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Increased mite parasitism as a cost of testosterone in male striped plateau lizards *Sceloporus virgatus*

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Summary

1. Testosterone (T) co-ordinates the seasonal and sex-specific expression of numerous physiological, behavioural and morphological traits that contribute to male reproductive success. However, increased susceptibility to parasitism has been proposed as a potential cost of elevated plasma T.

2. During the spring breeding season, male striped plateau lizards *Sceloporus virgatus* harbour significantly more ectoparasitic mite larvae (Acari: Trombiculidae) than females. Plasma T levels are also elevated in males at this time, suggesting that sex differences in mite parasitism may be driven by underlying sex differences in circulating T.

3. We tested this hypothesis experimentally by manipulating plasma T levels of yearling males via surgical castration and exogenous T implants. Upon recapture of free-living animals, we found significantly fewer mites on castrated males relative to either intact controls or castrated males that received T implants.

4. After removing variance attributable to treatment effects, we observed (1) a positive correlation between residual measures of plasma T and mite load, and (2) a negative correlation between residual measures of mite load and growth rate. These correlations suggest a growth cost associated with mite parasitism.

5. Previous studies have shown that exogenous T increases parasitism, but ours is one of the few to show that castration also reduces parasitism. This result, coupled with the fact that our induced plasma T levels remain within physiological limits, makes this one of the clearest demonstrations of a functional relationship between T and parasitism in any free-living vertebrate.

Key-words: castration, cost of reproduction, growth rate, immunocompetence handicap hypothesis, Trombiculidae.

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Introduction

The androgenic sex steroid testosterone (T) co-ordinates the seasonal and sex-specific expression of numerous behavioural, physiological, and morphological traits that contribute to male reproductive success. However, elevated T can also incur costs that reduce male fitness. Increased parasitism may represent one such cost that has significant implications for the evolution of vertebrate mating systems (Hamilton & Zuk 1982; Folstad & Karter 1992; Moore & Wilson 2002). Several recent comparative studies of vertebrates have shown that males often harbour more parasites than conspecific females, although the opposite may be true of some host–parasite systems (Poulin 1996; Schalk & Forbes 1997; McCurdy *et al.* 1998; Moore & Wilson 2002; Klein 2004). Experiments that have attempted to demonstrate a causal relationship between T and parasitism have yielded mixed results, but a recent meta-analysis of such studies found a significant overall increase in parasite load in response to exogenous T (Roberts, Buchanan & Evans 2004). This analysis also revealed that the effects of T are particularly strong in studies of reptile ectoparasites, suggesting that lizards and mites provide a promising host–parasite system in which to study the influence of T on parasitism.

In lizards, T stimulates activity, endurance, locomotor performance, territorial aggression, home range size and secondary sexual coloration (Marler & Moore 1988; Moore 1988; DeNardo & Sinervo 1994; John-Alder *et al.* 1996; Klukowski, Jenkinson & Nelson 1998; Klukowski, Ackerson & Nelson 2004; Cox, Skelly & John-Alder 2005a; Cox *et al.* 2005b). These effects of T presumably enhance male reproductive success, but elevated plasma T levels may also incur costs in the

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form of reduced energy acquisition (Marler & Moore 1988, 1989, 1991), increased energy expenditure (Marler *et al*. 1995) and reduced growth (Cox & John-Alder 2005; Cox *et al*. 2005a). Several experiments have also shown that T increases parasitism in male lizards (Salvador *et al*. 1996, 1997; Olsson *et al*. 2000; Klukowski, Ireland & Nelson 2001; Cox *et al*. 2005a). This may entail a substantial cost of T for male lizards, as parasitism has been linked to reductions in growth rate, body condition, fat storage, immune function, survival and reproductive success (Schall, Bennett & Putnam 1982; Schall 1983a,b; Schall & Dearing 1987; Saad, Khalek & el Ridi 1990; Sorci & Clobert 1995; Salvador *et al*. 1996; Sorci, Clobert & Michalakis 1996; Oppliger & Clobert 1997; Klukowski & Nelson 2001; Oppliger *et al*. 2004).

Although numerous experiments have demonstrated that exogenous T increases the prevalence and intensity of parasitism in lizards and other vertebrates, many of these studies have not explicitly addressed the physiological relevance of induced plasma T levels. Further, only five of 17 such experiments reviewed by Roberts *et al*. (2004) included both castration and exogenous T replacement, with no castration studies conducted on reptile hosts (but see Cox *et al*. 2005a). This methodological bias is no idle concern, given that significant effects of T on parasitism and immune function are more likely to be observed when exogenous T is administered in the absence of surgical castration (Roberts *et al*. 2004). The reasons for such experimental bias are not clear, but one concern is that exogenous T may often be administered in pharmacological doses, causing increases in parasitism that may be of questionable ecological relevance. Similar interpretations have been proposed for severe detrimental effects of exogenous T on growth and survival (Hews, Knapp & Moore 1994; Lerner & Mason 2001), but this issue has not been explicitly addressed in most studies of T and parasitism.

In the present study, we use both castration and replacement of exogenous T to determine the effect of physiologically relevant variation in plasma T on mite parasitism in the lizard *Sceloporus virgatus*. Previous studies have shown that males of this species harbour greater numbers of mites than females during the spring breeding season (Smith 1996), and that males and females differ substantially in plasma T levels during this time (Abell 1998b; Cox & John-Alder 2005). Further, we have previously shown that yearling females grow at twice the rate of males during the spring breeding season (Cox 2005), and that this dramatic sex difference in growth rate may result from an inhibitory effect of T on male growth (Cox & John-Alder 2005). These results suggest that elevated plasma T levels may be associated with energetic costs that constrain male growth and that increased mite parasitism may constitute such a cost. If elevated plasma T leads to increased mite parasitism, then we predict that castration should reduce mite loads relative to intact males, and that treatment of castrated males with exogenous T should restore mite loads to levels typical of intact males. To determine whether increased parasitism incurs a growth cost that could contribute to the inhibitory effect of T on male growth (Abell 1998a; Cox & John-Alder 2005), we also test for a relationship between parasitism and growth rate across experimental males.

Materials and methods

STUDY SPECIES AND EXPERIMENTAL DESIGN

We studied the striped plateau lizard *Sceloporus virgatus* Smith along a 2-km section of streambed in Cave Creek Canyon, located 1–3 km north-west of the American Museum of Natural History's South-western Research Station in the Coronado National Forest, Cochise County, Arizona (31°53–54′N, 109°13′W, elevation 1660–1760 m). We collected male yearlings (i.e. males born the previous September, $n = 66$) by hand-held noose in late April of 2004, near the onset of the mating season. At this point in the reproductive cycle, plasma T levels reach seasonal peaks in both yearling and older males, and are substantially elevated relative to levels typical of females or nonbreeding males (Abell 1998b; Cox & John-Alder 2005).

Upon capture, we measured snout–vent length (SVL) to the nearest 1 mm with a ruler, body mass to the nearest 0·1 g with a Pesola® spring scale, and gave each lizard a unique toe clip for identification. We then assigned males to one of three size-matched treatment groups: castrated males receiving a placebo implant (CAST), castrated males receiving a T implant (TEST), and intact control males receiving a placebo implant (CON). Implants were constructed of Silastic® tubing (Dow Corning: 1·47 mm inner diameter, 1·96 mm outer diameter) using a novel method that allowed us to control precisely the amount of T in each tubule. After sealing one end of each tubule with silicone adhesive, we used a Hamilton syringe to inject 3 μL of a solution of T (Sigma T-1500) dissolved in dimethyl sulphoxide (DMSO: Sigma D-8779, 100 μg T μL^{-1}) into the open end of each implant. We then sealed each tubule with silicone adhesive and waited several days for the DMSO to evaporate and diffuse through the tubing, leaving 300 μg of crystalline T within the lumen (*c*. 1·5 mm length) of each implant. Previous studies have shown that these implants reliably induce plasma T levels that are well within the normal physiological range for males of several *Sceloporus* species (see Cox & John-Alder 2005; Cox *et al*. 2005a,b).

We anaesthetized animals with an intramuscular injection of ketamine (Vetus Animal Health (Vetus Animal Health, MFA Inc., Columbia, MO, USA), 1·3 mg per 10 g body mass) and exposed the testes with a single ventral incision. We then bilaterally castrated CAST and TEST males by ligating each spermatic cord with surgical silk, ablating each testis, and cauterizing each ligated spermatic cord after removal of the testes. For CON males, we performed 'sham' surgeries in which we made identical incisions to expose and manipulate

the testes while leaving them intact. We then inserted either a T implant (TEST) or a placebo implant (CAST and CON) into the coelomic cavity and closed the incision with Nexaband® surgical glue (Veterinary Products Laboratories, Phoenix, AZ, USA). Survival from surgery was high (63 of 66, 96%), resulting in initial sample sizes of 21 CAST, 22 CON and 20 TEST.

We released animals at their location of capture and left them undisturbed until recapture in June (mean 42 days post-surgery), at which time mite parasitism is maximal in this population (Abell 2000). Of the 63 animals that we released following surgery, we recaptured nine CAST, 17 CON and 15 TEST. Animals that were not recaptured presumably died or emigrated, as we searched the study site thoroughly for 10 days in an attempt to locate all remaining males. Upon recapture, we used a magnifying lens to count the number of larval mites (i.e. chiggers, Acari: Trombiculidae) attached to each lizard. Previous studies have identified *Eutrombicula belkini* as the primary mite parasite in this population (Smith 1996), but we did not verify this identification in the present study. To compare our experimental data to levels of parasitism in the natural population, we counted mites on unmanipulated yearling males and females captured at the same time of year. We also recorded SVL of experimental males and calculated individual growth rates by dividing change in SVL by elapsed time (mm day^{-1}).

TESTOSTERONE ASSAY

At the conclusion of our experiment, we collected blood samples from a subset of experimental males ($n = 6$ CAST, 8 CON, 10 TEST) to measure circulating T levels and verify treatment effects. We also collected blood samples from unmanipulated yearling males and females to verify natural sex differences in plasma T levels. We collected 20–60 µL of blood from the post-orbital sinus within 2 min of capture using heparinized microhematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA, USA). We held samples on ice until they could be centrifuged (within 6 h of collection), and stored the separated plasma at –20 °C until subsequent assays. We performed radioimmunoassays (RIAs) by extracting plasma samples twice in diethyl ether (mean 81% extraction efficiency), drying them under a stream of ultrafiltered air, and reconstituting them in phosphate-buffered saline with gelatin. Reconstituted samples were assayed with ^{3}H-T as a radiolabel (PerkinElmer Life Sciences Inc., Boston, MA, USA) and T antiserum (1 : 18 000 initial dilution) developed in rabbits by A. L. Johnson (The University of Notre Dame, IN, USA). Limits of detection ranged from 1 to 5 pg T per assay tube. All samples were analysed in a single assay with 8% intra-assay variation. Typical interassay variation is 6% for this assay in our laboratory. Further details regarding RIA methodology are reported elsewhere (Smith & John-Alder 1999; Cox & John-Alder 2005; Cox *et al*. 2005a).

STATISTICAL ANALYSES

We used two-tailed *t*-tests to compare mite counts and plasma T levels between unmanipulated males and females. For experimental males, we compared mite counts and plasma T levels using ANOVA with treatment as the main effect and Tukey *post-hoc* tests. To investigate correlations between mite counts, plasma T levels, and growth rates among individuals, we used least-squares linear regression after removing the variance attributable to treatment from each measure. We did this by calculating residuals for each measure based on mean values for each treatment group. Mite counts were $\log_{10}$-transformed prior to statistical analysis. All statistical analyses were conducted using SAS (version 8·2, SAS Institute, Inc., Cary, NC, USA).

Results

PLASMA TESTOSTERONE LEVELS

Upon recapture (mean 42 days post-surgery), plasma T levels were low in CAST, intermediate and variable in CON, and slightly elevated in TEST (Fig. 1a). Plasma

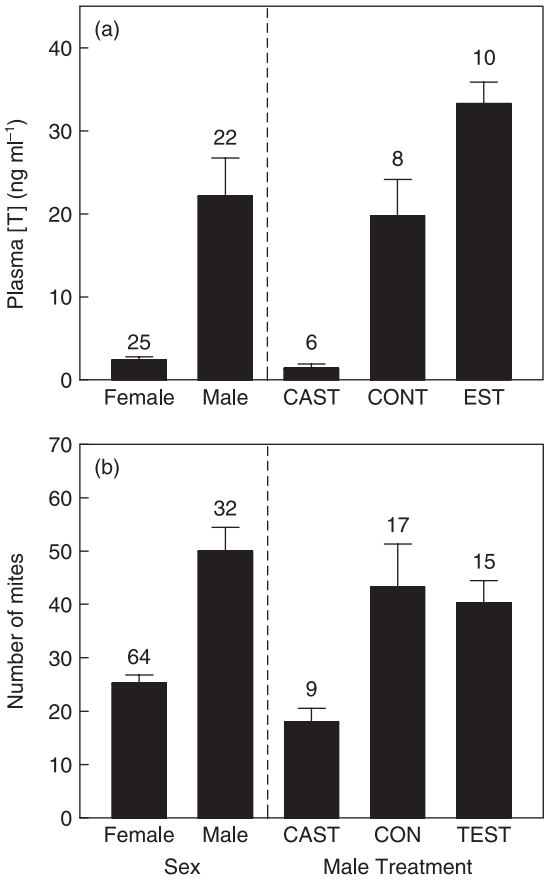

Fig. 1. Mean (± 1 SE) values for (a) plasma testosterone (T) concentration, and (b) number of mites counted on individual *S. virgatus* yearlings. Data are arranged by sex for unmanipulated males and females (left panels) and by treatment for experimental males (right panels). Numbers above each bar indicate sample size.

T levels were significantly different between all treatment groups ($F_{2,23}$ = 23·74; P < 0·001; Tukey *post-hoc* test). A detailed description of these experimentally induced plasma T levels and their relevance to natural ontogenetic patterns is provided elsewhere (Cox & John-Alder 2005). In short, plasma T levels of CON were nearly identical to mean values for unmanipulated males at the same time of year (June). Plasma T levels of TEST were slightly elevated relative to natural levels for June, but lower than peak values observed in April and May (mean 66 ng mL^{-1}; Cox & John-Alder 2005). Plasma T levels of CAST were uniformly low and similar to those of yearling females (Fig. 1a). Thus, our manipulations yielded variation in plasma T that was well within the natural physiological range of yearling males and females.

MITE PARASITISM

Each of the 137 individual lizards that we examined had a least one mite (range 1–115). Infestations were concentrated primarily in the nuchal 'mite pockets' characteristic of *Sceloporus* lizards (Arnold 1986), but we also frequently observed mites attached at other locations. Mite counts were highly variable among individuals, with substantial overlap between sexes and among male treatment groups. Despite this overlap, we counted nearly twice as many mites on males as on females (Fig. 1b), a difference that was highly significant (t = 4·10; P < 0·001; d.f. = 94). Among male treatment groups, we found significantly more mites on CON and TEST than on CAST (ANOVA; $F_{2,38}$ = 6·10; P = 0·005; Fig. 1b).

PARASITISM, TESTOSTERONE AND GROWTH

We found a positive relationship between $\log_{10}$ mite count and plasma T concentration across experimental males (r^2 = 0·347; F = 11·15; P = 0·003; Fig. 2a). However, this relationship includes variance due to overall treatment effects on each variable. After correcting for variance due to treatment effects, we still observed a positive relationship between residual plasma T and residual mite count (r^2 = 0·189; F = 4·81; P = 0·038; Fig. 2b). Therefore, males with relatively higher plasma T concentrations were more heavily parasitized, irrespective of overall treatment effects on either measure.

We also found a negative relationship between $\log_{10}$ mite count and growth rate across individual males (r^2 = 0·213; F = 10·53; P = 0·002; Fig. 3a). This correlation is driven by the significant negative relationships between $\log_{10}$ mite count and growth rate within both the CON group (r^2 = 0·239; P = 0·047) and the TEST group (r^2 = 0·286; P = 0·040; Fig. 3a). After removing variance due to treatment, the overall relationship between growth and parasite load remained essentially unchanged (r^2 = 0·209; F = 10·29; P = 0·003; Fig. 3b). Therefore, males with relatively fewer mites grew more quickly than those that were more heavily parasitized, irrespective of treatment effects on either measure. From the slope of our regression analysis using untransformed data, we estimated the growth cost of mite parasitism as an approximate reduction of 0·02 mm day^{-1} per 10 additional mites.

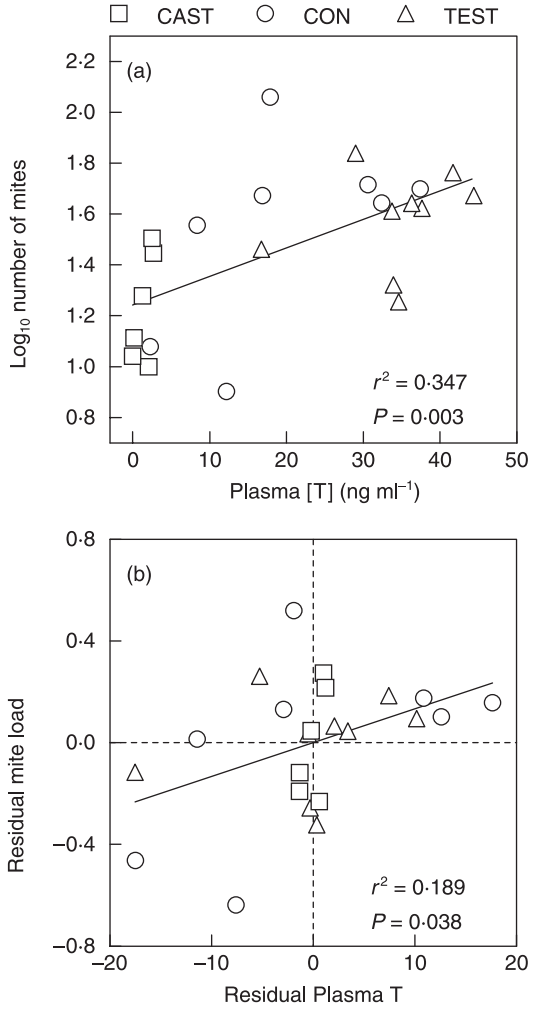

Fig. 2. Mite parasitism as a function of plasma testosterone (T) concentration (a) across individual males irrespective of treatment group, and (b) as residuals adjusted for the mean of each group to remove confounding treatment effects. Statistics are reported for relationships across all treatment groups.

Discussion

On average, we observed twice as many mites per lizard on males relative to females (Fig. 1a), and on CON and TEST relative to CAST (Fig. 1b). Whereas castration reduced mite parasitism to levels characteristic of females, treatment of castrated males with exogenous T elevated mite counts to levels characteristic of intact males. These results indicate that natural sex differences in mite parasitism are driven in part by sex differences in circulating T levels. This conclusion is strengthened by the positive relationship between plasma T and mite parasitism that we observed among experimental males after removing the variance attributable to overall treatment effects (Fig. 2). Thus, we conclude that increased

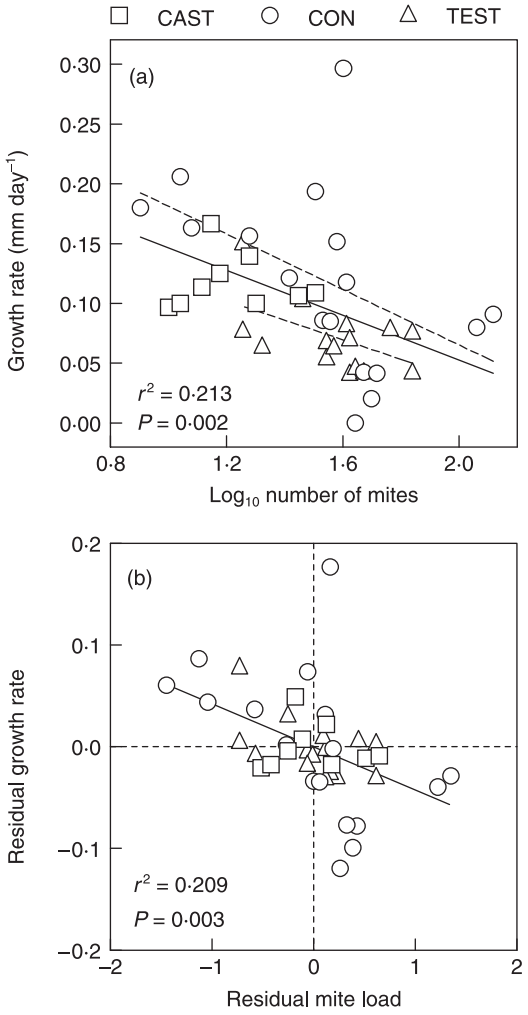

Fig. 3. Growth rate as a function of $\log_{10}$ mite count (a) across individual males irrespective of treatment group, and (b) as residuals adjusted for the mean of each group to remove confounding treatment effects. Dashed lines in panel (a) indicate significant correlations within the CON group (upper line) and the TEST group (lower line). Statistics are reported for relationships across all treatment groups.

mite parasitism represents a natural cost of elevated plasma T in breeding males of *S. virgatus*.

Our results are consistent with studies of other lizard species that have reported increased parasitism following administration of exogenous T to males (Salvador *et al*. 1996, 1997; Olsson *et al*. 2000; Klukowski & Nelson 2001; Cox *et al*. 2005a), although several studies have failed to find an effect of exogenous T on parasitism (Veiga *et al*. 1998; Oppliger *et al*. 2004). However, with the exception of our recent study of *S. undulatus* (Cox *et al*. 2005a), none of these previous studies included a castration treatment to remove the source of endogenous T. Further, inferences regarding the effects of T on parasitism have often been drawn in the absence of actual post-treatment measures of plasma T levels (Salvador *et al*. 1996; Veiga *et al*. 1998; Oppliger *et al*. 2004). Together, these limitations raise questions as to the physiological and ecological relevance of observed effects of exogenous T on parasitism. In other T-manipulation studies involving reptiles, investigators have expressed concerns about the possibility that their implants induced pharmacological T levels that severely impacted survival and growth of implanted animals (Hews *et al*. 1994; Lerner & Mason 2001). However, this issue has not been directly addressed in most studies of T and parasitism in lizards.

Our results clearly show that castration causes a decrease in the intensity of mite parasitism, whereas treatment of castrated males with exogenous T restores mite parasitism to levels typical of intact males. Further, our castration treatments reduced male plasma T to levels characteristic of females, and our implants restored plasma T to levels typical of breeding males. This indicates that effects of T on mite parasitism in *S. virgatus* are not simply artefacts of pharmacological manipulations, but responses to biologically relevant variation in plasma T. Interestingly, we would not have been able to demonstrate an effect of T on parasitism in the absence of our castration treatments, as both TEST and CON males had similarly high mite loads (Fig. 1b). This underscores the importance of an experimental design involving both removal of the primary source of endogenous T and subsequent treatment with exogenous T.

Although elevated plasma T levels are generally associated with increased parasitism (Roberts *et al*. 2004), the causal mechanisms underlying this pattern are not well understood. Two primary, nonexclusive hypotheses have been proposed to link T levels with parasite load (reviewed in Veiga *et al*. 1998; Klukowski & Nelson 2001; Uller & Olsson 2003). One possibility is that T increases the probability of encountering parasites by stimulating movement and extending daily activity period. Although exogenous T typically induces such behavioural responses in free-living lizards (e.g. Marler & Moore 1988, 1989; DeNardo & Sinervo 1994; Olsson *et al*. 2000; Cox *et al*. 2005a), there is little direct evidence to address whether these behavioural changes actually increase exposure to parasites. For example, male fence lizards *Sceloporus occidentalis* harbour more ticks than females, but infestation occurs primarily at night when the lizards are inactive in their burrows, not during diurnal activity (Lane, Kleinjan & Schoeler 1995; Schall, Prendeville & Hanley 2000).

Alternatively, T may increase susceptibility to infestation via suppression of the immune system (Klein 2004). This mechanism has received considerable attention in light of the immunocompetence handicap hypothesis (Folstad & Karter 1992). This hypothesis states that T suppresses immune function, resulting in costs (i.e. increased parasites and pathogens) that act as a handicap (Zahavi 1975) to limit the expression of T-mediated sexual traits to those males in good condition, thus rendering such traits honest signals of male quality (Folstad & Karter 1992). Subsequent studies have focused primarily on testing the mechanistic assumptions that T decreases immunocompetence and increases parasitism (reviewed in Roberts *et al*. 2004). A recent meta-analysis failed to find a consistent directional effect

of T on several measures of immune function and argued that the experimental support for the immunocompetence handicap hypothesis is currently weak (Roberts *et al*. 2004). As we did not measure immune function in our study, we cannot directly address the immunocompetence handicap hypothesis in *S. virgatus*. However, the effect of T on mite load that we observed is consistent with its prediction that T incurs costs such as increased parasitism.

Although T inhibits immune response in some lizards (Saad *et al*. 1990; Veiga *et al*. 1998; Olsson *et al*. 2000), some studies have found that reduced immune function following T manipulation does not necessarily lead to increased parasite load (Oppliger *et al*. 2004). Further, whereas T is generally immunosuppressive in many vertebrates (Klein 2004), some studies have shown that T has no effect or even stimulates immune function (reviewed in Veiga *et al*. 1998; Klukowski & Nelson 2001; Uller & Olsson 2003). Finally, although the immune system is clearly an important component of host defence against ectoparasitic arthropods (reviewed in Wikel 1996; Wiel, Martin & Nelson 2006), very few ecological studies have identified concrete physiological mechanisms linking host immune response to number of ectoparasites *per se*. However, several studies of mammals have demonstrated an acquired immune response to mites and chiggers and have shown that this response often reduces the duration of attachment or number of parasites on the host (reviewed in Wikel 1996; Wrenn 1996; Nisbet & Huntley 2006). A link between immune function and number of ectoparasites is therefore plausible, but definitive studies are lacking for reptiles.

Many deleterious effects of parasitism have been documented in lizards, including skin lesions, inflammation, disease transmission and reductions in stamina, growth rate, body mass, fat storage, tail regeneration, testis size, haematocrit and immune function (Schall *et al*. 1982; Schall 1983b; Saad *et al*. 1990; Goldberg & Bursey 1991; Dunlap & Mathies 1993; Sorci & Clobert 1995; Salvador *et al*. 1996; Sorci *et al*. 1996; Oppliger & Clobert 1997; Klukowski & Nelson 2001). These effects may have important fitness consequences, as evidenced by reduced survival and reproductive success associated with parasitism (Schall 1983a; Schall & Dearing 1987; Sorci & Clobert 1995; but see Abell 2000). In the present study, we found that heavily parasitized males grew more slowly than males with fewer mites, irrespective of treatment effects on either measure (Fig. 3). This inferred growth cost of parasitism would presumably have important fitness consequences, as male reproductive success increases with body size in *S. virgatus* (Abell 1997). This result also suggests that parasitism may provide a mechanism for observed growth inhibition by T in this species (Abell 1998a; Cox & John-Alder 2005), as well as for natural sex differences in growth rate (Cox 2005). Interestingly, the twofold sex difference in mite load that we observed in the present study is similar in magnitude to the twofold sex difference in yearling growth rate that we have previously documented during the spring mating season (Cox 2005).

Previous studies have shown that parasites suppress the growth of lizards and other vertebrate hosts (Schall 1983b; Nilsson 2003), but the precise energetic mechanisms for this effect are not clear. Host metabolic rate often increases in response to parasitism (Booth, Clayton & Block 1993; Kristan & Hammond 2000; Khokhlova *et al*. 2002; Nilsson 2003), suggesting that growth suppression may occur by virtue of an energy allocation trade-off. Potential metabolic costs of parasitism include increased immune function, induction of fever and support of parasite metabolism (reviewed in Nilsson 2003; Hawlena *et al*. 2006). In addition to its possible direct effects on host growth, parasitism may interact with T to inhibit growth. For example, *Lacerta vivipara* lizards that are pre-natally exposed to elevated T levels exhibit reduced post-natal mass gain following tick infestation (Uller & Olsson 2003). However, this effect of parasitism on growth is not observed in the absence of embryonic T manipulation, and pre-natal T exposure actually stimulates post-natal mass gain in the absence of tick infestation (Uller & Olsson 2003). Any such interaction could potentially exacerbate the effects of parasitism on growth of males with high plasma T levels, relative to females or castrated males with low plasma T levels.

Abell (2000) did not find any association between levels of mite parasitism and several measures of male fitness (i.e. mating success, survival, weight loss) in our population of *S. virgatus*. However, Smith (1996) reported a negative correlation between mite count and growth rate in free-living *S. virgatus* males and females from this same population. In the present study, we found a negative relationship between mite load and growth rate among experimental males. However, this correlation does not demonstrate a causal effect of parasitism on growth. One alternative is that T affects parasitism and growth independently, creating a negative association between the two in the absence of a direct causal relationship. For example, T might stimulate activity and movement (see Cox *et al*. 2005a), thereby simultaneously increasing encounters with mites and creating metabolic costs that are traded off against growth. Alternatively, T might suppress energy allocation to both immune function and growth in favour of allocation to reproductive behaviours. These examples are conjectural, but they illustrate the need for caution in attributing the reduced growth of *S. virgatus* males to elevated mite loads *per se*. To address this question directly, it would be necessary to manipulate mite loads and measure subsequent growth, preferably while controlling for confounding factors such as plasma T levels. While it remains uncertain whether increased mite parasitism directly impacts growth, we emphasize that our combined results from several complementary studies do provide direct experimental evidence for effects of T on both mite load and growth rate in *S. virgatus* and closely related *S. undulatus* (Cox & John-Alder 2005; Cox *et al*. 2005a; this study).

In summary, we have shown that yearling *Sceloporus virgatus* males harbour greater numbers of ectoparasitic

mite larvae than females during the spring mating season, when male plasma T levels naturally peak. We have also demonstrated that castration reduces male mite loads to levels typical of females, while chronic treatment of castrated males with exogenous T restores mite loads to levels typical of intact males. The physiological and ecological relevance of our experiment is strengthened by the close agreement between plasma T levels in castrated and T-implanted males and those of unmanipulated females and males. Finally, we have provided correlative evidence that growth rate decreases with increasing mite load among experimental males, suggesting a growth cost of parasitism that may help to explain the inhibitory effects of T on growth in this species. Future work should focus on determining the behavioural and physiological mechanism(s) that give rise to these observed effects of testosterone on parasite load (e.g. increased parasite encounter rates, T-mediated immunosuppression), as well as the ultimate effects of parasitism on host fitness.

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