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.

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; Martin 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; Saikeld 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 immunological and physiological 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-implemented male lizards should have heavier ectoparasite burdens than nonimplanted 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, ± 0.5 cm) and body mass (± 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-implemented (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 silicone 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 levels physiologically relevant. 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 α = 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 ≤ 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 ≤ 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 ≤ 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 ≤ 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
and Wingfield, ’97; Veiga et al., ’98; Duffy et al., 2000; Uller and Olsson, 2003; Bellure et al., 2004; Oppler 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-

Figure 1. Ectoparasite loads on testosterone-implanted (gray) and control (white) male western fence lizards (Sceloporus occidentalis). Values are reported as means ± 1 SEM. Asterisks indicate a significant difference between treatment groups (P ≤ 0.0001).
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 acarine 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 characterizing 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 Östfeld, 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.

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EXOGENOUS TESTOSTERONE AND ECTOPARASITE LOADS


