

Testosterone, Endurance, and Darwinian Fitness: Natural and Sexual Selection on the Physiological Bases of Alternative Male Behaviors in Side-Blotched Lizards

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The mechanistic bases of natural and sexual selection on physiological and behavioral traits were examined in male morphs of three colors of the side-blotched lizard, *Uta stansburiana*. Orange-throated males are aggressive and defend large territories with many females. Blue-throated males defend smaller territories with fewer females; however, blue-throated males assiduously mate guard females on their territory. Yellow-throated males do not defend a territory, but patrol a large home range. They obtain secretive copulations from females on the territories of dominant males. Males with bright orange throats had higher levels of plasma testosterone (T), endurance, activity, and home range size and concomitantly gained greater control over female home ranges than blue- or yellow-throated males. Experimentally elevating plasma T in yellow- and blue-throated males increased their endurance, activity, home range size, and control over female territories to levels that were seen in unmanipulated orange-throated males that had naturally high plasma T. However, the enhanced performance of orange-throated males is not without costs. Orange-throated males had low survival compared to the other morphs. Finally, some yellow-throated males transformed to a partial blue morphology late in the season and the endurance of these transforming yellow-throated males increased from early to late in the season. In addition, yellow-throated males that

transformed to blue also had significantly higher plasma T late in the season compared to the plasma T earlier in the season. T appears to play an important role in the physiological changes that all three color morphs undergo during the process of maturation. In some yellow males, T plays an additional role in plastic changes in behavior and physiology late in the reproductive season. We discuss natural and sexual selection on physiological and behavioral traits that leads to the evolution of steroid regulation in the context of alternative male strategies. © 2000 Academic Press

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The presence of aggressive territory holding males versus “satellite” or “sneaker” males is a widespread reproductive strategy found in a variety of vertebrates, including birds (Lank *et al.*, 1995), lizards (Sinervo and Lively, 1996), and fish (Gross, 1984; Cardwell and Liley, 1991; Gross, 1991; Brantley *et al.*, 1993). Behavioral morphs have been linked to the effects of gonadal steroids such as testosterone (T) or perhaps the regulation of gonadal steroids via gonadotropin or gonadotropin releasing hormone (Bass, 1996; Kindler *et al.*, 1989; Brantley *et al.*, 1993). Studies of the role of steroids on alternative male strategies often focus on the organizing effect that gonadal steroids such as T have on juvenile phases of the life history (e.g., Hews *et al.*, 1994; Bass, 1996). For example, Hews *et al.* (1994) found that experimentally elevated plasma T in hatch-

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ling lizards caused them to develop into aggressive territorial morphs at a higher frequency.

While the organizational effects of steroids are critical for the neurodevelopment of alternative male behaviors, steroids have activational effects that are manifest at maturity when males initiate reproduction. Later acting activational effects trigger changes in behavior and physiology in adults. Dramatic changes in behavior often leave the individual exposed to the efficient effects of natural and sexual selection. Selection on activational events is under "direct selection" in that the survival or reproduction of males is temporally associated with changes during maturation. In contrast, direct selection on earlier acting organizational effects is less likely as differentiation is not yet complete. However, the direct selection on activational events can lead to indirect selection on organizational effects if activational and organizational effects are governed by the same sets of genes. Thus, direct selection on activational events and indirect selection on organizational events may primarily take place during maturation when activational events are manifest. Understanding the interplay between natural selection and steroid regulation is critical to understanding microevolutionary changes in steroid regulation that might have led to larger macroevolutionary patterns found among extant classes of vertebrates.

The activational effects of gonadal steroids include changes in levels of aggression (Moore, 1986, 1988) and changes of morphology and physiology, such as muscle development necessary for sexual behavior (Regnier and Herrera, 1993). In this paper, we investigate later acting activational effects arising from variation in levels of plasma T of maturing lizards that lead to enhanced endurance, territorial behavior, and control of female home range. Experimental analysis of performance and fitness is facilitated by a comparative analysis that focuses on three color morphs of side-blotched lizards, *Uta stansburiana*, that differ in dominance, physiological capacity, and circulating levels of T. Male side-blotched lizards develop into one of three discrete throat color morphs (orange, blue, and yellow throats). Throat color only becomes differentiated in March, when lizards begin maturing at an age of 6 to 8 months (Sinervo and Lively, 1996). Throat color has a genetic basis (Sinervo *et al.*, 2000). Orange-throated males are considered ultradominant and actively defend a very large territory. They are very aggressive and vigorously attack other territory holding males that venture onto their territory. Blue-throated males are likewise territory holders, but they are less aggressive and tend to defend smaller territo-

ries. Yellow-throated males do not defend a territory and they will often mimic female behavior when they are confronted by territory holding males. Throat color and dorsal color patterning of yellow-throated males is also superficially similar to the phenotype of females. Yellow-throated males are furtive and do not perch on conspicuous rocks like the territorial orange- and blue-throated males.

Although links between T, behavior, and aggression have been established (Crews, 1974; Fox, 1983; Moore, 1986; DeNardo and Licht, 1993; DeNardo and Sinervo, 1994), these patterns are largely derived from "manipulative experiments" performed in the laboratory or in the wild. In addition, T has also been shown to have multiple effects on performance of males, but the majority of data are likewise based on manipulative laboratory studies (Fennell and Scanes, 1992; Young *et al.*, 1993; Rand and Herrera, 1993; Regnier and Herrera, 1993; Van Breda *et al.*, 1993; Staron *et al.*, 1994). The physiological and selective significance of "natural variation" in plasma T is rarely documented in nature, and it is rarer still for manipulative experiments to be directly compared to natural variation. We complement comparative analysis of differences in hormones, physiology, and behavior among the three alternative male morphs with experimental manipulations of T in laboratory and field experiments. "Phenotypic engineering" (Ketterson and Nolan, 1992, *sensu* "allometric engineering," Sinervo and Huey, 1990) is useful for testing cause and effect relations between hormones and their manifold behavioral and physiological effects. Moreover, natural differences in plasma T and physiology observed among morphs can be used as a metric against which we can compare effects of experimentally induced variation to ensure that pharmacological effects of hormones do not affect our conclusions (Sinervo and Svensson, 1998; Sinervo, 1998).

We correlated endurance as measured on a laboratory treadmill, a stringent measure of whole-organism physiological capacity (Bennett, 1978; Sinervo and Huey, 1990; Garland and Losos, 1994), with hormones and territorial behaviors of males. High endurance and high levels of aggression may be necessary to maintain a large territory and defend a large aggregation of females. The high endurance and aggression of orange-throated males compared to blue-throated males could be parsimoniously brought about by elevated plasma T, given demonstrated effects of T on muscle physiology (e.g., Saborido *et al.*, 1991). In keeping with their more modest territory size, blue-throated males presumably have lower performance than orange-throated males, but higher performance

than yellow-throated males. Low levels of T were expected in yellow-throated sneaker males, which superficially resemble females in throat morphology and behaviors. We also expected that yellow-throated males should have lower endurance relative to both types of territory holding males because yellow-throated males do not engage in territory defense.

However, some adult yellow-throated sneaker males undergo a throat color transformation during the second clutch and may acquire a distinct blue patch on the throat. Their behavior changes from that of sneaker to aggressive territorial males. Transforming males also had increased endurance. Implanting T into yellow-throated males tested the hypothesis that T contributes to the increased endurance of yellow-throated males when they acquire blue coloration. Likewise, implanting T into blue-throated males tested the hypothesis that the difference in T between orange-throated and blue-throated males contributes to differences in endurance observed between the two territorial morphs.

MATERIALS AND METHODS

Study Sites and Overview of Field and Laboratory Experiments

During 1990 through 1994, we studied activity, territorial behavior, and survival in a population of male side-blotched lizards (*U. stansburiana*) that inhabit rocky outcroppings of sandstone in the inner Coast Range of California (near Los Baños Grandes, Merced Co.). Males mature in early March at a size of 5 to 11 g and maturation of all three morphs is initiated in the first 8 months of life. Lizards in the Los Baños Grandes population mature at a size that is 50% larger than is the typical body size for *U. stansburiana* from other parts of the species range. DNA paternity evidence confirms that males present in the spring, regardless of size or morphotype, are all capable of siring progeny on the first clutch of season (Zamudio and Sinervo, in press). Females also mature in 8 months and at a size of 4 to 10 g (prelaying mass). Females produce up to four clutches during the reproductive season (March through August), at approximately 1-month intervals. Reproduction is synchronous and most females lay eggs within a 2-week period.

Territory defense by males was correlated with a discrete color polymorphism. At the beginning of the breeding season we scored males unambiguously according to the following criteria (Sinervo and Lively,

1996). Orange-throated males had bright orange on their throat and/or side. Blue-throated males had dark blue extending from posterior to anterior regions (nearly 100% coverage, with the occasional yellow flecks). Yellow-throated males had yellow stripes across the entire throat. If any blue was present, it was very pale in coloration and restricted to pale blue stripes between the yellow stripes. In addition, some yellow-throated males transformed from yellow to blue. Early in the season we caught them with yellow stripes, but late in the season they acquired a small blue patch on the posterior dewlap (less than 50% coverage by blue), when females were yolking their second clutch. Not all yellow-throated males transformed, and some retained yellow.

We followed the territorial behavior and survival of all males located on a 250-m-long rock outcropping of sandstone during the spring (approximately $N = 100$ males each year, see Sinervo and Lively, 1996). Yearly survival rates were estimated from the number of males marked at maturity that were recovered on the outcrop in the next reproductive season (1 year later). Males do not immigrate as adults (Doughty and Sinervo, 1994) and males often defend a territory in the same location in their second year as that defended in their first reproductive season (Sinervo, unpublished observations). After obtaining estimates of home range use and activity patterns, we brought animals into the lab to estimate endurance. We obtained estimates of plasma T on a second group of males located on an adjacent rock outcropping because plasma sampling via the postorbital sinus might affect territorial behavior. In addition, we investigated the effects of experimentally elevated plasma T on endurance in the laboratory and the effects of elevated T on territorial behavior in field trials on a third group of males located on an adjacent rock outcrop.

Activity Level and Home Range in the Wild

Activity level was indexed by the number of times an individual was observed during 16 complete passes of our study site during March 20, 1993, through April 19, 1993, and from March 29, 1994, through April 9, 1994. No effort was made to turn over rocks. Males, if active, are quite conspicuous on the surface of rocks. Home range area was computed (DeNardo and Sinervo, 1994a,b) from the minimum convex polygon that circumscribed all mapped locations for a male. Differences in territorial behavior among male morphs were compared by ANOVA. In addition, changes in territorial behavior of transforming yellow/blue-

throated males were compared to those of nontransforming yellow-throated males by repeated measures ANOVA. We measured home range size and territory size of males when females were yolking their first and second clutches. Measurements of activity and territory size were not confounded by male mortality. Data for an individual were used in statistical analyses if the male survived to the end of the sampling period.

Endurance

The endurance of free-ranging males was measured during a 1-day laboratory stay. Endurance was indexed by the elapsed time (minutes) spent running on a treadmill until exhaustion, which was scored by loss of the righting response (Sinervo and Huey, 1990). Lizards were motivated to run by gently tapping them on their hind legs. The treadmill measured 30 × 40 cm (width × length). The treadmill rotated at 0.5 km/h, which is an ecologically relevant speed approximating the speed used by males during patrols of their territory. Activity body temperature (35–38°C) of males on the treadmill was maintained with an incandescent lamp mounted over the track. The endurance of all males in our focal population was measured on June 16, 1993, and from April 18 through April 20, 1994, i.e., after we obtained estimates of activity and home range use. While estimates of endurance from 1993 may be from a select group of males that managed to survive to from winter emergence in March until June, this is not true of the group of males raced in April of 1994. The survival of males from March to April was quite high (N.B., comparable ANOVA comparisons for morphs between 1993 and 1994 indicate that measurement date has no effect on endurance among morphs and measurement of endurance is not confounded with mortality).

Male Survival

From 1990 to 1994, all males on a 250-m-long outcrop were marked with a permanent toe-clip mark. The survival of males from April of their first year of life to April of the following year was estimated from an intensive mark recapture study. Because our survival censuses of males takes place during our intensive sampling of home range use, it is unlikely that males surviving from the previous year would be overlooked during April censuses. All animals in a buffer zone that extends 600 m around our focal outcrop were captured in April and emigration does not affect estimates of adult mortality (Sinervo *et al.*, 1992;

Doughty *et al.*, 1994; Doughty and Sinervo, 1994). Differences in morph survival were compared using pairwise χ^2 tests.

Steroid Assays

We obtained plasma samples from a group of males located on a outcrop that was adjacent to the group of males used in experiments described above. Males were caught within 2 min of sighting. All plasma was collected within 2 min of capture and plasma T was not correlated with time required to capture the male. Plasma samples were collected between 900 and 1300 h, which coincides with the peak in territory activity of males. Plasma was sampled twice during the breeding season, which corresponded to the first (April 18) and second (May 8–13) clutches of the reproductive season. Blood was collected from the post-orbital sinus using two to three 50- μ l hematocrit tubes and kept on ice for 4–6 h, after which we centrifuged the blood to isolate the plasma. The plasma T (ng/ml) of field active males was measured by radio-immunoassay after chromatographic separation on celite microcolumns (Wingfield and Farner, 1975; Moore, 1986). Intra-assay coefficients of variation were obtained by running four standard tubes, which contained a known amount of steroid in distilled water, through all the same steps as the sample tubes. Intra-assay coefficients of variation were 0.067 and 0.004 for two different concentrations of standards. We analyzed (ANOVA) plasma T after log transformation because variance in plasma among morphs is heteroscedastic and sample variance increases with the sample size mean. However, we present means and SE for untransformed data in the figures.

Effects of Experimental Supplementation of Testosterone on Endurance

To examine the relationship between experimentally elevated T and endurance, we implanted blue- and yellow-throated males with T implants in laboratory trials. We administered T in silastic implants that were designed to elevate plasma levels by approximately 60 ng/ml (previously determined in laboratory and field trials used by DeNardo and Licht, 1993, and DeNardo and Sinervo, 1994b). Lizards used in implant studies were not sampled for plasma because we measured physiological traits (e.g., endurance) known to be affected by drawing a sample of blood (Sinervo and Miles, unpublished data). Implants consisted of a 3-mm length of 0.22- to 0.35-mm (inside–outside di-

ameter) Silastic medical grade tubing (Dow Corning 602-305) sealed at each end with 1 mm of silicone sealant (nontoxic). The implants provided a 1-mm length of fillable hormone. Silicone was allowed to cure for at least 48 h. Prior to implantation, the implants were soaked in sterile physiological saline for 24 h to allow the saline to enter the implants. Similar implant construction has been used to create hormone levels equivalent to the high physiological range of plasma T in *U. stansburiana* (DeNardo and Licht, 1993; DeNardo and Sinervo, 1994b). Implants are known to keep hormone levels elevated within physiological limits in excess of 3 months in both captive and free-ranging lizards (DeNardo and Licht, 1993), far beyond the length of studies reported in this paper (1 month maximum).

We measured the effects of experimentally enhanced plasma T on endurance of males that were maintained in the laboratory. Animals were raced on the day of capture (April 18, 1994), and $N = 6$ males received a sham implant (soaked in saline) and $N = 6$ males received a T implant. Endurance trials took place on days 1, 3, 7, 14, and 18. Because laboratory-maintained animals were restricted to 5-liter terraria throughout the trial, it is unlikely that effects of T on endurance arise from a "training effect" that might be observed in an experiment on free-ranging lizards (T implantation greatly increases home range size, DeNardo and Sinervo, 1994b). Changes in performance in laboratory trials were analyzed by repeated measures ANOVA.

Effects of Testosterone on Free-Ranging Territorial Behavior

To examine the relationship between experimentally elevated T and a male's access to females, we reanalyzed data from a previous study by DeNardo and Sinervo (1994b) that was conducted in March and April, 1992. We could not discriminate between yellow- and blue-throated males at the time of implantation in mid March because males do not yet have differentiated throats. Moreover, T is related to the transformation of yellow-throated males to blue and thus T implantation per se might alter the throat color of yellow males. Accordingly, we pooled the results of implant experiments on putative blue- and yellow-throated males. Space use of males and females was studied using methods described above. Male home range was estimated during a 10-day period prior to implantation and from 5 to 15 days after implantation.

A male was scored as having access to a female if the male was observed in the female's home range at least once (mean female home range area is $13.7 \pm 1.7 \text{ m}^2$). We also used another index of access to females that was based upon the percentage of overlap between male and female home ranges. The percentage of overlap gives a measure of the degree to which a male might be able to effectively exclude other males from females in his territory. Changes in access to females before versus after implantation were analyzed by repeated measures ANOVA. Females were included in statistical analysis if they survived to produce offspring.

RESULTS

Variation in Plasma Testosterone and Survival among Morphs

Orange-throated males ($N = 8$) had significantly higher levels of plasma T than blue-throated ($N = 17$)- or yellow-throated ($N = 15$) males (ANOVA, $P < 0.01$, Fig. 1A) during the first clutch of the season. Enhanced performance of orange-throated males is not without costs (Fig. 1B); they had lower yearly survival compared to blue-throated ($\chi^2 = 8.47$, $P < 0.004$) or yellow-throated ($\chi^2 = 8.88$, $P < 0.003$) males. Yearly survival rates for blue- and yellow-throated males were comparable ($P < 0.05$).

Males that start the breeding season with a yellow throat versus a blue throat were further distinguished in terms of seasonal shifts in plasma T based on the two seasonal samples, endurance, and territorial behavior. Males that began the breeding season with a blue throat have levels of plasma T (April: $120.3 \pm 6.7 \text{ ng/ml}$, mean \pm SE, $N = 8$) comparable to those of blue-throated males that were sampled 1 month later (May: $108.1 \pm 28.2 \text{ ng/ml}$) (paired t test, $P < 0.5$). Males that began the season with a yellow throat had low levels of plasma T (April: $92.0 \pm 14.7 \text{ ng/ml}$, $N = 6$); however, 1 month later the plasma T of yellow-throated males that were transforming to the blue morphology (May: $149.7 \pm 24.9 \text{ ng/ml}$; $P < 0.05$, paired t test) was significantly higher than that of yellow-throated males in the earlier sample. Plasma levels of yellow-throated males that did not transform remained low (May: $48.7 \pm 9.9 \text{ ng/ml}$, $N = 3$); however, the sample size of nontransforming males captured late in the season was limited to three males. Likewise, late in the season, we could only obtain samples for two orange males, but levels of plasma T

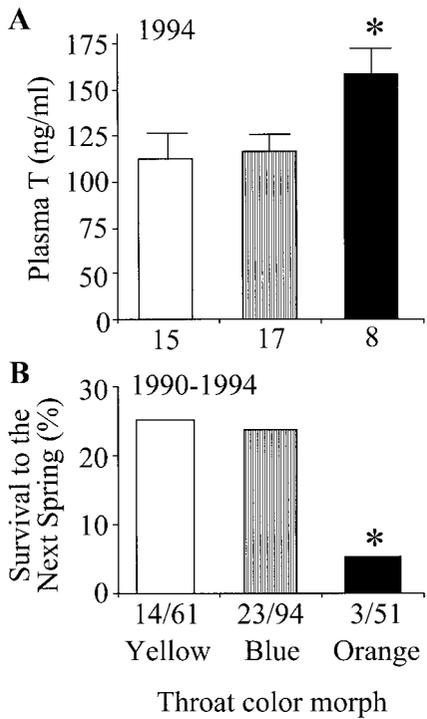


FIG. 1. (A) Variation in plasma testosterone (ng/ml) of throat color morphs of male side-blotched lizards during the first clutch of the reproductive season. Histograms reflect means \pm SE. (B) Annual survival probability of throat color morphs from one breeding season to the next. The sample size is provided below each histogram.

were very high in these two males (203 ± 25.3 ng/ml, $N = 2$), suggesting that orange-throated males maintain high T late in the season. Orange-throated males typically begin disappearing at a high rate after the first clutch, which also coincides with the transformation of some yellow-throated males to a blue throat.

Seasonal Variation in Endurance and Territorial Behavior among Morphs

Orange-throated males had higher endurance (Fig. 2A) and activity (Fig. 2B) and larger territories (Fig. 2C) than blue-throated males. Early in the season, yellow-throated males had low plasma T and low endurance, and they did not defend a territory. However, yellow males did range across a large area (Fig. 2C), albeit with relatively low levels of activity (Fig. 2B). Yellow-throated males that transformed to blue also had significantly higher endurance (Fig. 2A, 1994) than yellow-throated males that did not transform. As yellow-throated males transformed to a blue-throated

morphology, their relatively large and undefended home range was reduced to a more compact territory. Territory size of transformed yellow/blue-throated males was similar to territory size used by males that began the breeding season with a blue throat (Fig. 2C) (N.B., No traits were significantly correlated with body mass (g), $P > 0.05$, ANCOVA).

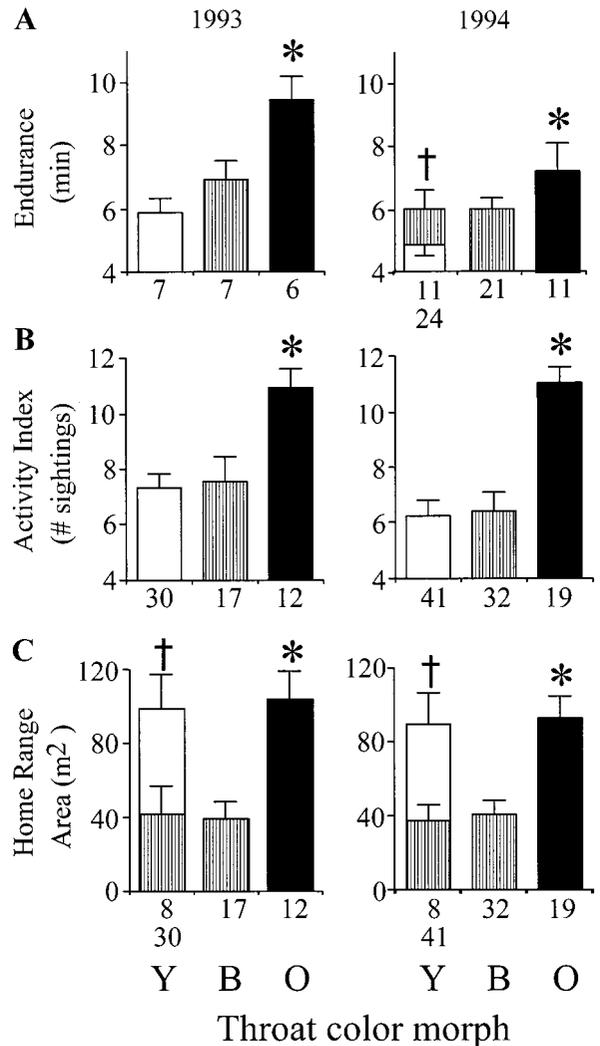


FIG. 2. Variation in (A) endurance on a laboratory treadmill (minutes), (B) activity in the wild, and (C) home range area (m²) of throat color morphs in 1993 and 1994. Histograms reflect means \pm SE. The sample size is listed below the histograms. An asterisk denotes morphs that were significantly different from the other two morphs ($P < 0.05$). Two histograms are supplied when data were available on yellow males (white) that transformed to blue throat later in the season (hatched). Significant differences in nontransforming yellow and transforming yellow-blue morphs are denoted by †.

Experimentally Induced Variation in Endurance, Territorial Behavior, and Access to Females

Males that received exogenous T had significantly higher endurance (49% higher) within 18 days of implantation than controls ($P < 0.02$, Fig. 3A). The enhanced endurance is not an effect of increased activity that would lead to a "training effect" (Gleeson, 1979), which might arise from an elevated patrol rate during daily territorial defense. Males were not allowed to patrol territories because they were maintained in small terraria in the laboratory.

Free-ranging males that received T implants ($N = 8$) secured access to significantly more female territories ($P < 0.04$) than sham-implanted males ($N = 11$, Fig. 3B). T-implanted males also overlapped with significantly ($P < 0.02$) more of a female's home range (percentage of overlap) than sham-implanted males (Fig. 3C). No differences were seen between males in each group prior to treatment (access to females, $P < 0.49$; percentage of overlap $P < 0.75$).

DISCUSSION

Effects of Plasma Testosterone on Physiology and Behavior in Side-Blotched Lizards

A proximate link between physiology, performance, behavior, and fitness should arise from the endocrine system (Marler and Moore, 1988; Ketterson *et al.*, 1991; Sinervo and Licht, 1991a,b; Sinervo and DeNardo, 1996; Sinervo and Svensson, 1998). The adaptive significance of hormonal and physiological traits can be assessed with a "selectionist's" paradigm (Arnold, 1983) that entails measurement of a dose response curve between hormones that govern expression of physiological or behavioral traits and "fitness gradients" between physiological or behavioral traits and survival or reproductive success. Deriving dose response curves for all the physiological and behavioral traits influenced by gonadal steroids is essential to understanding the selection that occurs on the endocrine regulation of these traits.

Elevated physiological capacity is presumably important during prolonged battles that are essential for maintaining a territory and acquiring access to females. High plasma T should enhance endurance. This hypothesis was confirmed with T implants. Blue- and yellow-throated males that received exogenous T in a silastic implant had 49% higher endurance than controls (Fig. 3A). Values for endurance of T-implanted blue- and yellow-throated males were similar to those

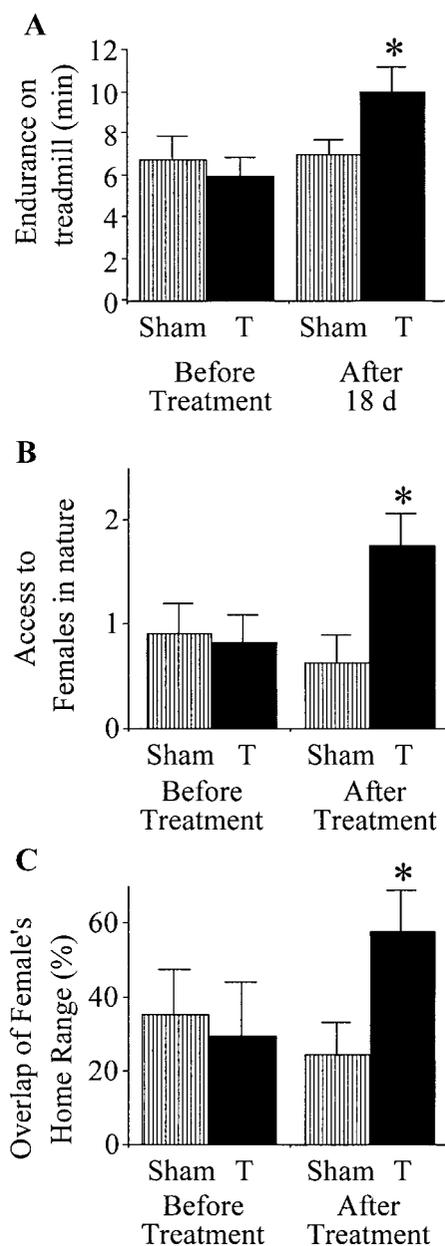


FIG. 3. (A) Pre- and posttreatment changes in the endurance of blue- and yellow-throated males that received exogenous T (treated) or empty (sham) Silastic implants in laboratory experiments. (B) Pre- and posttreatment changes in the number of females in the home range of blue- and yellow-throated males that received exogenous T (treated) or empty (sham) implants in the wild. (C) Pre- and posttreatment changes in percentages of overlap of female home ranges with those of blue- and yellow-throated males that received exogenous T (treated) or empty (sham) implants in the wild.

of the orange-throated males that have naturally high plasma T. Orange-throated males had 46–48% higher endurance than unmanipulated blue- and yellow-

throated males, values remarkably similar to the induced effects of T on the endurance of blue- and yellow-throated males compared to controls (cf. Figs. 2A and 3A). Blue- and yellow-throated males with experimentally elevated plasma T also became more active and acquired territory from neighboring sham-manipulated males (DeNardo and Sinervo, 1994b) and concomitantly gained greater access to females' territories (Figs. 3B and 3C). It is noteworthy that blue- and yellow-throated males implanted with T defended $\sim 100\text{-m}^2$ territories (see DeNardo and Sinervo, 1994b), which is nearly equal to the natural territory size of orange-throated males ($\sim 100\text{ m}^2$). Thus, the higher endurance and territory size of orange-throated males compared to blue- and yellow-throated males appeared to be related to their higher plasma T.

Both orange- and blue-throated male *U. stansburiana* are behaviorally dominant lizards and actively defend their territories against intruding males. However, orange-throated males are much more aggressive than blue-throated males. In staged field experiments where an "intruding" male is introduced onto a resident's territory, orange-throated males invariably escalate to an attack within 1 min (Sinervo and Miles, unpublished data). Blue-throated males often escalate to attack, but latency to attack is longer than that observed for orange-throated males. Blue-throated males are more likely to engage in "bluff" displays without attack. In this regard, we view orange-throated males as "ultradominant" and blue-throated males as "dominant" in territorial contests. The third male morph of *U. stansburiana* has a yellow throat and is not territorial. Early in the season they use a very large home range that spans an average of five dominant male territories (Sinervo and Lively, 1996). Yellow-throated males often mimic a "female rejection display" in staged encounters with dominant males (Sinervo and Miles in preparation). It is noteworthy that prereceptive females in this population also have yellow throats. As territorial males disappear from attrition some yellow-throated sneakers transform, acquire a blue throat, and adopt territorial behaviors of dominant males (Fig. 2C). Physiological changes in endurance of yellow-throated males during transformation were correlated with seasonal changes in plasma T (e.g., 92 versus 150 ng/ml in pre- and post-transformation yellow-throated males. It is noteworthy that a 49% increase in endurance was induced by exogenous T within 12–18 days (Fig. 3A).

The precise mechanism whereby T enhances endurance in *U. stansburiana* is not known and will require further histological and biochemical analysis of mus-

cle. T supplementation is known to increase muscle mass (Fennell and Scanes, 1992; Young *et al.*, 1993) and muscle fiber size (Rand and Herrera, 1993; Regnier and Herrera, 1993), shift muscle fiber toward an oxidative type (Van Breda *et al.*, 1993; Staron *et al.*, 1994), and increase the action of enzymes such as calcium ATPase and lactate dehydrogenase (Devi and Almon, 1984; Saborido *et al.*, 1991). While the effect of T may be mediated by direct action via receptors in the muscle (Bleisch *et al.*, 1982), muscle growth may also arise from interaction with growth hormone (GH) (Hunter *et al.*, 1965). Many of these effects might be classified as the activational effect of steroids on males at maturity (Moore and Thompson, 1990). However, it is noteworthy that orange-throated male *U. stansburiana* are significantly ($P < 0.0001$) heavier at maturity (7.65 ± 0.12 g, mean \pm SE) than blue-throated (6.61 ± 0.21 g) or yellow-throated ($6.82 \pm .16$ g) males. The fact that orange-throated males are heavier at maturity and more massive per unit of body length (unpublished data) suggests that the morphs diverge early in ontogeny, and this leaves open the possibility that some morph differences in endurance might also be due to the organizing effect of steroids that act during hatching development (Moore and Thompson, 1990; Hews *et al.*, 1994). We did not find that male size had any effects on endurance in this study, but orange-throated males may have other histological differences in muscle or motor neurons that contribute to elevated endurance.

Linking Studies of Experimentally Induced and Natural Variation in Plasma Hormones

Phenotypic engineering via hormone implants (Ketterson and Nolan, 1992; Raouf *et al.*, 1997) is useful to test cause and effect relations between hormones, physiology, and fitness. Moreover, variation among genetically determined morphs can be used as a metric to ensure that pharmacological effects of hormones are not influencing the findings (Sinervo and Svensson, 1998; Sinervo, 1998). In this study, we complemented experimental implant studies with a study of the similarly "discrete" natural variation observed among genetically distinct morphs within a single population. The effects of natural variation closely matched the effect of hormone treatments. Similarly, study of the experimental effects of reproductive hormones of female side-blotched lizards (Sinervo and DeNardo, 1996; Sinervo, 1998) has corroborated the natural survival costs of reproduction. Experimental manipulation of hormones should be referenced to

natural variation, whenever possible, to understand the ecological and evolutionary significance of hormone action.

We targeted the T manipulations to correspond to the natural variation in T found among three morphs of side-blotched lizards. Implants used in this study were designed to elevate the plasma T of males by 60 ng/ml (DeNardo and Licht, 1993). Orange-throated males had significantly higher plasma T than blue- or yellow-throated males during the course of the reproductive season. The difference in plasma T between orange-throated males and blue/yellow-throated males was 40 ng/ml. Similarly, when some of the yellow-throated males transformed and acquired a blue patch their plasma T increased from 92 to 150 ng/ml, a difference of 57 ng/ml. Experimentally elevated and natural variation in plasma T had comparable effects on endurance and territorial behavior. Many physiological and behavioral differences among the three alternative morphs are parsimoniously explained in terms of genetic variation in the regulation of plasma T at maturity.

Proximate Bases of Variation in the Development of Alternative Male Strategies

We have estimated significant heritability of throat color between *U. stansburiana* fathers and their free-ranging progeny ($h^2 = 0.86$, DNA paternity studies, Zamudio and Sinervo, in press) and for dams and their free-ranging daughters ($h^2 = 0.48$, Sinervo et al., 2000). Given a genetic mechanism underlying morph determination in *U. stansburiana*, it is likely that steroid hormones might also organize male morphs during egg or hatchling development, as has been implicated in *Urosaurus ornatus* (Hews et al., 1994). High heritability appears to be incompatible with the plasticity of yellow-throated males, which can transform to a blue throat. However, all yellow-throated males begin the season with sneaker behaviors and physiology, and transformation to a dominant phenotype involves the mortality of a territory holding male, which is usually an orange male. Moreover, not all yellow-throated males transform. Males that have the opportunity to acquire territory when the resident dies are more likely to undergo transformation. Finally, the throat color (blue patch with <50% coverage of the dewlap) that sneakers acquire late in the breeding season is qualitatively different from the throat color of a male that begins the season with a blue throat (~100% coverage of the dewlap).

The female mimicry exhibited by yellow-throated

males disappears after they transform to the blue-throated morphology. We have never observed a yellow-throated male use mimicry or sneaker behaviors after it has transformed to a blue-throated male. We hypothesize that activational events associated with elevated plasma T may permanently alter behaviors generated by the central nervous system such that transformation from sneaker to territory holder is irreversible. Whereas sneaker males can transform to become a dominant male, dominant males cannot transform to sneakers. Plasticity in alternative male morphs may be permissible in directions that correspond to normal sexual differentiation. The plasticity in alternative male morphs in other dioecious and sequential hermaphroditic vertebrates seems to follow the pattern seen in *Uta* (e.g., Brantley et al., 1993; Cardwell and Liley, 1991; Gross, 1984, 1991; Sinervo and Lively, 1996; Warner, 1988). Generalized vertebrate development seems to have a ground state that is female-like in pattern and that require activational events associated with T to trigger a change to the male patterns.

The three-morph mating system of *U. stansburiana* (ultradominant orange-throated males, dominant blue-throated males, sneaker yellow-throated males) is more complex than the two-morph mating systems described in *Sceloporus undulatus erythrocheilus* (Rand, 1990, 1992) and *U. ornatus* (Thompson and Moore, 1991; Thompson and Moore, 1992). Whereas sneaker males in *U. ornatus* have orange throats and are larger than dominant blue-throated males, sneaker males in *U. stansburiana* have yellow throats and are smaller than the ultradominant orange-throated males, but similar in size to dominant blue-throated males. The color of sneakers is fixed in *U. ornatus*, in contrast to the marked phenotypic plasticity seen in the yellow-throated sneaker *U. stansburiana*. The yellow-throated sneaker *U. stansburiana* can partially change to a dominant blue-throated morphology in appropriate social contexts. However, orange-throated males in *U. stansburiana* are fixed throughout life; none have been observed to change color.

Recent work has advanced our understanding of the relationship between hormones and ecologically relevant behaviors, such as dominance and the developmental organization of such variation (Moore and Thompson, 1990). In two lizards, *S. undulatus erythrocheilus* (Rand, 1990, 1992) and *U. ornatus* (Thompson and Moore, 1991a,b), elevated plasma T has been shown to be associated with polymorphism in male throat color. The polymorphism in *U. ornatus* is fixed in adults and color does not change in response to T

implants (Thompson and Moore, 1991a,b); however, manipulations of T in hatchling *U. ornatus* have successfully altered throat color development (Hews and Moore, 1985). Throat color serves as a “badge” which reliably signals status among male *U. ornatus* (Thompson and Moore, 1991b) as well as in *U. stansburiana* (Y. Brandt and B. Sinervo, in preparation). In *U. ornatus*, blue- or orange-blue-throated males are dominant over orange-throated males (Thompson *et al.*, 1993). Only males with blue throats hold territories, whereas orange-throated males are floaters (Thompson and Moore, 1991b; Thompson and Moore, 1992). Males with blue throats are more aggressive and smaller than orange-throated males in *U. ornatus* (Thompson and Moore, 1992; Thompson *et al.*, 1993) and manipulation of T early in ontogeny suggests the polymorphism is fixed before sexual maturity (Hews *et al.*, 1994).

Natural History Context of Steroid Regulation and Rock-Paper-Scissors Games in Side-Blotched Lizards

Natural history observations provide the context for interpreting the mechanistic links between steroid regulation, physiology, performance, and behavior that are presented above. Bright orange sides in *U. stansburiana* represent a conspicuous “badge” of status (Maynard Smith, 1982) that signals both high aggression and physiological capacity (Figs. 2A and 2B) to conspecific males (Y. Brandt and B. Sinervo, submitted for publication) and perhaps high mate quality to females. Our results extend previous studies on the effects of T because we describe the link between plasma T and endurance. We also show how such proximate mechanisms translate into ecologically relevant behaviors such as territory defense, which influence potential mating success. In addition, plasma T can act over very short time scales and enhance endurance within 11 days. A very rapid elevation in endurance in response to T may be selectively advantageous when yellow-throated males change to blue morphology. Transforming males must not only defeat rival transforming males, but they must be able to hold off the incursions of dominant males in adjacent territories.

Differences in morph physiology and behavior have interesting effects on the evolutionary dynamics of morph frequency across years. Each morph is successful against one of the other morphs, but less successful against the third, which resembles a “rock–paper–scissors” game (see below). Sexual selection arising

from territorial contests and mate acquisition favors orange-sided males in *U. stansburiana* under some social contexts (e.g., variation in morph frequency). Notably, when orange-throated males are at low frequency they obtain a large advantage in mate access at the expense of neighboring blue-throated males, who lose territory and mates to orange-throated males (Sinervo and Lively, 1996). The asymmetry in plasma T and endurance between orange- and blue-throated males would result in a concomitant difference in Resource Holding Potential or fighting ability (Maynard Smith, 1982), which favors orange-throated males in territorial contests. Orange-throated males increase from low to high frequency in subsequent years and blue-throated males decline in frequency. Low survival of orange-throated males relative to the other two morphs (Fig. 1B) may counterbalance some of the advantages accrued by sexual selection. Moreover, survival costs exhibited by orange-throated males that have naturally high T provide comparative support for manipulative experiments in lizards (Marler and Moore, 1988) that document survival costs associated with chronically elevated exogenous T.

As orange-throated males increase in frequency, yellow-throated males obtain greater access to females on orange-throated males’ territories. Yellow-throated males mimic females and thereby avoid agonistic encounters with orange-throated males. The low plasma T of yellow-throated males during the early reproductive season may be necessary to allow these males to mimic female-like behaviors. Yellow-throated males do not actively defend a territory, but have a large home range that encompasses on average five dominant male territories (Sinervo and Lively, 1996) and they fertilize females on territories of dominant males. An important aspect of the yellow-male strategy entails transformation to a blue throat. The proximate cue for transformation is likely to be the disappearance of a dominant orange-throated male. Yellow-throated males typically transform to blue with the death of territory holders (Sinervo and Zamudio, manuscript). If a transforming male can successfully defend females on the recently usurped territory, the transformed male would gain a fitness advantage through production of second-clutch hatchlings (Sinervo and Dougherty, 1996). Yellow-throated males that rapidly transform to a territory holding blue would be favored and elevated T should promote rapid changes in physiology.

The frequency of orange-throated males declines owing to cuckoldry by yellow-throated males. Orange-throated males are nearly eliminated (~5% fre-

quency), while at the same time the frequency of yellow climbs to nearly 50%. The blue-throated males gain an advantage over yellow-throated males now that orange is nearly eliminated. Evidence from DNA paternity studies (Zamudio and Sinervo, in press) indicates that the large territory size of orange-throated males allows yellow-throated males to sneak copulations from females because orange-throated males cannot guard all the females in their territory. In contrast, the modest territory size of blue-throated males allows them to effectively mate guard females from sneaker copulations. Blue-throated males guard their females and they have higher plasma T and endurance than yellow-throated males, which allows them to acquire greater access to females when yellow-throated males are common (and orange is rare). Finally, as blue-throated males increase in frequency and yellow-throated males decline in frequency, orange-throated males again acquire an advantage over blue-throated males and the cycle in morph frequency is renewed. The cycle takes 4 to 5 years to complete.

Effects of plasma T on the behavior and physiology of side-blotched lizards provides the mechanistic bases for a "rock-paper-scissors" game of alternative male strategies. In the children's rock-paper-scissors game, rock beats scissors, paper beats rock, and scissors beat paper. In nature, the ultradominant orange-throated strategy (rock) beats the mate guarding strategy of blue-throated males (scissors). However, the orange male strategy (rock) is susceptible to the sneaker strategy of yellow males (paper). The yellow-throated strategy (paper) is in turn thwarted by the mate guarding strategy of blue-throated males (scissors). Strong and cyclical frequency-dependent selection as found in the rock-paper-scissors game perpetually refines regulatory mechanisms controlling plasma T, behavior, and physiological traits like endurance because of mechanistic links among these phenotypic traits and fitness.

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