged ticks (*Ixodes pacificus*) (Eisen and Eisen, '99; Eisen et al., 2004; Slowik and Lane, 2009) as well as *Eutrombicula* (Allred and Beck, '62; Klukowski, 2004) and *Geckobiella* mites (Allred and Beck, '62; Schall et al., 2000; Schall and Smith, 2006). These acarines commonly accumulate on lizards in a small skin fold adjacent to the tympanic membrane, termed the nuchal pocket, although they attach to other regions as well, such as around the eyes (Arnold, '86; Goldberg and Bursey, '91; Dunlap and Mathies, '93). Descriptive studies on this species have shown that lizard body size, sex (males typically have higher burdens), habitat, and other variables impact tick load (Lane and Loye, '89; Tälleklint-Eisen and Eisen, '99; Schall et al., 2000; Eisen et al., 2001; Lumbad et al., 2011). Although substantial numbers of subadult *I. pacificus* may feed on *S. occidentalis*, these hosts are reservoir-incompetent for the causative agent of Lyme disease in the United States, *Borrelia burgdorferi* sensu stricto, transmitted by *I. pacificus* in the western states (Burgdorfer et al., '85; Manweiler et al., '92). A borreliacidal factor in the blood of *S. occidentalis* appears to reduce spirochete populations in infected nymphs (Lane and Quistad, '98; Kuo et al., 2000). However, *S. occidentalis* may still increase the risk of human exposure to *Borrelia* by increasing the density of infected subadult ticks in some areas (Swei et al., 2011). While it is therefore known that the host-parasite relationship between this lizard and tick may be important in Lyme disease ecology, little is known about how physiological factors in host lizards, such as sex hormone concentrations, affect tick burdens.

We examined whether T influences tick and mite loads in free-ranging *S. occidentalis*. We hypothesized that elevated T is a driving factor responsible for high burdens of ectoparasites on male lizards. We manipulated T concentrations in free-ranging male *S. occidentalis*, released them and recaptured them after one month, then recorded the tick and mite loads on each lizard. If T is responsible for higher ectoparasite loads on lizards, then T-implanted male lizards should have heavier ectoparasite burdens than nonmanipulated male control lizards.

METHODS

We collected 54 male *S. occidentalis* on April 4–5, 2009 from Poly Canyon on the campus of the California Polytechnic State University, San Luis Obispo, California, USA. This area is characterized by oak woodland with several man-made structures. Upon capture by noose, snout-vent length (SVL, $\pm$ 0.5 cm) and body mass ($\pm$ 0.5 g) were recorded and each lizard received a unique toe-clip for identification. Lizards were randomly placed into one of two treatment groups, T-implanted ($n = 27$) or control ($n = 27$). Treatment groups did not significantly differ in SVL (two-tailed $T = 0.17$, $P = 0.864$) or body mass (two-tailed $T = 1.06$, $P = 0.299$). Implants were made from 5-mm pieces of silastic diffusion tubing (Dow Corning, Clarkesville, TN, USA: 1.47 mm inner diameter, 1.96 mm outer diameter), capped and sealed with silicon caulking, and filled with either 3 mm of crystalline testosterone propionate (Sigma-Aldrich, St. Louis, MO, USA)

(T implants) or silicon caulking (control). In the field, lizards were placed on ice until cold-induced surface anesthesia prevented the foot-withdrawal reflex. Implants were placed into the coelomic cavity via a small ventrolateral incision that was then closed with absorbable suture. Lizards were immediately warmed in the sunlight and released back at their original capture sites. Study procedures were approved by the California Polytechnic State University, San Luis Obispo, Institutional Animal Care and Use Committee (Protocol # 806) and the California Department of Fish and Game California Scientific (Permit # 801072-05).

Four weeks later, we recaptured as many lizards as possible (14 T implanted and 12 control lizards). In this experiment, we did not measure circulating concentrations of T to evaluate the efficacy of our implants. However, we did so in another experiment conducted concurrently (Pollock et al., 2012), where the implants doubled circulating concentrations of T (control males = 21.1 ± 2.8 ng/mL; T-implanted males = 35.7 ± 2.0 ng/mL). The T-implanted average is at the high end of natural concentrations of T observed during spring (Taylor et al., unpublished data). We therefore feel confident that our implants successfully increased circulating T to high but physiologically relevant levels. Lizards were then transported back to the laboratory at California Polytechnic State University in cloth bags. Immediately upon return, the lizards were placed into individual 13 × 8 × 8 (cm) metal mesh cages elevated above tubs of water to collect all ectoparasites (mites and ticks) infesting lizards as they dropped from their hosts. Tubs were 30 × 16 × 8 (cm) and filled with 4 cm of water, such that any ticks and mites dropping off the host lizards would fall into the tubs and float until they were retrieved daily by the investigators. The sides of the tubs were coated with Fluon (Bioquip, Rancho Dominguez, CA, USA) to prevent tick and mite escape. Tubs were placed in environmental chambers (27°C, 8:16 light:dark). Water was offered *ad libitum* and 2–3 crickets were offered per day. Numbers of replete tick larvae, replete tick nymphs, and total mites were quantified daily as they dropped off the lizard host and into the water until all ticks had dropped off. At the termination of the experiment, the total numbers of replete tick larvae, replete tick nymphs, total replete ticks (larvae plus nymphs), and mites that had dropped off lizards were calculated for each lizard. Lizards were also visibly inspected for any remaining mites, which were removed and added to the total mite load for each lizard. However, it was not possible to determine if all mites, especially larval mites, dropped off the lizards because some trombiculids may remain on the host for long periods of time (Klukowski, 2004) and are difficult to see.

Parasite loads (replete tick larvae, replete tick nymphs, total ticks, and mites) of the two treatment groups were individually compared using two-sample Poisson rate tests. To examine the effects of body size on these measurements of parasite load, we conducted analyses of covariance (ANCOVA) with log-transformed SVL as the covariate, comparing log-transformed parasite loads between treatments (T-implanted and control). The homogeneity of slopes assumption of all ANCOVAs was met.

All P-values were considered significant at the $\alpha = 0.05$ level. Statistical analyses were performed using Minitab Statistical Software version 10 (State College, PA, USA).

RESULTS

Testosterone-implanted males had significantly more replete tick larvae than control males (mean tick larvae: T-implanted males = 17.1 ± 6.4, control males = 9.0 ± 3.0; $Z = 5.70$, $P \leq 0.0001$; Fig. 1). Testosterone-implanted males also had significantly more replete tick nymphs than control males (mean tick nymphs: T-implanted males = 12.5 ± 2.6, control males = 6.1 1.6; $Z = 5.33$, $P \leq 0.0001$; Fig. 1). Therefore, T-implanted males had significantly higher total number of ticks (larvae and nymphs combined) than control males (mean total ticks: T-implanted males = 29.6 ± 7.2, control males = 15.1 ± 3.9; $Z = 7.79$, $P \leq 0.0001$; Fig. 1). Control males had significantly higher mite loads than T-implanted males (mean mites: T-implanted males = 14.8 ± 2.2, control males = 23.5 ± 9.4; $Z = -4.85$, $P \leq 0.0001$; Fig. 1). Snout-vent length did not significantly affect replete tick larval loads ($F = 1.05$, $P = 0.316$), replete tick nymphal loads ($F = 4.19$, $P = 0.053$), total tick loads ($F = 3.32$, $P = 0.082$), or mite loads ($F = 1.98$, $P = 0.173$).

DISCUSSION

We found that experimental elevation of T concentrations in *S. occidentalis* led to increased loads of both subadult life stages of the tick *I. pacificus*, supporting the hypothesis that T is a driving factor responsible for high burdens of ectoparasites on male lizards. Positive relationships between T and ectoparasite loads have been demonstrated in several other lizard species. Salvador et al. ('96) showed that experimental elevation of T in a free-living population of the large Psammodromus lizard (*Psammodromus algirus*) increased tick loads. With respect to naturally occurring concentrations of T, higher mite loads were observed in male eastern fence lizards (*Sceloporus undulatus*) (Klukowski and Nelson, 2001) and striped plateau lizards (*Sceloporus virgatus*) (Cox and John-Alder, 2007) with higher circulating T concentrations. However, some studies have found no effect on parasite loads on lizards (Salvador et al., '97; Oppliger et al., 2004). Fuxjager et al. (2011) suggest opposing effects of T on different parasite species. The studies by Oppliger et al. (2004) and Fuxjager et al. (2011), however, were conducted during the nonreproductive season, raising the possibility that T implantation may produce different results depending on the reproductive state of the host.

Although numerous studies suggest a positive relationship between parasitism and T concentrations, the precise mechanisms that drive this phenomenon remain unclear. Testosterone, as a key driver of reproductive coloration (Rand, '92; Cox et al., 2005b; Cox et al., 2008), territorial behaviors (Moore, '86; Marler and Moore, '89; Klukowski and Nelson, '98), and home range size (DeNardo and Sinervo, '94; Cox et al., 2005a), may increase parasite burdens through a physiological trade-off in which immune function is diminished (Grossman, '85; Saad et al., '90; Zuk et al., '95; Hillgarth
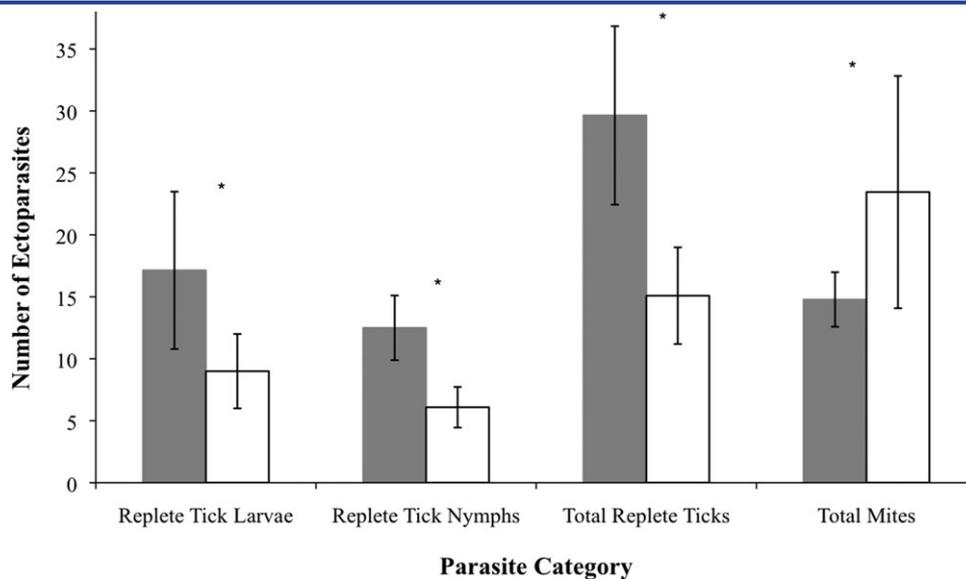
Figure 1. Ectoparasite loads on testosterone-implanted (gray) and control (white) male western fence lizards (*Sceloporus occidentalis*). Values are reported as means $\pm$ 1 SEM. Asterisks indicate a significant difference between treatment groups ($P \leq 0.0001$).

and Wingfield, '97; Veiga et al., '98; Duffy et al., 2000; Uller and Olsson, 2003; Belliure et al., 2004; Oppliger et al., 2004; Roberts et al., 2004). Although immune function is clearly important in defense against ectoparasites (Ribeiro, '89; Wikel et al., '94; Wikel, '96; Brossard and Wikel, 2004; Wiel et al., 2006), few studies connect immune function to ectoparasite loads. Several studies in mammals have demonstrated a humoral immune response to tick salivary gland components (Wheeler et al., '89; Canals et al., '90; Galbe and Oliver, '92). A link between T, immune function, and ectoparasite load therefore seems plausible. However, host immune reactivity to ticks is relatively poorly understood in reptiles. One study by Galbe and Oliver ('92) showed broad-headed skinks (*Plestiodon laticeps*) did not develop increased resistance after repeated infestations of *Ixodes scapularis* ticks. Further studies, however, are needed to examine the interplay between hormones and the immune response to reptile ectoparasites.

An alternate mechanism through which T may increase parasite burdens could be through altered behavioral patterns and daily activity periods, which could increase the host's probability of encountering ectoparasitic acarines (Foufopoulos, '99; Klukowski and Nelson, 2001; Nunn and Dokey, 2006; Fuxjager et al., 2011). Olsson et al. (2000) demonstrated that male sand lizards (*Lacerta agilis*) displayed increased movement when T concentrations were elevated, which led to increased tick burdens. When T was experimentally elevated in free-ranging male *S. undulatus*, movement, home range size, and ectoparasite loads all increased (John-Alder et al., 2009). Lane et al. ('95) suggested that infestation of *S. occidentalis* by *I. pacificus* primarily occurs

when lizards are nocturnally inactive beneath a light layer of soil or leaf litter; however, a later study found that wood substrates, such as tree trunks and logs, had substantially higher numbers of nymphal *I. pacificus* than leaf litter (Lane et al., 2007). Ectoparasite burden differences observed in T-treated lizards in this study may be the consequence of movement or microhabitat use alterations that affect exposure to host-seeking parasites. In addition to host physiological and behavioral factors, habitat, host periodicity, and ectoparasite host-seeking behavior need to be taken into consideration to better understand local variations in parasite burdens.

As has been found with ticks, studies examining the effect of T on mite ectoparasitism typically demonstrate a positive relationship between circulating T concentration and host mite burdens. Studies by Cox and John-Alder (2007) and Klukowski and Nelson (2001) showed that testosterone implantation in male *S. undulatus* resulted in increased mite parasitism. It is therefore intriguing that T-implanted lizards had significantly lower mite loads than control lizards in the present study. One potential explanation for this could be interspecific competition between ticks and mites (Combes, 2001; Poulin, 2007), such that ticks outcompete mites for optimal host attachment sites. Reptile mites frequently have preferred feeding sites, particularly regions where the skin layers are thin (Wharton and Fuller, '52; Delfino et al., 2011). Both ticks and mites infesting *S. occidentalis* frequently attach inside the nuchal pocket, which probably reduces dislodgement during host movement (Arnold, '86; Cunha-Barros et al., 2003). During our study, perhaps increased nuchal pocket tick densities of T-

implanted lizards displaced mites, resulting in overall lower mite burdens than control lizards. This reasoning is mostly speculative; however, evidence supports interspecific competition for optimum feeding sites between seabird mites (Choe and Kim, '89), rodent fleas (Krasnov et al., 2005), and also between rodent fleas and two species of ixodid ticks (Krasnov et al., 2010). Future studies could further elucidate the role of interspecific competition between ticks and mites by experimentally infesting hosts with both of these ectoparasites, determining if attachment success is impacted by the presence of the other group, and evaluating the relationship between attachment site and feeding success.

Alternatively, because the feeding duration and behavior of parasitic mites and ticks differ, these acarines may elicit somewhat distinct immune responses, and could respond differently to host T concentrations. For example, trombiculid mites typically persist on reptilian hosts for longer periods of time than ticks (Arnold, '86; Curtis and Baird, 2008), periodically feeding and developing while on the host; thus, mites may provoke a reduced immune response (Wrenn, '96) compared to ticks. Furthermore, T may have different effects on immune responses that are relevant to different parasites. Fuxjager et al. (2011) demonstrated that elevated T increased trombiculid mite abundance, decreased the abundance of one species of gastrointestinal nematode, but had no effect on a second gastrointestinal nematode species. In our study it is intriguing to consider that the host immune response may have been dampened in T-implanted lizards in a manner that increased the permissiveness of tick attachment and feeding, but decreased mite feeding success on these same males. Future studies should focus on characterization of the reptile host immune response to feeding by ticks versus mites. The role of T in these potentially different responses and its impact on ectoparasitism should also be examined.

In summary, administration of exogenous T to free-ranging male lizards during the spring mating season increases tick loads and decreases mite loads. In addition to abiotic factors that mediate local tick abundance and prevalence on hosts, an understanding of how host-based physiological factors can mediate tick burdens on lizards may improve our ability to predict seasonal tick population dynamics on hosts, which ultimately contributes to a better understanding of the ecology of tick-borne diseases such as Lyme disease (Glery and Ostfeld, 2007; Salkeld and Lane, 2010; Swei, 2011). Future studies should focus on determining the behavioral and physiological mechanisms that give rise to the observed effects of T on parasitism, as well as potential mechanisms of interspecific competition among ectoparasites.

ACKNOWLEDGMENTS

LITERATURE CITED

Allred DM, Beck DE. 1962. Ecological distribution of mites on lizards at the Nevada atomic test site. Herpetologica 18:47–51.

Anthony CD, Mendelson JR III, Simons RR. 1994. Differential parasitism by sex on Plethodontid salamanders and histological evidence for structural damage to the nasolabial groove. Am Midl Nat 132:302–307.

Arnold EN. 1986. Mite pockets of lizards, a possible means of reducing damage by ectoparasites. Biol J Linn Soc 29:1–21.

Aubret F, Bonnet X, Harris M, Maumelat S. 2005. Sex differences in body size and ectoparasite load in the ball python, *Python regius.* J Herpetol 39:312–315.

Bakker TCM, Mazzi D, Zala S. 1997. Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. Ecology 78:1098–1104.

Baudoin M. 1975. Host castration as a parasitic strategy. Evolution 29:335–352.

Belliure J, Smith L, Sorci G. 2004. Effect of testosterone on T cell-mediated immunity in two species of Mediterranean lacertid lizards. J Exp Zool 301A:411–418.

Brooks DR. 1979. Testing the context and extent of host-parasite coevolution. Syst Zool 28:299–307.

Brossard M, Wikel SK. 2004. Tick immunobiology. Parasitology 129: S161–S176.

Brown SG, Kwan S, Shero S. 1995. The parasitic theory of sexual reproduction: parasitism in unisexual and sexual geckos. Proc R Soc Lond B 260:317–320.

Burgdorfer W, Lane RS, Barbour AG, Gresbrink RA, Anderson JR. 1985. The western black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi.* Am J Trop Med Hyg 34:925–930.

Canals A, Oleaga A, Pérez R, Domínguez J, Encinas A, Sánchez-Vízcaino JM. 1990. Evaluation of an enzyme-linked immunosorbent assay to detect specific antibodies in pig infested with the tick *Ornithodoros erraticus* (Argasidae). Vet Parasitol 37:145–153.

Choe JC, Kim KC. 1989. Oecologia Microhabitat selection and coexistence in feather mites (Acari: Analgoidea) on Alaskan seabirds. Oecologia 79:10–14.

Christian KA, Bedford GS. 1995. Physiological consequences of filarial parasites in the frillneck lizard, *Chlamydosaurus kingii*, in northern Australia. Can J Zool 73:2302–2306.

Clobert J, Oppliger A, Sorci G, Ernande B, Swallow JG, Garland T. 2000. Trade-offs in phenotypic traits: endurance at birth, growth, survival, predation and susceptibility to parasitism in a lizard, *Lacerta vivipara.* Funct Ecol 14:675–684.

Combes C. 2001. Parasitism: the ecology and evolution of intimate interactions. Chicago, IL: University of Chicago Press.

Cooper WE Jr, Mendonca MT, Vitt LJ. 1987. Induction of orange head coloration and activation of courtship and aggression by

testosterone in the male broad-headed skink (*Eumeces laticeps*). J Herpetol 21:96–101.

Cotgreave P, Clayton DH. 1994. Comparative analysis of time spent grooming by birds in relations to parasite load. Behaviour 131:171–187.

Cox RM, John-Alder HB. 2007. Increased mite parasitism as a cost of testosterone in male striped plateau lizards *Sceloporus virgatus*. Funct Ecol 21:327–334.

Cox RM, Skelly SL, John-Alder HB. 2005a. Testosterone inhibits growth in juvenile male eastern fence lizards (*Sceloporus undulatus*): implications for energy allocation and sexual size dimorphism. Physiol Biochem Zool 78:531–545.

Cox RM, Skelly SL, Leo A, John-Alder HB. 2005b. Testosterone regulates sexually dimorphic coloration in the eastern fence lizard, *Sceloporus undulatus*. Copeia 2005:597–608.

Cox RM, Zilberman V, John-Alder HB. 2008. Testosterone stimulates the expression of a social color signal in Yarow's spiny lizard, *Sceloporus jarrovii*. J Exp Zool A 309A:505–514.

Cunha-Barros M, Van Sluys M, Vricibradic D, Galdino CAB, Hatano FH, Rocha CFD. 2003. Patterns of infestation by chigger mites in four diurnal lizard species from a restinga habitat (Jurubatiba) of southeastern Brazil. Braz J Biol 63:393–399.

Curtis JL, Baird TA. 2008. Within-population variation in free-living adult and ectoparasitic larval trombiculid mites on collared lizards. Herpetologica 64:189–199.

Delfino MMS, Ribeiro SC, Furtado IP, Anjos LA, Almeida WO. 2011. Pterygosomatidae and Trombiculidae mites infesting *Tropidurus hispidus* (Spix, 1825) (Tropiduridae) lizards in northeastern Brazil. Braz J Biol 71:549–555.

DeNardo DF, Sinervo B. 1994. Effects of steroid hormone interaction on activity and home-range size of male lizards. Horm Behav 28:273–287.

Dence WA. 1958. Studies on ligula-infected common shiners (*Notropis cornutus frontalis agassiz*) in the Adirondacks. J Parasitol 44:334–338.

Duffy DL, Bentley GE, Drazen DL, Ball GF. 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. Behav Ecol 11:654–662.

Dunlap KD, Mathies T. 1993. Effects of nymphal ticks and their interaction with malaria on the physiology of male fence lizards. Copeia 1993:1045–1048.

Eisen L, Eisen RJ. 1999. Abundance of ticks (Acari: Ixodidae) infesting the western fence lizard, *Sceloporus occidentalis*, in relation to environmental factors. Exp Appl Acarol 23:731–740.

Eisen RJ, Eisen L, Lane RS. 2001. Prevalence and abundance of *Ixodes pacificus* immatures (Acari: Ixodidae) infesting western fence lizards (*Sceloporus occidentalis*) in northern California: temporal trends and environmental correlates. J Parasitol 87:1301–1307.

Eisen L, Eisen RJ, Lane RS. 2004. The roles of birds, lizards, and rodents as hosts for the western black-legged tick *Ixodes pacificus*. J Vector Ecol 29:295–308.

Evans MR, Goldsmith AR, Norris SRA. 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). Behav Ecol Sociobiol 47:156–163.

Folstad I, Nilssen AC, Halvorsen O, Anderson J. 1989. Why do male reindeer (*Rangifer t. tarandus*) have higher abundance of second and third instar larvae of *Hypoderma tarandi* than females? Oikos 55:87–92.

Foufopoulos J. 1999. Host–parasite interactions in the mountain spiny lizard, Sceloporus jarrovi [PhD dissertation]. Madison: University of Wisconsin.

Fuxjager MJ, Foufopoulos J, Diaz-Uriarte R, Marler C. 2011. Functionally opposing effects of testosterone on two different types of parasite: implications for the immunocompetence handicap hypothesis. Funct Ecol 25:132–138.

Galbe J, Oliver JH Jr. 1992. Immune response of lizards and rodents to larval *Ixodes scapularis* (Acari: Ixodidae). J Med Entomol 29:774–783.

Glery ST, Ostfeld RS. 2007. The role of lizards in the ecology of Lyme disease in two endemic zones of the northeastern United States. J Parasitol 93:511–517.

Giorgi MS, Arlettaz R, Christe P, Vogel P. 2001. The energetic grooming costs imposed by a parasitic mite (*Spinturnix myoti*) upon its bat host (*Myotis myotis*). Proc R Soc Lond B 268:2071–2075.

Goldberg SR, Bursey CR. 1991. Integumental lesions caused by ectoparasites in a wild population of the side-blotched lizard (*Uta stansburiana*). J Wildlife Dis 27:68–73.

Grossman CJ. 1985. Interactions between the gonadal steroids and the immune system. Science 227:257–260.

Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384–387.

Hart BL. 1992. Behavioral adaptations to parasites: an ethological approach. J Parasitol 78:256–265.

Hillgarth N, Wingfield JC. 1997. Parasite-mediated sexual selection: endocrine aspects. In: Clayton DH, Moore J, editors. Host-parasite evolution: general principles and avian models. New York, NY: Oxford University Press. p 78–84.

Holmes JC, Bethel WM. 1972. Modification of intermediate host behavior by parasites. Zool J Linn Soc Lond 51:123–149.

Hughes VL, Randolph SE. 2001. Testosterone increases the transmission potential of tick-borne parasites. Parasitology 123:365–371.

John-Alder HB, Cox RM, Haenel GJ, Smith LC. 2009. Hormones, performance, and fitness: natural history and endocrine experiments on a lizard (*Sceloporus undulatus*). Integr Comp Biol 49:393–407.

Karvonen A, Seppala O, Valtonen ET. 2004. Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. Parasitology 129:159–164.

Klukowski M. 2004. Seasonal changes in abundance of host-seeking chiggers (Acari: Trombiculidae) and infestations on fence lizards, *Sceloporus undulatus*. J Herpetol 38:141–144.

Klukowski M, Nelson CE. 1998. The challenge hypothesis and seasonal changes in aggression and steroids in male northern fence lizards (*Sceloporus undulatus hyacinthinus*). Horm Behav 33:197–204.

Klukowski M, Nelson CE. 2001. Ectoparasite intensities in free-ranging northern fence lizards, *Sceloporus undulatus hyacinthinus*: effects of testosterone and sex. Behav Ecol Sociobiol 49:289–295.

Krasnov BR, Burdelova NV, Irina S. 2005. Larval interspecific competition in two flea species parasitic on the same rodent host. Ecol Entomol 30:146–155.

Krasnov BR, Stanko M, Morand S. 2010. Competition, facilitation or mediation via host? Patterns of infestation of small European mammals by two taxa of haematophagous arthropods. Ecol Entomol 35:37–44.

Kristan DM, Hammond KA. 2000. Combined effects of cold exposure and sublethal intestinal parasites on host morphology and physiology. J Exp Biol 203:3495–3504.

Kuo MM, Lane RS, Gicias PC. 2000. A comparative study of mammalian and reptilian alternative pathway of complement-mediated killing of the Lyme disease spirochete (*Borrelia burgdorferi*). J Parasitol 86:1223–1228.

Lane RS, Kleinjan JE, Schoeler GB. 1995. Diel activity of nymphal *Dermacentor occidentalis* and *Ixodes pacificus* (Acari: Ixodidae) in relation to meteorological factors and host activity periods. J Med Entomol 32:290–299.

Lane RS, Loye JE. 1989. Lyme disease in California: interrelationship of *Ixodes pacificus* (Acari: Ixodidae), the western fence lizard (*Sceloporus occidentalis*), and *Borrelia burgdorferi*. J Med Entomol 26:272–278.

Lane RS, Mun J, Peribáñez MA, Stubbs HA. 2007. Host-seeking behavior of *Ixodes pacificus* (Acari: Ixodidae) nymphs in relation to environmental parameters in dense-woodland and woodland-grass habitats. J Vector Ecol 32:342–357.

Lane RS, Quistad GB. 1998. Borreliacidal factor in the blood of the western fence lizard (*Sceloporus occidentalis*). J Parasitol 84:29–34.

Lefcort H, Blaustein AR. 1991. Parasite load and brightness in lizards: an interspecific test of the Hamilton and Zuk hypothesis. J Zool 224:491–499.

Lopé de F, Møller AP, Cruz de la C. 1998. Parasitism, immune response, and reproductive success in the house martin *Delichon urbica*. Oecologia 114:188–193.

Lumbad AS, Vredevoe LK, Taylor EN. 2011. Season and host affect ectoparasite load in western fence lizards (*Sceloporus occidentalis*) on the California central coast. Southwest Nat 56:369–377.

Manweiler SA, Lane RS, Tempelis CH. 1992. The western fence lizard *Sceloporus occidentalis*: evidence of field exposure to *Borrelia burgdorferi* in relation to infestation by *Ixodes pacificus* (Acari: Ixodidae). Am J Trop Med Hyg 47:328–336.

Marler CA, Moore MC. 1988. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. Behav Ecol Sociobiol 23:21–26.

Marler CA, Moore MC. 1989. Time and energy costs of aggression in testosterone-implanted free-living male mountain spiny lizards (*Sceloporus jarrovi*). Physiol Zool 62:1334–1350.

Mitchell LG. 1989. Myxobolid parasites (Myxozoa: Myxobolidae) infecting fishes of western Montana, with notes on histopathology, seasonality, and intraspecific variation. Can J Zool 67:1915–1922.

Møller AP. 1997. Immune defence, extra–pair paternity, and sexual selection in birds. Proc R Soc Lond B 264:561–566.

Møller AP, Christie P, Lux E. 1999. Parasitism, host immune function, and sexual selection. Q Rev Biol 74:3–20.

Moore J. 2002. Parasites and the behavior of animals. New York, NY: Oxford University Press.

Moore MC. 1986. Elevated testosterone levels during non-breeding season territoriality in a fall-breeding lizard, *Sceloporus jarrovi*. J Comp Physiol A 158:159–163.

Mooring MS, Blumstein DT, Stoner CJ. 2004. The evolution of parasite-defence grooming in ungulates. Biol J Linn Soc 81:17–37.

Nunn CL, Dokey ATW. 2006. Ranging patterns and parasitism in primates. Biol Letters 2:351–354.

Oetinger DF, Nickol BB. 1981. Effects of acanthocephalans on pigmentation of freshwater isopods. J Parasitol 67:672–684.

Olsson M, Wapstra E, Madsen T, Silverin B. 2000. Testosterone, ticks, and travels: a test of the immunocompetence-handicap hypothesis in free ranging male sand lizards. Proc R Soc B 267:2339–2343.

Olsson M, Madsen T, Wapstra E, Silverin B, Ujvari B, Wittzell H. 2005. MHC, health, color, and reproductive success in sand lizards. Behav Ecol Sociobiol 58:289-294.

Oppliger A, Giorgi MS, Conelli GA, Nembrini M, John-Alder HB. 2004. Effect of testosterone on immunocompetence, parasite intensity, and metabolism in the common wall lizard (*Podarcis muralis*). Can J Zool 82:1713–1719.

Pollock NB, Vredevoe LK, Taylor EN. 2012. How do host sex and reproductive state affect host preference and feeding duration of ticks? Parasitol Res. Available online ahead of print: DOI: 10.1007/s00436-012-2916-8.

Poulin R. 1996. Sexual inequalities in helminth infections: a cost of being a male? Am Midl Nat 147:287–295.

Poulin R. 2007. Evolutionary ecology of parasites. Princeton: Princeton University Press.

Proctor H, Owens I. 2000. Mites and birds: diversity, parasitism and coevolution. Trends Ecol Evol 15:358–364.

Ramachandra RN, Wikel SK. 1992. Modulation of host-immune responses by ticks (Acari: Ixodidae): effect of salivary gland extracts on host macrophages and lymphocyte cytokine production. J Med Entomol 29:818–826.

Rand MS. 1992. Hormonal control of polymorphic and sexually dimorphic coloration in the lizard *Sceloporus undulatus erythrocheilus*. Gen Comp Endocrinol 88:461–468.

Ribeiro JMC. 1989. Role of saliva in tick/host interactions. Exp Appl Acarol 7:15–20.

Roberts ML, Buchanan KL, Evans MR. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. Anim Behav 68:227–239.

Saad AH, Khalek NA, El Ridi R. 1990. Blood testosterone level: a season-dependent factor regulating immune reactivity in lizards. Immunobiology 180:184–194.

Saino N, Møller AP, Bolzern AM. 1995. Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. Behav Ecol 6:397–404.

Salkeld DJ, Schwarzkopf L. 2005. Epizootiology of blood parasites in an Australian lizard: a mark-recapture study of a natural population. Int J Parasitol 35:11–18.

Salkeld DJ, Lane RS. 2010. Community ecology and disease risk: lizards, squirrels, and the Lyme disease spirochete in California, USA. Ecology 91:293–298.

Salvador A, Veiga JP, Martin J, Lopez P. 1997. Testosterone supplementation in subordinate, small male lizards: consequences for aggressiveness, color development, and parasite intensity. Behav Ecol 8:135–139.

Salvador A, Veiga JP, Martin J, Lopez P, Abelenda M, Puerta M. 1996. The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasite infestation. Behav Ecol 7:145–150.

Schall JJ, Dearing MD. 1987. Malarial parasitism and male competition for mates in the western fence lizard, *Sceloporus occidentalis*. Oecologia 73:389–392.

Schall JJ, Marghoob AB. 1995. Prevalence of a malarial parasite over time and space: *Plasmodium mexicanum* in its vertebrate host, the western fence lizard *Sceloporus occidentalis*. J Anim Ecol 64:177–185.

Schall JJ, Prendeville HR, Hanley KA. 2000. Prevalence of the tick *Ixodes pacificus*, on western fence lizards, *Sceloporus occidentalis*: trends by gender, size, season, site, and mite infestation. J Herpetol 34:160–163.

Schall JJ, Smith TC. 2006. Detection of a malaria parasite (*Plasmodium mexicanum*) in ectoparasites (mites and ticks), and possible significance for transmission. J Parasitol 92:413–415.

Schwanz LE. 2006. Schistosome infection in deer mice (*Peromyscus maniculatus*): impacts on host physiology, behavior and energetics. J Exp Biol 209:5029–5037.

Sinervo B, Miles DB, Frankino WA, Klukowski M, DeNardo DF. 2000. Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. Horm Behav 38:222–233.

Slowik TJ, Lane RS. 2009. Feeding preferences of the immature stages of three western North American Ixodid ticks (Acari) for avian, reptilian, or rodent hosts. J Med Entomol 46:115–122.

Soler JJ, Martínez JG, Soler M, Møller AP. 2001. Coevolutionary interactions in a host-parasite system. Ecol Lett 4:470–476.

Sorci G. 1996. Patterns of haemogregarine intensity, aggregation, and prevalence as a function of host age in the lizard *Lacerta vivipara*. J Parasitol 82:676–678.

Swei A, Ostfeld RS, Lane RS, Briggs CJ. 2011. Impact of the experimental removal of lizards on Lyme disease risk. Proc Roy Soc B 278:2970–2978.

Tälleklint-Eisen L, Eisen RJ. 1999. Abundance of ticks (Acari: Ixodidae) infesting the western fence lizard, *Sceloporus occidentalis*, in relation to environmental factors. Exp Appl Acarol 23:731–740.

Teel PD, Hopkins SW, Donahue WA, Strey OF. 1998. Population dynamics of immature *Amblyoma maculatum* (Acari: Ixodidae) and other ectoparasites on meadowlarks and northern bobwhite quail resident to the coastal prairie of Texas. J Med Entomol 35:483–488.

Theodoropoulos G, Koutsotolis K, Nikolaou E, Kalogiannis D, Petrakos G. 1998. Seasonal variation of gastrointestinal nematodes of sheep in the region of Joannina, Greece. Int J Parasitol 28:1287–1292.

Uller T, Olsson M. 2003. Prenatal exposure to testosterone increases ectoparasite susceptibility in the common lizard (*Lacerta vivipara*). Proc Roy Soc B 270:1867–1870.

Veiga JP, Salvador A, Merino S, Puerta M. 1998. Reproductive effort affects immune response and parasite infection in a lizard: a phenotypic manipulation using testosterone. Oikos 82:313–318.

Wharton GW, Fuller HS. 1952. A manual of the chiggers. The biology, classification, distribution, and importance to man of the larvae of the family Trombiculidae (Acarina). Mem Entomol Soc Wash 4:1–185.

Wheeler CM, Coleman JL, Habicht GS, Benach JL. 1989. Adult *Ixodes dammini* on rabbits: development of acute inflammation in the skin and immune responses to salivary gland, midgut, and spirochetal components. J Infect Dis 159:265–273.

Wiel ZM, Martin LB II, Nelson RJ. 2006. Interactions among immune, endocrine, and behavioural response to infection. In: Morand S, Krasnov BR, Poulin R, editors. Micromammals and macroparasites: from evolutionary ecology to management. New York, NY: Springer-Verlag. p 443–474.

Wikel SK. 1996. Host immunity to ticks. Annu Rev Entomol 41:1–22.

Wikel SK. 1999. Tick modulation of host immunity: an important factor in pathogen transmission. Int J Parasitol 29:851–859.

Wikel SK, Ramachandra RN, Bergman DK. 1994. Tick-induced modulation of the host immune response. Int J Parasitol 24:59–66.

Wingfield JC, Hahn TP. 1994. Testosterone and territorial behaviour in sedentary and migratory sparrows. Anim Behav 47:77–89.

Wrenn WJ. 1996. Immune responses to mange mites and chiggers. In: Wikel SK, editor. The immunology of host-ectoparasitic arthropod relationships. Wallingford: Cab International. p 259–289.

Zang X, Maizels RM. 2001. Serine proteinase inhibitors from nematodes and the arms race between host and pathogen. Trends Biochem Sci 26:191–197.

Zuk M. 1987. Seasonal and individual variation in gregarine parasite levels in the field crickets *Gryllus veletis* and *Gryllus pennsylvanicus*. Ecol Entomol 12:341–348.

Zuk M. 1990. Reproductive strategies and sex differences in disease susceptibility: an evolutionary viewpoint. Parasitol Today 6:231–233.

Zuk M, Johnsen TS, Maclarty T. 1995. Endocrine-immune interactions, ornaments, and mate choice in red jungle fowl. Proc R Soc Lond B 260:205–210.

Zuk M, McKean KA. 1996. Sex differences in parasitic infections: patterns and processes. Int J Parasitol 26:1009–1024.