

Hormones, performance and fitness: Natural history and endocrine experiments on a lizard (*Sceloporus undulatus*)

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Synopsis We used the “morphology–performance–fitness” paradigm (Arnold, 1983) as our framework to investigate endocrine control of performance and fitness in *Sceloporus undulatus* (Eastern Fence Lizard). Focusing on males, we used the “natural experiments” of seasonal, sexual, and developmental variation in growth and in exercise endurance to identify testosterone and corticosterone as potential modulators of performance and related traits of interest. We followed with experimental manipulations of testosterone to investigate functional relationships, both in the laboratory and in the field. Further, we used focal observations and demographic studies, coupled with genetic determination of paternity, to test associations between performance and fitness, measured as reproductive success. We found that in males, endurance and plasma concentrations of testosterone and corticosterone are at their peaks in the spring breeding season, when lizards are most actively engaged in patrolling home ranges and in reproductive behavior. At that time, plasma concentrations of testosterone are correlated with body size; plasma concentrations of corticosterone and parameters of home range, including area and the number of overlapped females, are correlated with home-range overlap between males and females. During prereproductive development, males (but not females) experience a maturational increase in plasma testosterone. At about the same time, they become more active, expand their home ranges, and grow less quickly than do females, suggesting a trade-off in the allocation of energy, mediated by testosterone. Experimentally, testosterone has positive effects on fitness by stimulating endurance and reproductive activity and increasing home-range area, but it exacts costs in fitness by increasing ectoparasitism, decreasing growth, and decreasing survivorship. We found evidence of selection on body size, endurance, and home-range size (and thus access to potential mates). Despite having positive effects on performance traits, plasma concentrations of testosterone were not correlated with number of offspring sired by males. However, we found a strong correlation between the level of plasma corticosterone and the number of offspring sired. We also found evidence of size-assortative mating, indicating that for males, both the number and the size (and thus, fecundity) of their mates increase with body size. Our studies exemplify the power of natural history combined with experimental endocrine manipulations to identify hormonal regulators of performance and linkages to fitness. Furthermore, our results illustrate ecological and evolutionary significance of individual variation in endocrine traits.

Introduction

The fundamental goal of integrative biology is to understand the adaptive significance of phenotypic traits (e.g., morphology, physiology, behavior) through their functional linkages to components of fitness (e.g., survival, reproduction). Nearly 30 years ago, building on Walter Bock's phased analyses of structure, function, and fitness (Bock 1980), Steve Arnold melded this conceptual goal into a specific statistical framework to illustrate how lower-level functions can be linked to fitness through selection on performance, which he defined as “some ecologically relevant activity” (Arnold 1983).

This “morphology–performance–fitness” paradigm has been profoundly influential (see Irschick et al. 2008), as evidenced by 477 citations of Arnold's original paper (Google Scholar, May 21, 2009) as well as the impetus behind at least three symposia sponsored by the Society for Integrative and Comparative Biology (formerly American Society of Zoologists) alone (San Antonio, 1990: “Ecological Morphology: Integrative Approaches in Organismal Biology”; Toronto, 2003: “Selection and Evolution of Performance in Nature”; Boston, 2009: “Hormonal Regulation of Whole-Animal Performance: Implications for Selection”).

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Interpretation of the term “performance” has been revised in the ensuing years to include ecologically relevant processes beyond those involving activity (Bradley 1994; Kingsolver and Huey 2003; Husak et al. 2009). As such, one can think of performance as the organismal interface between lower-level morphological and physiological functions and the ecological context in which these traits influence fitness. Regardless of variants in interpretation, however, performance comprises complex functional traits of organisms supported by subordinate processes integrated into suites of correlated traits (Garland and Kelly 2006). “Performance” is thus replete with allusions to endocrine mechanisms, but hormones—biological agents of coordination—have only recently been explicitly incorporated into the paradigm (Sinervo and Calsbeek 2003; Breuner et al. 2008). Inclusion of hormones is long overdue; as the processes mediated by these substances are the biological analogues of statistical relationships espoused by Arnold (see McGlothlin and Ketterson 2008).

To clarify the adaptive significance of performance, the “ultimate goal is to understand how suites of traits and trade-offs between competing functions respond to natural selection” (Ghalambor et al. 2003). Stearns (1989) observed that “endocrine regulation is an obvious aspect of intermediate structure with impact on phenotypic correlations”, where “intermediate structure” denotes “mechanisms under endocrinological control” that “modulate the expression of genetic trade-offs”. Thus, any instance of phenotypic covariance in Arnold’s statistical paradigm could hypothetically represent an endocrine mechanism of correlation or trade-off (i.e., hormonal pleiotropy; Ketterson and Nolan 1992; Finch and Rose 1995; Flatt and Kawecki 2004; Zera et al. 2007; McGlothlin and Ketterson 2008). Furthermore, because the “endocrine system interprets environmental variation to produce a range of phenotypes from the same genotype” (Dufty et al. 2002), hormones can be invoked to address the purported “technical difficulty” that “environmental variation will hopelessly complicate the measurement of selection” (Arnold 1983). After all, “animals do not achieve fitness in a vacuum but in a physical and social environment to which their hormones must respond appropriately” (Adkins-Regan 2005). In this context, an explication of hormonal mechanisms will provide biological insight into complex, condition-dependent functions that link underlying traits through performance to fitness.

In the present contribution, we synthesize case studies of *Sceloporus undulatus* (Eastern Fence Lizard) to illustrate how endocrine experiments, informed by demography and natural history, provide insight into functional relationships involving hormones, performance, and fitness. Specifically, we capitalize on two “natural experiments” to investigate linkages from hormones to fitness: (1) seasonal and sexual variation in exercise performance, testosterone, and corticosterone and (2) sexual divergence in plasma testosterone and development of sexual dimorphisms in body size and behavior. We complement our descriptive studies of natural history with experiments involving manipulation of testosterone designed to mimic natural variation and to test specific hypotheses of underlying mechanisms of variation in performance. We then discuss fitness in terms of reproductive success, which we estimated via DNA fingerprinting, and finally we close with statistical evidence of selection at two levels: (1) at the level of performance, measured as exercise endurance and home-range behavior and (2) at the level of underlying physiology in terms of plasma concentrations of hormones. We focus on testosterone because of its role in the regulation of reproduction and aggression, and on corticosterone because adrenal glucocorticoids are purportedly an essential component of appropriate organismal responses to environmental challenges (Romero 2002). We caution the reader, however, not to infer that the absence of evidence on other endocrine systems is evidence of their absence from biological regulation of performance (see John-Alder 1984 for a specific example and Sinervo and Calsbeek 2003 for a sense of complexity).

Natural history: Home-Range behavior

“Natural history is the principle source of information about organisms living under natural conditions”; it provides a comprehensive and all-inclusive view of a species’ biology and “allows an investigator to phrase questions with precision” (Bartholomew 1986). Thus, natural history is the requisite starting point to identify performance traits of ecological relevance and to yield insight into their functional relationships with hormones.

We have been particularly interested in hormones and performance traits that enhance reproductive success in a population of *S. undulatus* in the pinelands of New Jersey. We have focused on exercise endurance and growth, but, as revealed through natural history, these cannot be isolated from other performance traits that may facilitate males’ efforts

to gain access to females, including behavior and coloration. Lizards in this population hatch in late summer and reach reproductive maturity at ~ 20 months of age in the spring of their second full activity season (Haenel and John-Alder 2002). Males and females are initially indistinguishable in body size and live on equally small home ranges. By the age of reproductive maturity, females are $\sim 10\%$ larger (length) than males because of faster growth during the first full activity season (Haenel and John-Alder 2002), and home-range area is about $10\times$ greater in males than in females (Haenel et al. 2003a; Fig. 1). The population density of *S. undulatus* is relatively low in the New Jersey

pinelands, and as a consequence, home ranges of males are much larger than in other populations (Haenel et al. 2003a). Sexual divergence in body size and home range area begins at ~ 9 months of age, in concert with sexual divergence in coloration and behavior and in association with the onset of maturational divergence in plasma testosterone (Skelly and John-Alder 2002; Cox et al. 2005b). These observations raise the possibility that testosterone may modulate growth, coloration, and behavior.

Following the attainment of reproductive maturity, home-range area continues to increase with age and body size in males (but not in females), although area *per se* is not the resource of biological significance (Haenel et al. 2003a). Males acquire home ranges in search of females: the size and shape of home ranges of males depend on the abundance and locations of females (Fig. 1), as has been noted in other populations (Ferner 1974; Jones and Droge 1980) and in other species of *Sceloporus* (Davis and Ford 1983; Smith 1985). Thus, the size-dependence of home ranges indicates that larger/older males move greater distances while searching for females.

Males of *S. undulatus* tolerate extensive overlap of home range with other males (Haenel et al. 2003a; Fig. 1). They do not defend home range *per se*, but they exhibit territorial behavior in agonistic contests over females (see Martins 1994). In these encounters, aggressiveness and body size are clearly advantageous: winners of the contests are larger and more aggressive than the losers (Haenel et al. 2003a) and are differentiated by the winner's dorsal coloration becoming "lighter" than that of the loser (Smith and John-Alder 1999; Haenel et al. 2003a). In other species of lizards, physiological control of color (see Cooper and Greenberg 1992) serves as an indicator of social status (Zucker 1994). The same might be occurring in *S. undulatus*.

Home range and territorial behavior can be energetically expensive, especially when males are actively seeking mates. In *Sceloporus jarrovi*, for example, Ruby (1978) surmised that males incur "significant" energetic costs while patrolling their entire territories every day, and he speculated that "reproductive success ... is related to those phenotypic and behavioral characteristics necessary to obtain and defend a territory" (Ruby 1981). Ruby's conjecture has subsequently been confirmed experimentally by Marler and Moore (1989), who demonstrated testosterone-induced energetic costs of aggression in *S. jarrovi*. In *Sceloporus virgatus*, the sister species of *S. undulatus* (Wiens 1993),

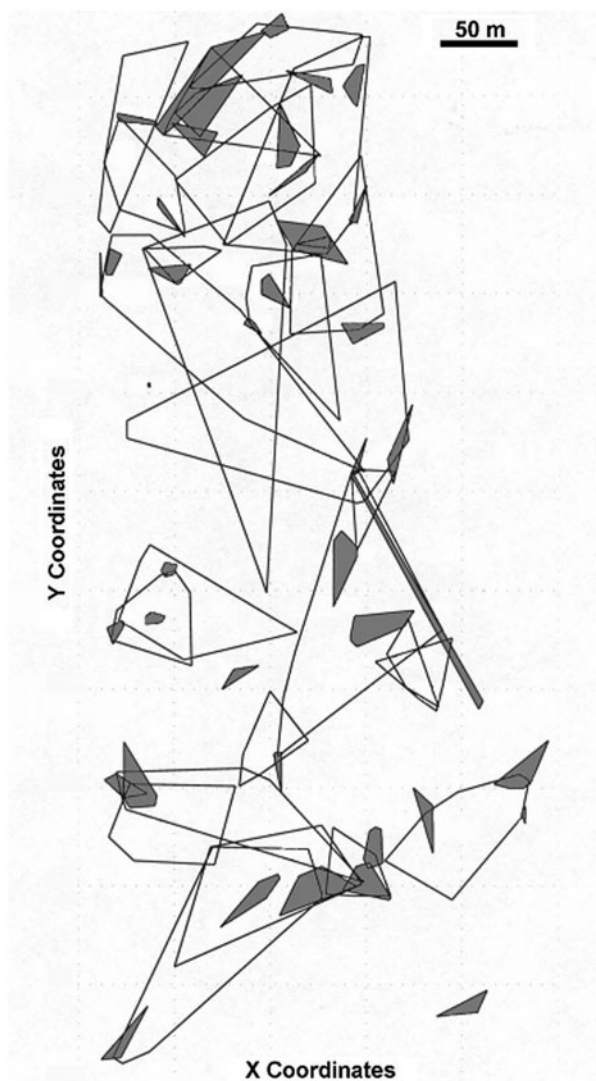


Fig. 1 Minimum convex polygon estimates of adult male (open) and female (shaded) home ranges. Home ranges are much larger for males than for females and overlap extensively with those of other males and of females. Reprinted with permission from Haenel et al. (2003a).

total daily energy expenditure increases substantially in males engaged in home-range patrol and territoriality associated with reproduction (Merker and Nagy 1984). It follows that reproductive activity in male lizards might be facilitated by an animal's ability to sustain activity, and endurance might be especially important in low-density populations where males cover great distances. This conjecture is supported by reports that in other species success in competition between males is correlated with exercise endurance (Robson and Miles 2000; Perry et al. 2004).

Natural history: Exercise endurance

To further explore this hypothesis, we used the method of John-Alder and Joos (1991) to measure exercise endurance of reproductively mature males and females at monthly intervals throughout two consecutive activity seasons. We subsequently measured endurance in males at the beginning and end of the breeding season for two more years for a total of four consecutive years. Exercise endurance, measured as running time to exhaustion on a treadmill, is a significantly repeatable measure of individual performance in *S. undulatus* between replicate trials (John-Alder et al. 1996) and over extended periods of time in the field, consistent with previous reports (Van Berkum and Tsuji 1987; Garland 1988; Tsuji et al. 1989; Brodie and Russell 1999). We found that relative endurance is also significantly correlated between the beginning and end of the breeding season (~ 35 days; $R^2 = 0.646$, $F_{1,16} = 29.211$, $P < 0.0001$), even though performance of individuals declined significantly over this interval (see below). In other words, while endurance is a labile performance trait (John-Alder et al. 1996), individuals retain their rank relative to other males in the population. Furthermore, this ranking of endurance, or relative performance, measured early in the breeding season remains correlated over a period of years (1996–97: $R^2 = 0.402$, $F_{1,9} = 6.056$, $P = 0.0361$; 1997–98: $R^2 = 0.784$, $F_{1,4} = 14.538$, $P = 0.0189$). Thus, regardless of the causes of variation among individuals, exercise endurance is a biologically informative measure of performance. Variation in endurance reflects coordinated variation in underlying physiological support and in sustained biological differences between individuals.

Exercise endurance in males is higher during the breeding season than at any other time of the year and is higher in males than in females (Fig. 2A). Endurance is highest in males at the time of year when they are most actively engaged in reproductive

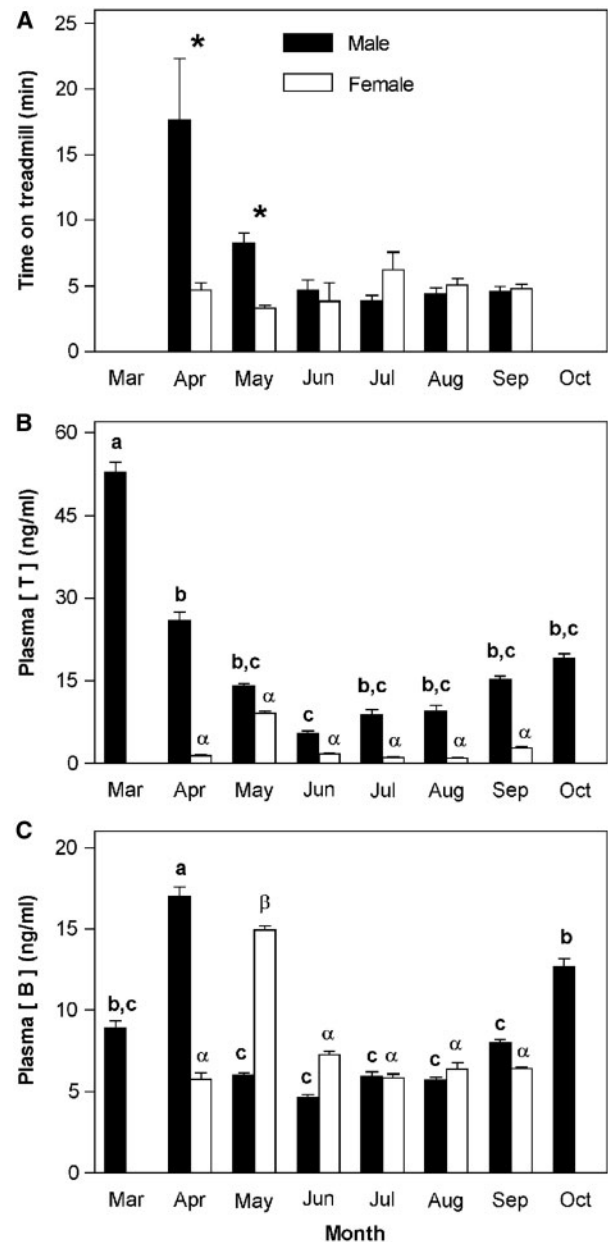


Fig. 2 Seasonal and sexual differences in (A) exercise endurance, (B) plasma testosterone levels, and (C) plasma corticosterone levels. Asterisks (A) indicate significant ($P < 0.01$) differences between males and females within a given month. Lowercase Arabic letters (B and C) denote statistical separation across months within males, whereas lowercase Greek letters (B and C) denote statistical separation across months within females. No females were located for sampling in March, suggesting that males emerge from hibernation earlier than do females.

behavior (home-range patrol, agonistic interactions, and courtship), and the difference in endurance between males and females is associated with the 10-fold sexual difference in home-range area. Following this general description of seasonal variation, we confirmed in the subsequent 2 years that

endurance in males is significantly higher early in the breeding season than later [i.e., late April: 12.4 ± 1.4 min ($n = 60$) versus early June: 6.4 ± 0.7 min ($n = 29$); $F = 7.96$, $P < 0.01$; note that for $\alpha = 0.01$, $F_{1,27} = 7.677$]. We measured hematocrit during one of those years and found it to be significantly higher in males than in females during the breeding season (♂ : 34 ± 0.5 versus ♀ : 29 ± 0.6 ; $F_{1,65} = 35.28$, $P < 0.0001$), suggesting a concomitantly greater capacity for oxygen delivery during activity.

Seasonal variation in endurance has been reported previously in two other species of lizards [*Dipsosaurus dorsalis*, John-Alder 1984; *Ctenophorus (Amphibolurus) nuchalis*, Garland and Else 1987]. In each species, variation in performance capacity was correlated with variation in underlying physiology, including aerobic capacity both at the organismal and tissue levels. These findings suggest that the intensity of home-range behavior and territoriality may generally be supported by high performance in endurance-type activity. As such, this would represent a more general phenomenon of seasonal variation in performance capacity associated with reproductive cycles (see Wells 2007) and would suggest that exercise endurance may be a target of sexual selection (see Garland et al. 1990; Robson and Miles 2000; Perry et al. 2004; Husak et al. 2006). In contrast, exercise endurance in females, which do not patrol large home ranges and do not actively search for mates, is uniformly low throughout the year (Fig. 2A).

Natural history: Hormones

In other populations of *S. undulatus*, males emerge from hibernation with large, spermatogenic testes, and high concentrations of plasma testosterone. Subsequently, testosterone decreases markedly in association with testicular regression in mid-summer, and increases in association with testicular recrudescence at the end of the activity season (McKinney and Marion 1985). A similar pattern occurs in males in the pinelands population under study (Fig. 2B). Plasma testosterone is highest immediately upon emergence from hibernation, $\sim 10\times$ higher than its summer nadir. It declines over the next several months to its lowest point just at the end of the breeding season, and gradually increases up to an intermediate level by the end of the activity season. In males, seasonal changes in plasma testosterone closely parallel changes in endurance, especially through the breeding season. In females, plasma concentrations of testosterone

are consistently lower than in males and do not undergo significant seasonal variation.

Dorsal lightening in winners of contests between males (above) occurs only during the breeding season (Smith and John-Alder 1999), when concentrations of plasma testosterone are high. Similarly, the tendency of dominant males to increase their intensity of territorial behavior in response to social challenge occurs only in the breeding season and not later in the summer (Klukowski and Nelson, 1998). Experiments *in vitro* indicate that dorsal lightening in *S. undulatus* is caused by sympathetic activation of α -adrenergic receptors (Tsao, Gleba, Smith, and John-Alder, unpublished data), and Cox et al. (2005a) demonstrated that male-specific dorsal color patterning is dependent on testosterone. In *Anolis carolinensis*, plasma catecholamines increase rapidly following aggressive interactions between males (Summers and Greenberg 1994). Plasma concentrations of norepinephrine (α -agonist) are higher in winners than in losers, and this sympathetic response is blunted by removal of the testes. Thus, dorsal lightening (and increased territorial behavior) in winners of the contests between males of *S. undulatus* may reflect α -adrenergic-mediated sympathetic arousal, and sympathetic responsiveness may depend in part on plasma concentrations of testosterone.

In most reptiles, plasma concentrations of corticosterone exhibit seasonal variation that peaks during the breeding season (Romero 2002). A similar pattern occurs in *S. undulatus* (Fig. 2C). In males, plasma concentrations of corticosterone reach their highest level in late April, by which time plasma testosterone is considerably lower than its earlier peak immediately after emergence from hibernation. Like testosterone, corticosterone declines to low concentrations in the plasma by the end of the breeding season and remains low until an increase to an intermediate level late in the activity season. Seasonal changes in plasma corticosterone are associated with variation in steroidogenic properties of adrenocortical cells (Carsia and John-Alder 2003). Indeed, the rapid decrease in plasma testosterone after emergence from hibernation may release adrenocortical cells from inhibition and allow a delayed peak production of corticosterone (Carsia et al. 2008). Plasma concentrations of corticosterone reach their peak at the time of year that presumably is the most energetically demanding, when males are most actively engaged in home-range patrol and in the social interactions of courtship and aggression.

In females, plasma corticosterone undergoes a pattern of seasonal change similar to that of males, except that it is offset by 1 month (Fig. 2C). Peak concentrations of corticosterone occur in May, the month of maximal vitellogenic activity, consistent with the seasonal pattern reported for females of *S. undulatus* in another population (Phillips and Klukowski 2008). In this regard, the correspondence between peak corticosterone and vitellogenic activity is similar to that reported for *Uta stansburiana* (Wilson and Wingfield 1992) and for various other groups of animals, suggesting that high corticosterone may reflect the energetic cost of reproduction.

Our data indirectly support the conjecture that home-range behavior may be energetically costly in *S. undulatus*. In males, plasma corticosterone is correlated with the extent of overlap of home ranges between males and females (Haenel et al. 2003a; Fig. 3A), a correlation that is not apparent for testosterone (Fig. 3B). In other words, the greater the percentage of his home range overlapped by home ranges of females, the higher is a male's plasma concentration of corticosterone. This measure of overlap of home ranges can be interpreted as proxy for the behavioral intensity of activity associated with home-range patrol, agonistic encounters, and courtship. Thus, the correlation between corticosterone and overlap in home range may reflect the energetic demands of those activities, which could be tested via experimental manipulation of the number of conspecifics overlapping a male's home range. This interpretation is consistent with the "energy mobilization hypothesis" (Romero 2002), which posits that variation in plasma corticosterone is functionally tied to the mobilization of substrate that supports activity. If true, then in both males and females of *S. undulatus*, peaks in plasma corticosterone, offset as they are by 1 month, coincide with their respective peak energy demands associated with reproduction.

Despite the parsimonious appeal of the energy mobilization hypothesis, we cannot exclude the "behavior hypothesis" as an interpretation of seasonal variation in plasma corticosterone (Romero 2002). This hypothesis posits that the most important role for corticosterone is the support of behavior, but this interpretation is not consistent with experimental evidence. Although staged social interactions cause a transient increase in plasma corticosterone in males of *S. undulatus* (Smith and John-Alder 1999), physiological effects of this response have not been identified. However, administration of exogenous corticosterone reduces

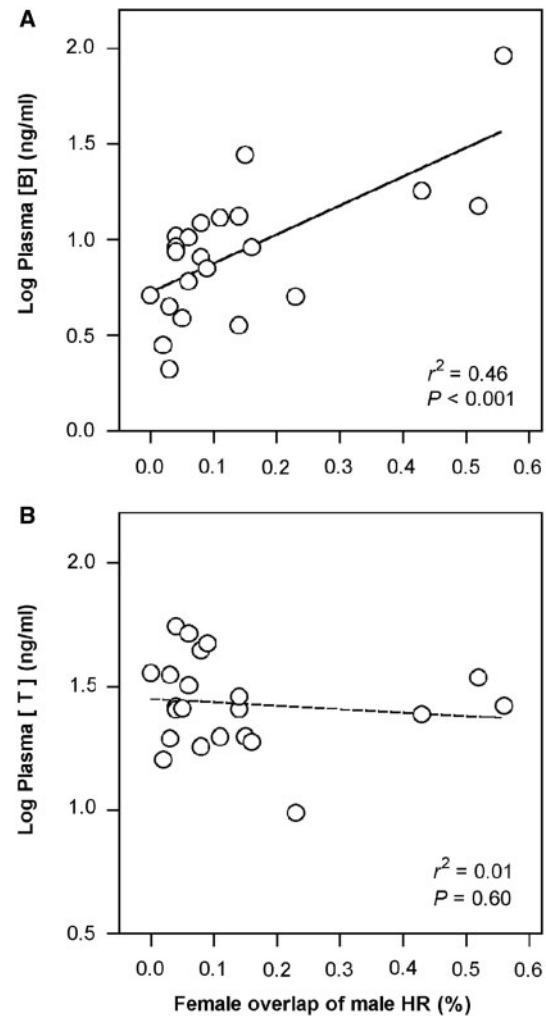


Fig. 3 Plasma concentrations of (A) corticosterone (B; \log_{10} -transformed) and (B) testosterone (T; \log_{10} -transformed) versus percent of males' home ranges (HR) overlapped by females' home ranges. Plasma was collected from free-living males in late April, during the period of peak reproductive activity. See Haenel et al. (2003a) for further procedural details. Plasma corticosterone was not correlated with body mass ($r^2 < 0.01$), whereas plasma testosterone was proportional to (body mass) $^{1.3 \pm 0.4}$ ($r^2 = 0.41$, $P = 0.001$; see John-Alder et al. 1996).

aggressive behavior and home-range activity in other lizards (Tokarz 1987; DeNardo and Licht 1993; DeNardo and Sinervo 1994). In light of these reports, seasonal elevations in plasma corticosterone would be predicted to be associated with behavioral depression, contrary to the association evident in males of *S. undulatus*. Clearly, further experimental studies are required to close the gap in understanding between social inducement of corticosterone and physiological/behavioral effects of exogenous corticosterone.

Hormonal manipulations: Endurance and home-range behavior

The natural history of seasonal and sexual variation in hormones and performance suggests that endurance and home-range behavior may be stimulated or supported by testosterone and/or corticosterone in reproductively mature males. To date, however, the functional role of corticosterone is unclear. In the only preliminary experiment we have conducted, we found no effect of exogenous corticosterone on endurance (John-Alder et al. 1997), effectively splitting the difference between a recent report that exogenous corticosterone improved endurance in *U. stansburiana* (Miles et al. 2007) but decreased sprint speed in *Lacerta vivipara* (Meylan and Clobert 2004). Further experimental analyses of this issue are desperately needed.

Our experiments have instead focused on testosterone. We have investigated effects of testosterone on exercise endurance and supporting physiology in reproductively mature lizards (John-Alder et al. 1996, 1997) as well as the effects of testosterone on movement and home-range behavior in developing yearlings.

In one set of experiments on adults, we influenced endogenous androgenic activity by manipulating the social environment of captive male *S. undulatus* (John-Alder et al. 1996). Males were held in captivity for 8–11 weeks, either in complete isolation or with groups of other males. One group of isolated males was surgically castrated to mimic the androgen-suppressing effect of social crowding, and both of the intact groups received sham surgeries. These treatments caused endurance to be 2- to 3-fold higher in intact isolates than in males from crowded groups or in castrated isolates, although the experimental design failed to identify the group(s) within which endurance changed. Thus, both social crowding and castration suppressed exercise endurance relative to intact isolates and caused apparent reductions in androgenic activity. These socially-induced differences in endurance were also associated with differences in mass of the heart, suggesting a difference in aerobic capacity and a partial physiological basis for the difference in performance.

An appealing feature of those experiments is that differences in exercise endurance were induced simply by holding captive male lizards under different social conditions, so the regulatory factors must have been endogenous. However, social conditions were not representative of field-active conditions, and regardless of the effect of surgical castration

and the strength of correlated responses that implicated testosterone, the experimental design precluded a definitive identification of the causal agent.

In a second experiment on adults, we measured exercise endurance after surgically castrating male lizards with or without testosterone replacement. Replacement of testosterone via implanted Silastic tubules caused a significant increase in exercise endurance in comparison to both castrates and controls (Fig. 4A), similar to the effect of testosterone on endurance in certain morphs of *U. stansburiana* (Sinervo et al. 2000). This increase in endurance was similar in magnitude to the difference between males measured early and late in the breeding season (Fig. 2A). In a related experiment, Klukowski and Nelson (1998) reported that exogenous testosterone caused an increase in sprint speed of free-living *S. undulatus*. Taken together, these experiments strengthen support for the hypothesis that seasonal and sexual variation in exercise performance (endurance and sprint speed) is regulated by testosterone.

We did not investigate the underlying physiological mechanisms in *S. undulatus*. However, in a separate experiment on *Anolis sagrei*, testosterone replacement in surgically castrated males caused significant increases in running endurance, cardiac muscle mass, and oxidative capacity of hind leg muscle, suggesting that endurance is constrained by aerobic capacity at several levels of organization (John-Alder 1994; John-Alder et al. 1997).

In yearling males of *S. undulatus*, male-specific coloration begins to appear at about the same time that plasma concentrations of testosterone become higher in males than in females (Cox et al. 2005a). During the same developmental period, males expand their home ranges and begin to exhibit movement patterns characteristic of reproductive activity (Skelly and John-Alder 2002; Cox et al. 2005b). We investigated effects of testosterone on movement and home-range behavior in yearlings that were surgically castrated with and without testosterone replacement. Experiments were conducted under field conditions in an enclosed area of natural habitat adjacent to the Rutgers Pinelands Research Station and were designed in accordance with the natural history of *S. undulatus* to insure physiological relevance (Cox et al. 2005b).

Hourly movement was estimated from successive focal observations of experimental lizards, and area of the home range was estimated by minimum convex polygons for each lizard (Cox et al. 2005b). Distance moved and area of the home range were

Testosterone stimulates performance measures that may increase reproductive success:

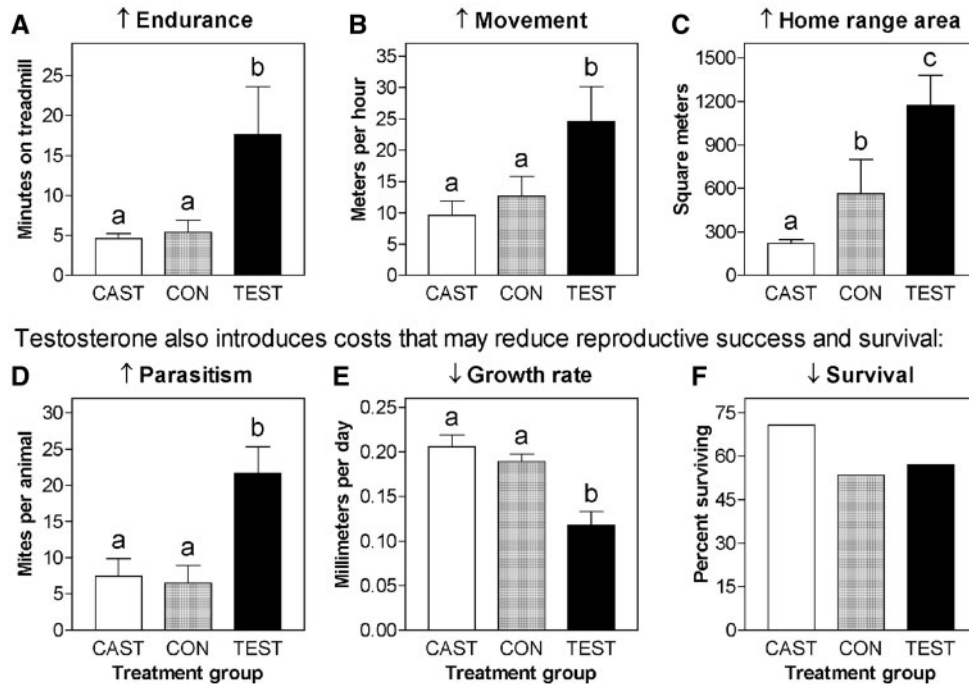


Fig. 4 Effects of experimental castration and exogenous testosterone on (A) endurance, (B) movement, (C) home-range area, (D) parasite load, (E) growth rate, and (F) survival in *S. undulatus* males. Adults were used for endurance, and yearlings for everything else. CAST = castration surgery and empty implant; CON = sham surgery and empty implant; TEST = castration surgery and exogenous testosterone implant. Data are means (+1SE) from studies described by Cox et al. (2005b). All treatment effects are statistically significant except survival data in panel F. See text for further details.

significantly increased by testosterone (Fig. 4B and C), and area of the home range was decreased by surgical castration. Thus, assuming that yearlings respond in a similar fashion as do adults (Fig. 4A), enhanced endurance is associated with greater movement and increased area of the home range, thereby providing the ecological relevance of the physiological response. Our results are consistent with experimental reports on other lizards, in which exogenous testosterone stimulated activity, territorial aggression, and home-range size (Moore and Marler, 1987; Marler and Moore, 1988; Moore, 1988; DeNardo and Sinervo, 1994; Klukowski et al. 1998). These results lend powerful support to hypotheses of functional linkages between hormones and performance based on descriptive natural history (above). Collectively, our descriptive and experimental data suggest that testosterone may regulate both physiological and behavioral performance traits that facilitates males' efforts to locate and gain access to females.

Hormone manipulations: Fitness costs of testosterone

During the same developmental period in which yearlings increase their daily movement and the

area of their home ranges, females grow about twice as fast as males. These observations suggest that energetic requirements of activity may be traded off against growth, and that testosterone may mediate this trade-off (Cox et al. 2005b). The experiments described above demonstrate that testosterone stimulates performance traits that may enhance the reproductive success of males. At the same time, testosterone has several potential fitness costs.

We found that testosterone caused an increase in ectoparasitism (Fig. 4D), a reduction in growth rate (Fig. 4E), and a nonsignificant tendency toward reduced survivorship (Fig. 4F), all of which would be expected to diminish reproductive success. Increased susceptibility to ectoparasitism is clearly a fitness cost of testosterone. Similar effects of testosterone on ectoparasitism have been reported in other studies of *S. undulatus* (Klukowski and Nelson 2001), and natural sex differences in ectoparasitism in closely related *S. virgatus* are driven in part by natural sexual differences in levels of plasma testosterone (Cox and John-Alder 2005). Deleterious effects of parasitism in lizards include reductions in endurance and in the growth rate (Schall et al. 1982; Cox and John-Alder 2007) and

are associated with reduced survival and reproductive success (Schall and Dearing 1987; Sorci and Clobert 1995).

The adaptive significance of the testosterone-induced decrease in growth rate is more difficult to interpret. On the one hand, yearling males with lower growth rates have greater survival over winter (Haenel and John-Alder 2002), suggesting that natural selection for moderate to slow growth would be favored, at least in this age class, and that testosterone may inhibit growth even while enhancing other performance traits used by males to gain access to females. On the other hand, large body size of adult males confers two clear reproductive advantages: (1) dominance in head-to-head competition for access to females (Haenel et al. 2003a) and (2) increased reproductive output due to assortative mating with large, fecund females (below). As such, sexual selection should favor large body size and, because age at first reproduction is inflexible (Haenel and John-Alder 2002), rapid growth in males. Thus, it is difficult to determine whether inhibition of growth by testosterone is likely to impose a fitness cost (i.e., reduced mating success) or provide a benefit (i.e., increased overwinter survival).

Given the mating advantage of large size and the positive effects of testosterone on behavioral, physiological, and morphological traits that facilitate reproductive success (Cox et al. 2005a, 2005b), it seems unlikely that inhibition of growth by testosterone confers a selective advantage. Instead, inhibition of growth may reflect an energetic trade-off resulting from increased activity and ectoparasitism. In our experiments, exogenous testosterone increased the length of the activity period, daily movement, home-range area, and ectoparasitism at the same time that it decreased growth. We estimated that the energetic cost of testosterone-induced activity was equivalent to ~80% of the energy savings of reduced growth (Cox et al. 2005b). Increased ectoparasitism may also have imposed a significant growth cost (Cox and John-Alder 2007), potentially mediated by an additional trade-off in the allocation of energy. Thus, growth costs of testosterone can be explained through allocation trade-offs without invoking lower-level mechanisms. At the level of molecular regulation, testosterone may actually have had a positive effect on the growth-regulating endocrine axis (i.e., GH/IGF-1 axis), consistent with known molecular effects of testosterone in other species (Borski et al. 1996; Riley et al. 2002). In the face of limiting energy, however, organismal growth would not have been stimulated

proportionately. In light of the mating advantage of body size, this would seem to be the most parsimonious explanation of our results.

Selection on performance traits

During 1 year of our study, we used DNA fingerprinting to determine paternity and thus to characterize reproductive success of males (36 males, 26 clutches, and 148 hatchlings) (Haenel et al. 2003b). All but one clutch could be definitively resolved to a single father. The number of clutches sired by a male was highly skewed, varying from 0 to 6, and the number of eggs per clutch ranged from 6 to 12. Most commonly, reproductively successful males sired only one clutch. This variation in number of clutches sired and number of offspring per clutch yielded a skewed distribution of reproductive success in which the majority of males did not sire any offspring, whereas one exceptional male sired an estimated 64 offspring. This male was known to be ≥ 4 -years old and was larger (SVL = 66 mm) than all but one other male in the population (range: 56–68 mm).

Our estimates of highly skewed current reproductive success indicate that the majority of offspring are sired by a small number of males and suggest that some males may never successfully reproduce. Alternatively, current reproductive success may be misleading, and an analysis of lifetime reproductive success may reveal that unsuccessful males in any particular year have opted to minimize current costs and delay reproduction with the prospect of increasing their likelihood of surviving to reproduce the following year. We do not have data on reproductive success to evaluate this alternative hypothesis, but survivorship of males in this population is estimated to be only 0.20 from age 24 to 36 months and only 0.09 from 36 to 48 months (Haenel and John-Alder 2002). Thus, in light of the strong probability that reproductively mature males will not survive into the next year, one would predict strong competition for females and great potential for sexual selection among males within any single year.

We used number of offspring sired to estimate linear selection differentials for selection on size (measured as snout-vent length), endurance, home-range area, and circulating levels of testosterone and corticosterone. We estimated directional selection as the regression coefficient ($\beta \pm 1\text{SE}$) for relative fitness (an individual's reproductive success divided by the population mean) as a function of trait values standardized to the population mean in units of standard deviation (Lande and Arnold 1983;

Arnold and Wade 1984). The selection differentials obtained by this method are valid descriptions of sexual selection regardless of the skewed distribution of reproductive success, but tests of statistical significance depend upon the distributional assumptions of parametric analysis. Therefore, as a supplemental method, we dichotomized reproductive success into males that sired at least one clutch ($n=17$) and males that did not sire any clutches ($n=19$). We then compared traits' distributions between fitness categories using Wilcoxon rank-sum tests. These nonparametric tests were used instead of logistic regression (Janzen and Stern 1994) because the distributions of males' traits, particularly endurance, were also skewed.

Selection gradient analyses revealed positive directional selection on snout-vent length ($\beta=0.686 \pm 0.290$; $P=0.024$; Fig. 5A) and endurance ($\beta=1.302 \pm 0.239$; $P<0.001$; Fig. 5B). However, these analyses were both highly influenced by the single male who sired 64 offspring and was also an extreme outlier with respect to endurance (4.84 SD above the mean). Exclusion of this male weakened the strength of selection on male size ($\beta=0.194 \pm 0.189$; $P=0.31$) and actually reversed the direction of selection on endurance ($\beta=-0.321 \pm 0.398$; $P=0.43$). Males who sired clutches were slightly larger than those who did not ($\chi^2=2.26$; $P=0.13$; Fig. 5A), but no such trend was evident for endurance ($\chi^2=0.05$; $P=0.82$; Fig. 5B). Thus, there is a weak trend for selection favoring larger male size, but the relationship between endurance and reproductive success is driven entirely by one individual with extreme values for each measure.

Selection gradient analysis did not reveal a significant relationship between home-range area and reproductive success ($\beta=0.863 \pm 0.562$; $P=0.14$), but males that sired at least one clutch had significantly larger home ranges than did males that failed to sire a clutch ($\chi^2=4.26$; $P=0.039$; Fig. 5C). This reflects the fact that the number of females whose home ranges overlap a males' home range increases with males' home-range area; the number of clutches sired by a male increases with the number of home ranges of females that his home range overlaps (Haenel et al. 2003b).

Through both direct and indirect mechanisms, testosterone promotes home-range acquisition and therefore enhances reproductive success. For example, testosterone promotes aggressiveness (Klukowski and Nelson 1998), dorsal lightening (Cox et al. 2005a), and expansion of home range itself (Cox et al. 2005; Fig. 4C), and through its

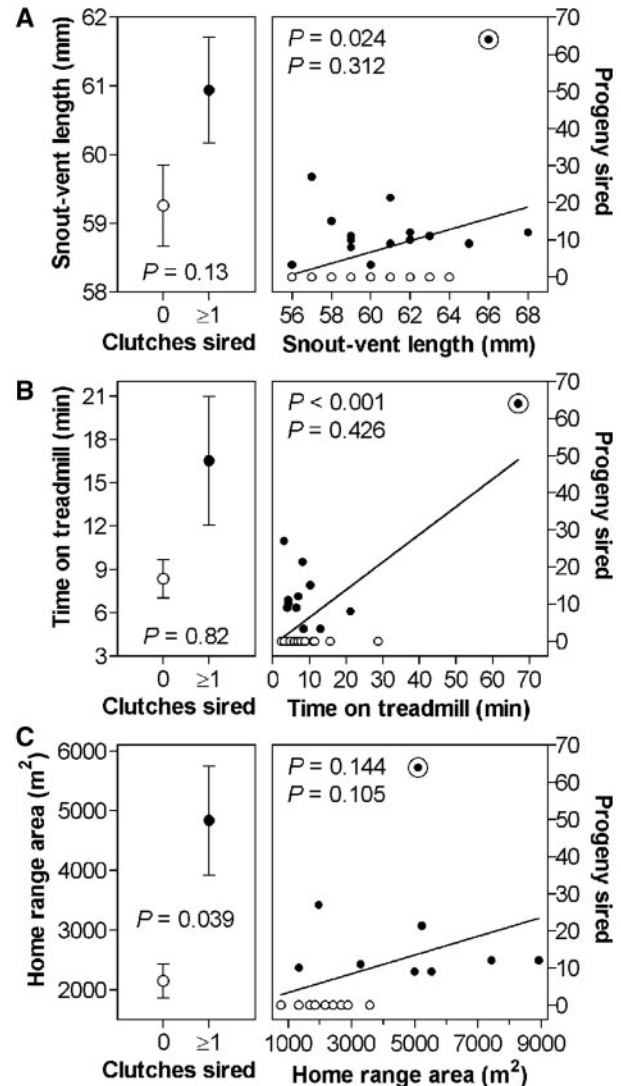


Fig. 5 Reproductive success, estimated from DNA fingerprinting data, as a function of (A) snout-vent length, (B) endurance, and (C) home-range area for free-living *S. undulatus* males. Left panels report mean ($\pm 1SE$) trait values for males that failed to sire any clutches versus males that sired at least one clutch. Significance values are reported for nonparametric Wilcoxon rank-sum tests comparing these two classes of males. Right panels report total number of progeny sired per male as a function of morphological and performance traits. The circled data point is a statistical outlier with respect to reproductive success and endurance. Significance values are reported for least squares regressions including (top) and excluding (bottom) this data point. See text for associated estimates of selection differentials.

effect on exercise endurance (Fig. 4A), testosterone supports the energetic requirements of home-range patrol and social interactions. All males have higher endurance during the breeding season than at any other time of the year, but endurance itself is not directly correlated, on an individual basis, with reproductive success (Fig. 5B). The failure of

endurance to predict reproductive success may be due to a motivational component of endurance that cannot be adequately assayed in trials using laboratory treadmills (see Losos et al. 2002; Sabhapandit et al. 2008).

Despite the functional relationship between testosterone and traits related to the acquisition of a home range (Fig. 4A–C), plasma testosterone levels in the breeding season did not predict male reproductive success (Fig. 6A). This is perhaps not surprising, given that any single measurement of circulating testosterone may fail to provide a realistic picture of an individual's typical hormone profile. However, plasma corticosterone was strongly correlated with male reproductive success ($\beta = 1.774 \pm 0.321$; $P < 0.0001$) and was significantly

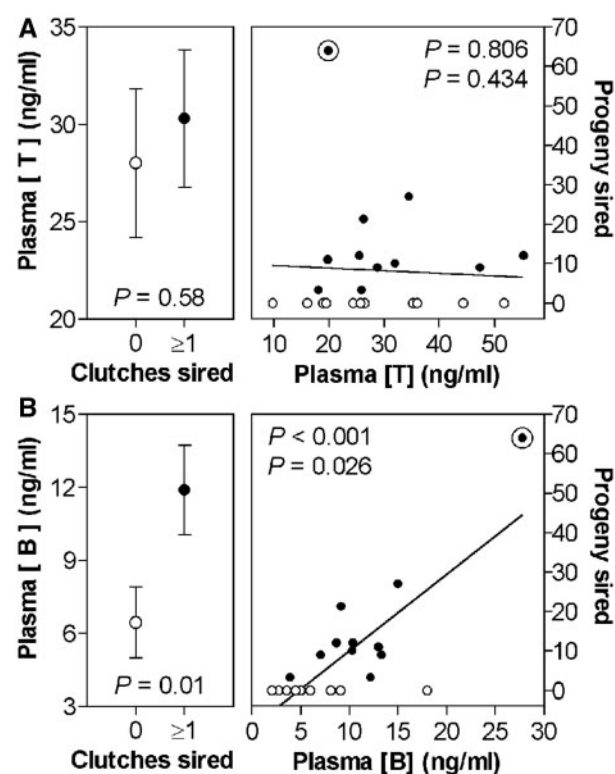


Fig. 6 Reproductive success as a function of (A) the level of plasma testosterone, and (B) the level of plasma corticosterone for free-living male *S. undulatus*. Left panels report mean (± 1 SE) values of hormones for males that failed to sire any clutches versus males that sired at least one clutch, excluding the outlier value of B. Significance values are reported for nonparametric Wilcoxon rank sum tests comparing these two classes of males. Right panels report total number of progeny sired per male as a function of circulating hormone levels measured at the beginning of the breeding season (April). The circled data point is a statistical outlier with respect to reproductive success. Significance values are reported for least squares regressions including (top) and excluding (bottom) this data point. See text for associated estimates of selection differentials.

greater in males that sired at least one clutch than in males that failed to sire a clutch ($\chi^2 = 6.43$; $P = 0.011$; including outlier: $\chi^2 = 4.01$; $P = 0.045$; Fig. 6B). This relationship is expected, given that circulating corticosterone levels and male reproductive success are both correlated with home-range area (Figs. 3A and 5C). (One outlier with plasma corticosterone of 91 ng/ml was omitted from the correlation analysis. This point had a tremendously disproportional influence on the regression, as evidenced by an *R*-student, or externally studentized residual, value of 21.6). As we suggested earlier, plasma corticosterone may reflect the demands of social interactions with females and with other males (see Natural history: Hormones above). If true, then the correlation between corticosterone and reproductive success provides evidence that reproductive success is scaled on a male's energetic investment in reproductive behaviors.

The relationship between body size and reproductive success may reflect components of both sexual selection and selection for higher fecundity. In the former case, relatively large body size is advantageous to males in agonistic encounters (Smith and John-Alder 1999; Haenel et al. 2003a) and therefore facilitates acquisition of a home range and access to mates. Thus, large males gain access to a greater number of potential mates. In the latter case, body size is correlated between mated males and females, such that larger males tend to mate with larger females (Fig. 7A). Because clutch size is correlated with body size in females (Haenel et al. 2003b; Fig. 7B), large males gain a fecundity advantage by mating with large females (Fig. 7C). Thus, large males tend to sire more clutches than do small males, and large males also tend to sire relatively more offspring per clutch by mating with large, fecund females.

Conclusion

Twenty-five years ago, Arnold (1983) introduced his statistical “morphology–performance–fitness” paradigm, and Stearns (1989) invoked hormones as biological agents of phenotypic correlation and covariance. Much more recently, evolutionary biology and endocrinology have been melded into the emergent empirical and experimental field of evolutionary endocrinology (Zera et al. 2007), and comparative endocrinologists have embraced in earnest the potential goldmine of information contained in individual variation of endocrine function (Williams 2008). In an obvious and long-overdue step forward, hormones and endocrine mechanisms

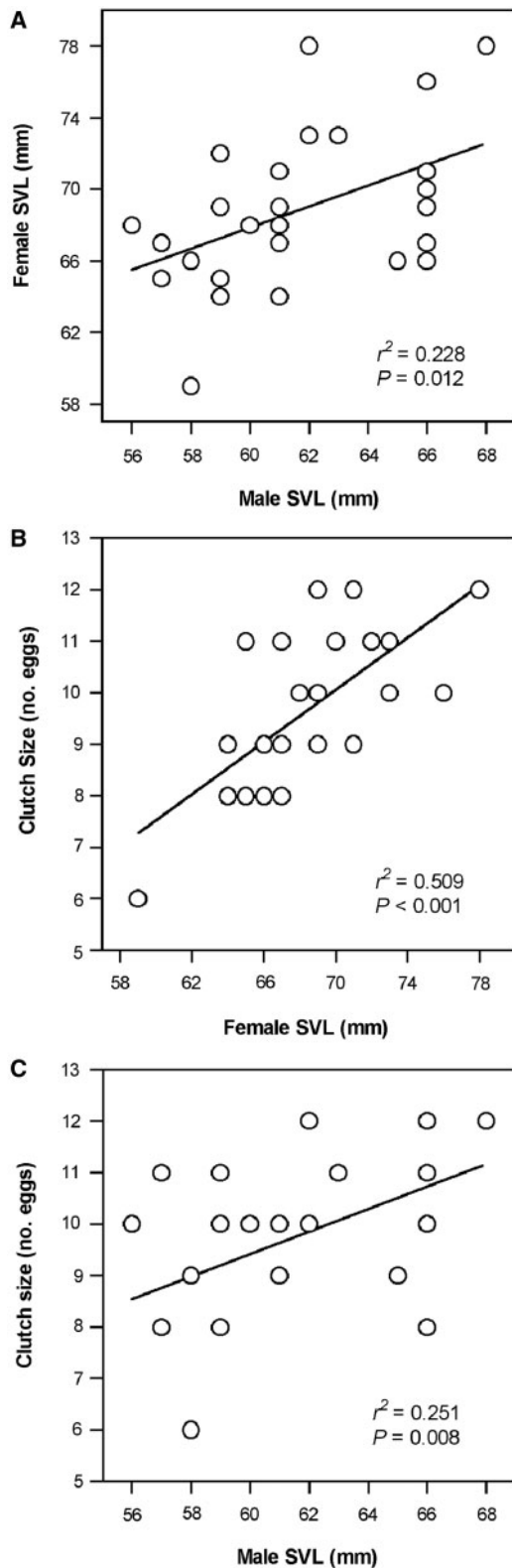


Fig. 7 Selection for fecundity favors large body size in males and females. (A) Among mating pairs, large males tend to mate with large females. (B) Fecundity increases with female size. (C) As a result, large males tend to sire relatively more offspring per clutch.

have been incorporated into Arnold's model (Breuner et al. 2008; McGlothlin and Ketterson 2008). Our present contribution reveals how Arnold's paradigm can serve as the conceptual framework for experimental studies steeped in natural history to explicate the functional roles and adaptive significance of hormones in natural populations of animals.

In his symposium publication, Arnold (1983) discussed complicating effects of ontogenetic and environmental variation in the context of the measurement of selection. From our point of view, these and other sources of variation represent "natural experiments", which can help to reveal relationships between hormones and performance traits and can serve to inform specific hypotheses for experimental investigation (see Bartholomew 1986). By combining natural history and demographic studies with experimental manipulations of hormones and estimates of fitness, we have been able to demonstrate functional associations and fitness effects of variation in concentrations of hormones, occurring among individuals, between sexes, and on both developmental and seasonal time scales.

Our report provides evidence of sexual selection on endurance and on correlated traits whose expression is coordinated by common hormones (i.e., hormonal pleiotropy) (see Husak et al. 2006). Moreover, through the use of physiologically relevant experimental manipulations carefully informed by natural history, we have been able to demonstrate how a hormone can mediate antagonistic suites of correlated traits. We are now at a time when further integration of traditional endocrinological techniques with the quantitative statistical frameworks of evolutionary biology will continue to provide insight into how hormones mediate the evolution of complex suites of performance traits.

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