Gonadotropin Hormone Modulation of Testosterone, Immune Function, Performance, and Behavioral Trade-Offs among Male Morphs of the Lizard *Uta stansburiana*

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ABSTRACT: Sexual selection predicts that trade-offs maintain trait variation in alternative reproductive strategies. Experiments often focus on testosterone (T), but the gonadotropins follicle-stimulating hormone and luteinizing hormone may provide a clearer understanding of the pleiotropic relationships among traits. We assess the activational role of gonadotropins on T and corticosterone regulation in traits expressed by polymorphic male side-blotched lizards *Uta stansburiana*. Gonadotropins are found to enhance and suppress multiple physiological, morphological, and behavioral traits independently, as well as indirectly via T, and we demonstrate selective trade-offs between reproduction and survival. The OBY locus, a genetic marker in our model vertebrate mating system, allows characterization of the interaction between genotype and hormone treatment on male traits. Our results suggest that *oo*, *ob*, and *bb* males are near their physiological and behavioral capacity for reproductive success, whereas *yy* and *by* males are maintained below their physiological maximum. Both *by* and *yy* morphs show trait plasticity, and we demonstrate that gonadotropins are likely proximate effectors that govern not only trait differences between alternative mating strategies but also morph plasticity. Gonadotropins clearly represent an important mechanism maintaining variation in physiological, morphological, and behavioral traits, as well as potentially maintaining the immunosuppression costs of male sexual signals.

Keywords: follicle-stimulating hormone, luteinizing hormone, survival, reproduction.

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Understanding proximate mechanisms and selective trade-offs that maintain physiological variation in alternative reproductive strategies is a major goal in evolutionary biology (Zera and Harshman 2001). Physiological trade-offs provide key mechanisms underlying tests of sexual selection theory (e.g., Rolff and Siva-Jothy 2002; Peters et al. 2004). Variation in male reproductive strategies is common in many animal taxa (e.g., Insecta, Osteichthyes, Amphibia, Reptilia, and Aves), and these mating systems have been used to document trade-offs in life-history traits (Eberhard 1980; Warner and Hoffmann 1980; Shuster and Wade 1991; Sinervo and Lively 1996), which are intimately related to the expression of alternative mating behaviors, thus forging explicit links among physiological trade-offs and sexual selection theory. In this article, we simultaneously consider endocrine regulation of traits that contribute to mate competition and attraction, two phases of sexual selection that are typically considered in isolation. In this regard, steroid hormones such as testosterone (T) have been implicated in governing physiological, morphological, and behavioral trade-offs involved in mate competition as well as mate attraction (Ketterson and Nolan 1992). However, sex steroid regulation is governed by

gonadotropins; thus, the proximate cause of male trade-offs involving T may be governed by gonadotropin hormones.

One hypothesis explaining trade-offs between current and future reproduction is the immunosuppressive effects of plasma T and concomitant effects on survival or reproductive success (e.g., Marler and Moore 1988; Verhulst et al. 1999; S. C. Mills, A. Grapputo, I. Jokinen, E. Koskela, T. Mappes, and T. Puttonen, unpublished manuscript). T is predicted to exert immunosuppressive effects either directly (Folstad and Karter 1992) or indirectly by redirecting resources away from immune function toward sexual behavior (Wedekind and Folstad 1994). Elevated levels of the vertebrate stress hormone corticosterone (CORT) may also depress immune function (Sapolsky et al. 2000), which could potentially lead to lower survival. However, evidence supporting this hypothesis is inconclusive (Roberts et al. 2004), and relationships between T and CORT themselves, as well as their interaction on immune function (such as the immunoenhancing effects of CORT), are often contrary to predictions and thus complicate the hypothesis (Evans et al. 2000; Roberts et al. 2007). Thus, any assessment of the physiological effects of T or its endocrine regulation must consider the effects on CORT, given that these are two routes by which immune function can become compromised.

Here we consider activational effects of steroid hormones T and CORT (Moore 1991; Moore et al. 1998) that arise during sexual maturation (Sinervo et al. 2000*a*). Direct selection on reproductive success should act via endocrine factors that govern activation of T (Sinervo et al. 2000*b*). We assess fitness consequences of gonadotropin and steroid interactions in terms of the physiological, morphological, and behavioral male traits expressed in a model vertebrate mating system exhibited by the common side-blotched lizard *Uta stansburiana* (fig. 1).

Gonadotropins are secreted by the anterior pituitary, which is regulated by gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus (fig. 1; Phillips et al. 1987). The gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are essential for reproduction in their regulation of gonadal function. In mammals, LH stimulates T secretion in the testes (e.g., Saez 1994; Habert et al. 2001), and FSH stimulates sperm production (e.g., Simoni et al. 1997). However, little information is available regarding the role of gonadotropins in reptiles. LH increases androgen production in immature water snakes *Nerodia sipedon* (Krohmer 1986), while FSH stimulates androgen secretion in the turtle *Chelonia mydas* (Licht and Papkoff 1985). The interactive effects of LH and FSH in the expression of male physiological and behavioral traits and their effects on male trade-offs in reptiles and other vertebrates remain to be elucidated.

Male side-blotched lizards express one of three genetically determined color morph phenotypes (orange, blue, and yellow; referred to as "OBY"; see app. A in the online edition of the *American Naturalist*; Sinervo and Lively 1996; Sinervo et al. 2001). The three color alleles (*o*, *b*, and *y*) are tied to alternative male strategies (Sinervo and Lively 1996). Males with an orange throat have two *o* alleles (O = *oo*) or an *o* and a *b* allele (O = *bo*); they are large and territorial and express high levels of T (Sinervo et al. 2000*b*), which allows them to usurp territories (Calsbeek et al. 2002) from males with two *b* alleles (B = *bb*). The O male strategy is vulnerable to cuckoldry by nonterritorial sneaker males with *y* alleles (Y = *yy*, *by*; Zamudio and Sinervo 2000). A schematic of interactions illustrates that regulation of traits by T spans many functional modules (Wagner and Altenberg 1996) for behavioral, morphological, and performance traits (fig. 1). For example, in side-blotched lizards, T affects performance traits such as male endurance (Sinervo et al. 2000*b*) and male mating behaviors (DeNardo and Licht 1993), and given the strong documented differential, density-dependent social effects on immune function between female morphs (Svensson et al. 2001*a*, 2001*b*), T perhaps should likewise affect immune function differentially between male morphs. In order to test the effects of gonadotropin hormones, it is useful to have a genetic marker like the OBY locus for behavioral syndromes (Sih et al. 2004). If baseline gonadotropin levels are related to allele expression, throat color morphs may be regulated by gonadotropins, and they may vary in their response to gonadotropin treatment. The OBY locus can be used to test different responses to exogenous hormone application, enabling the simultaneous study of genetic and endocrine regulation of morphs and salient male trade-offs (Sinervo and Licht 1991*a*, 1991*b*; Sinervo and Basolo 1996). This approach is analogous to medical research into drug treatment in which genetic predispositions in physiological processes may alter the impact of a response to a novel drug (e.g., clearance rates, receptor sensitivity). In the case of a probe of alternative male strategies with reproductive hormones, one can uncover the endocrine basis of life-history trade-offs that structure the differences among morphs (Svensson et al. 2001*a*, 2001*b*, 2002).

In this study, we examine the individual and interactive effects of LH and FSH on male physiology in the polymorphic side-blotched lizard both directly and indirectly via the downstream regulation of T and CORT. LH and/or FSH are predicted to increase plasma T, and based on earlier work (Sinervo et al. 2000*b*), we expect a subsequent enhancement of performance (endurance and sprint speed), male courtship, and blue throat color but suppressed immune function. LH and/or FSH are predicted to increase plasma CORT. The multiple effects of steroid

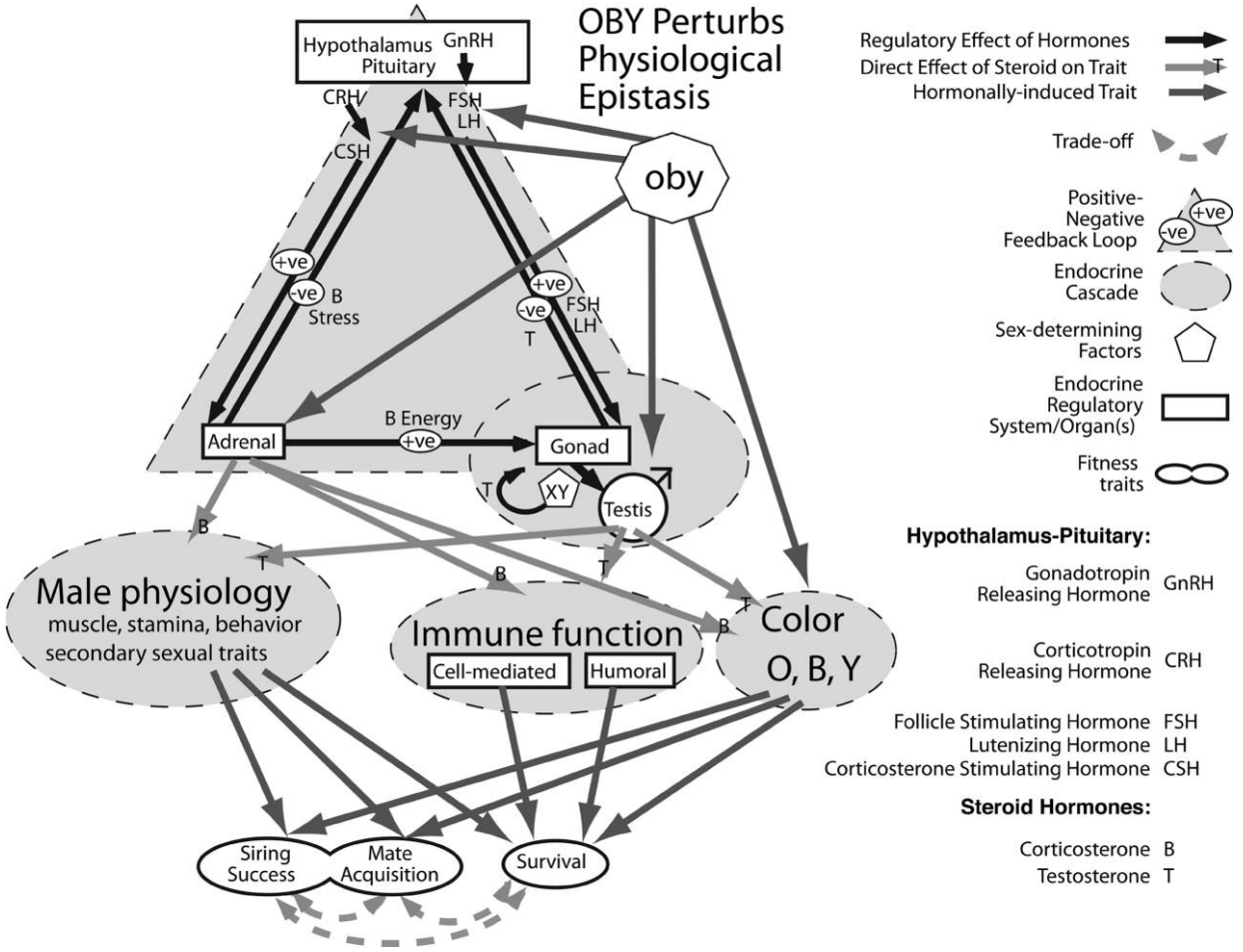

Figure 1: Schematic of interactions that make up endocrine regulation of luteinizing hormone, follicle-stimulating hormone, and testosterone spanning the functional modules for behavioral, morphological, and physiological traits in interaction with the OBY locus of the side-blotched lizard *Uta stansburiana*. This graph is modified from Sinervo and Calsbeek (2003).

and gonadotropin hormones, combined with pleiotropic effects of many loci with the OBY locus, are thought to cause the trait differences between morphs in both male and female side-blotched lizards (Sinervo and Svensson 2002). Therefore, effects of gonadotropins are predicted to show interactions with morph genotype. Furthermore, we investigate the consequences of elevated T and decreased CORT on immune function and performance. In order to determine which hormones mediate trade-offs, we determined the relative contribution of each gonadotropin (FSH vs. LH) and steroid hormone (T vs. CORT) in those analyses that revealed a trade-off. Finally, steroids have been implicated in plasticity observed in specific morph genotypes. In late-season clutches, some yellow males with a *by* genotype transform to a blue morphology and also exhibit higher endurance and higher T levels (Sinervo et al. 2000*b*). Here we also test whether gonadotropin hormones might be the upstream proximate trigger of the inducing effects of plasma T on behavior and physiology of any plastic changes in these sneaker male morphs and plastic throat color changes in the other sneaker genotype that is homozygous for the *y* allele (i.e., *yy*).

Material and Methods

Animals and Husbandry

In March 2003, 80 male and 40 female lizards were collected by noose or by hand from a population of the side-blotched lizard *Uta stansburiana* on Billy Wright Road near Los Baños Grandes, Merced County, California. Virgin female lizards were collected before the breeding season. Male lizards were collected between March 28 and March

30, during peak copulatory and aggressive behavior, so that male morphotype could be determined from visual inspection of fully formed throat and flank color. Three solid throat colors are expressed in putative homozygous males: orange (*oo*), dark blue (*bb*), and yellow (*yy*). Blue-yellow heterozygotes (*by*) have yellow and pale blue stripes, and blue-orange heterozygotes (*bo*) have blue and orange stripes on the throat. A three-class score was constructed for color on both a yellow and a blue axis (1.0 was assigned to *bb* and *yy* males and 0 to *oo* males); it is referred to as a genotypic value because it reliably predicts progeny color (Sinervo et al. 2006). In the laboratory, lizards were individually housed in standard conditions as described by Sinervo and Licht (1991*a*). A detailed time schedule of the experiment is provided in figure 2.

Hormone Treatment

After pretreatment parameters had been measured (body mass, snout-vent length, sprint speed, endurance, and throat colors; methods described below), males were randomly assigned to one of four hormone treatment groups: control, LH, FSH, and both (LH, FSH). Males were given a 50-μL injection of hormone treatment daily, intraperitoneally into their right flank. A concentration of 1 unit/mL was used for ovine LH (Sigma L-5269) and ovine FSH (Sigma F-8174) and 1 unit/mL each of LH and FSH for the both (LH, FSH) treatment. The control was 0.9% saline. Hormone treatment began on April 15, 2003, and continued daily between 1800 and 2100 hours for 13 days.

Blood Sampling

After 10 days of daily hormone treatment, blood samples were taken using three 50-μL hematocrit tubes (Sinervo et al. 2000*b*). Blood samples were centrifuged at 2,000 rpm for 5 min, and plasma was extracted for analysis of plasma T, plasma CORT, and antibody production (methods described below).

Endocrinology

Plasma T was measured using a radioimmunoassay kit (TESTO-CTK, DiaSorin, Byk-Sangtec Diagonstica, Dietzenbach, Germany) as described in detail elsewhere (Mills et al. 2007). We screened plasma from *U. stansburiana* for parallelism with the kit's standard curve, using a series of six dilutions in quadruplicate. The dilutions run parallel to the standard curve (homogeneity of slopes for sample vs. standard ANCOVA: $F = 3.205$, df $= 1, 23$, $P = .089$), thus validating the use of this kit. This technique also enabled us to determine that a dilution factor of 1 : 10 for lizard plasma samples corresponds to 50% of antibody bound.

Plasma CORT of males was measured using a radioimmunoassay kit (ImmuChem ^{125}I Corticosterone RIA, Tamro MedLab Oy, Vantaa, Finland) as follows. First, 100 μL of antiserum (B-3-caroxymethyloxime bovine serum albumin raised in rabbits) was added to tubes containing 50 μL of either seven standards or blood plasma samples diluted 1 : 100 and 100 μL of ^{125}I-labeled CORT. After a 2-h incubation at 22°–25°C, antibody-bound antigens were precipitated and pelleted after centrifuging (Heraeus Megafuge 1.0R) at 1,000 × relative centrifugal force for 15 min, and the supernatant was aspirated. The radioactivity of the precipitate was measured in a gamma counter (RackGamme, LKB Wallac, Sweden). It is inversely related to the amount of unlabeled CORT in the samples or standards. Sample CORT concentration was determined by interpolation from the standard calibration curve. We screened plasma from *U. stansburiana* for parallelism with the kit's standard curve using a series of 10 dilutions in quadruplicate. The dilutions ran parallel to the standard curve for *U. stansburiana* (homogeneity of slopes for sam-

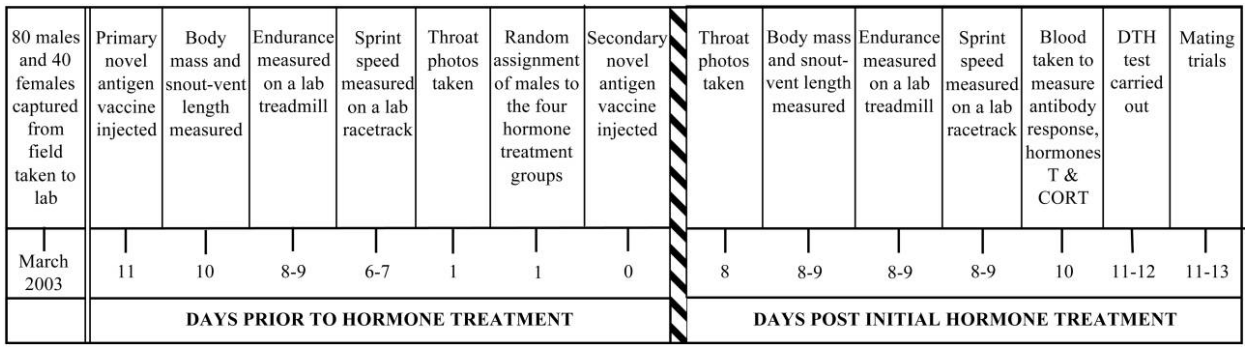

Figure 2: Experimental schedule. Hatched shading indicates day 1 of daily hormone treatment. *T* = testosterone; *CORT* = corticosterone; *DTH* = delayed-type hypersensitivity.

ple vs. standard ANCOVA: $F = 0.15$, df $= 1, 41$, $P = .904$), thus validating the use of this kit. The interplate coefficient of variation was 20%. This technique also enabled us to determine that a dilution factor of 1 : 90 for *U. stansburiana* plasma samples corresponds to 50% of antibody bound.

Multiple measurements were taken from the lizards within a relatively short period of time; however, because circulating titers of CORT are known to return to baseline levels within 24 h of handling (B. Sinervo and T. Comendant, unpublished data), we kept a minimum delay of 24 h between each measurement. Furthermore, the CORT levels in this study (fig. 3*d*–3*f*) were comparable to those previously measured from both control (means range from 8 to 18 ng/mL) and implanted (means range from 35 to 55 ng/mL) laboratory experimental males (DeNardo and Licht 1993).

Immune Function

The immune system is composed of three primary components: innate immunity, humoral immunity, and cell-mediated acquired immunity (Norris and Evans 2000). Immune function may be measured using monitoring or challenge techniques; however, the disadvantage of monitoring techniques is that they reflect the immune response to current infections (Norris and Evans 2000). We used challenge techniques, whereby a component of the immune system is exposed to a novel antigen and the subsequent immune response quantified. In order to account for possible differential investment in different components of the immune system, we used two tests: the first measured adaptive humoral immunity, and the second measured both innate and adaptive cell-mediated components of the immune system.

Antibody Production (Adaptive Humoral Response). All males received a primary immunization with a novel antigen 11 days before hormone treatment and were revaccinated (boostered) on the first day of hormone treatment. Ten days later, all males were bled to measure antibody production. They were injected with 50 μL of the novel antigen diphtheria-tetanus (DT) toxoid vaccine (2 mg/mL $Al(OH)_3$), which has previously been used with *U. stansburiana*, and antibody production was measured using a standard enzyme-linked immunosorbent assay protocol (Svensson et al. 2001*a*, 2001*b*, 2002).

Delayed-Type Hypersensitivity (Innate and Cell Mediated). The delayed-type hypersensitivity (DTH) test has recently been found to represent a multifaceted index of cutaneous innate and adaptive immune responses, including the infiltration of basophils, eosinophils, heterophils, lymphocytes, macrophages, and thrombocytes (Martin et al. 2006). The method we use is based on that previously used in lizards (Belliure et al. 2004; López and Martin 2005), which was considered a standard assay of cell-mediated immune responsiveness. We assessed DTH after 11–12 days of daily hormone treatment. The right front foot pad was injected with 50 μL phytohemagglutinin solution containing 50 mg of phytohemagglutinin-P lectin from red kidney bean *Phaseolus vulgaris* (Sigma) in 10 mL sterile phosphate-buffered solution (PBS). The left front foot pad was injected with 50 μL of sterile PBS as a control. Repeatability was calculated for foot pad measurements ($n = 75$ individuals) recorded in triplicate using ANOVAs (Lessells and Boag 1987; repeatability of all foot pads = 0.566, $F = 4.920$; preinjection: left = 0.490, $F = 3.886$; right = 0.515, $F = 4.190$; postinjection: left = 0.606, $F = 5.609$; right = 0.544, $F = 4.582$).

Physiology and Performance

Hematocrit. A hematocrit level (volume percentage of packed red blood cells) was taken from the average of the three blood samples taken after 10 days of hormone treatment. Because of their influence on oxygen uptake, hematocrit values are a measure of metabolic activity preceding blood sampling.

Condition. Measurements of body mass (g) and snout-vent length (SVL; mm) were taken 10 days before hormone treatment and after 8–9 days of daily hormone treatment. We log transformed and standardized (mean of 0, SD of 1.0) mass and SVL. We regressed (ordinary least squares) mass on SVL for the whole population and used the residuals as a measure of body condition (Schulte-Hostedde et al. 2005).

Sprint Speed. Lizards were raced on a 2-m-long racetrack equipped with photoelectric cells using standard protocols (Miles and Smith 1987). The fastest sprint time over 1 m during four trials at optimal body temperature (36°C; Sinervo and Huey 1990) was used as an estimate of maximum velocity. Lizards were measured 6–7 days before hormone treatment and after 8–9 days of daily hormone treatment.

Endurance. The endurance of males at optimal body temperature was indexed by the elapsed time (s) spent running on a treadmill (0.50 km/h belt speed) until fatigued. Lizards were induced to run by gently tapping the flanks; they were considered fatigued when their righting response was lost (Garland and Losos 1994; Robson and Miles 2000; Sinervo et al. 2000*b*). The endurance of all males was

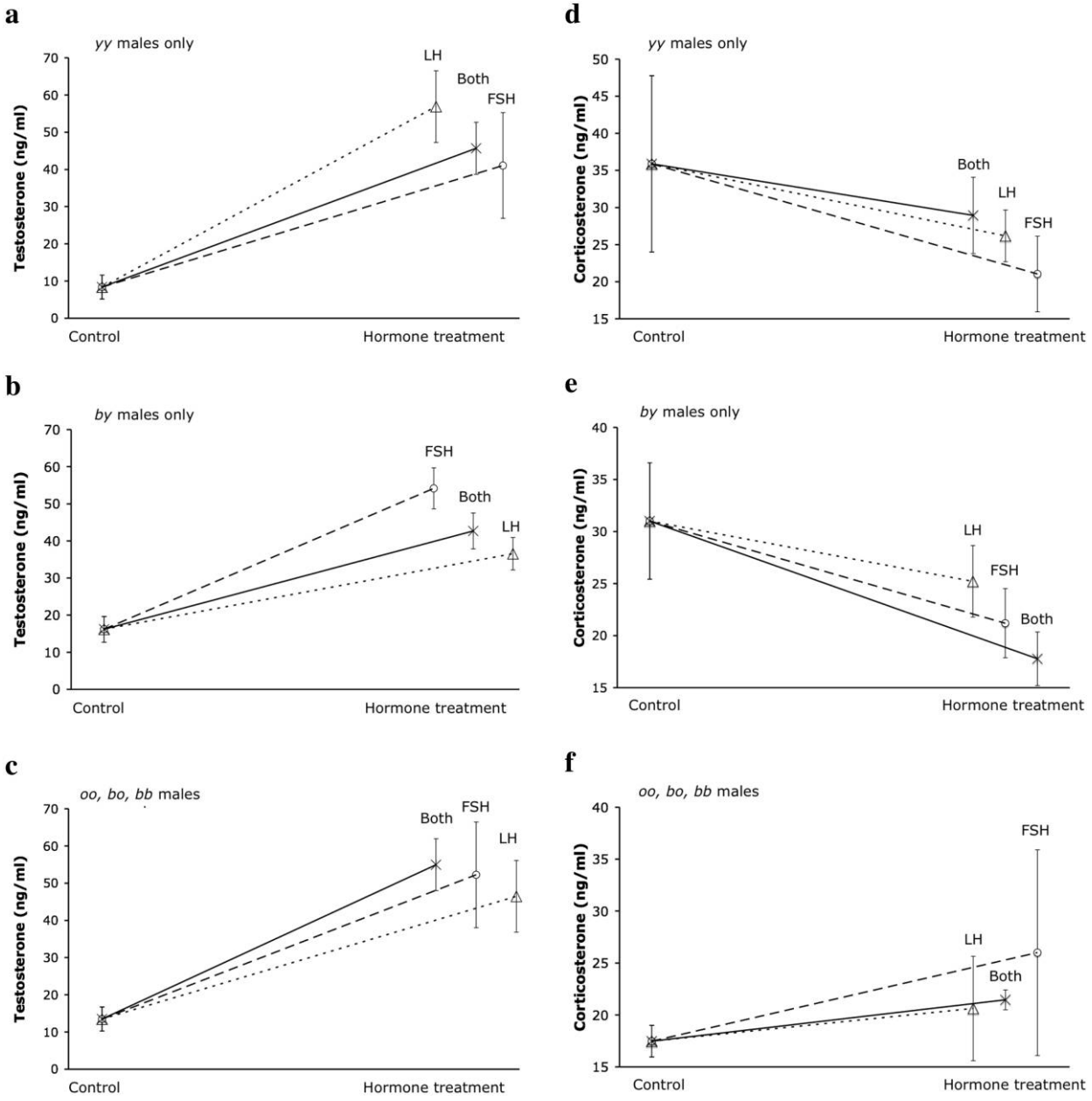

Figure 3: Mean (±SE) results of gonadotropin treatment on (*a*) plasma testosterone (T) for *yy* males only; (*b*) T for *by* males only; (*c*) T for *oo*, *bo*, and *bb* males only; (*d*) plasma corticosterone (CORT) for *yy* males only; (*e*) CORT for *by* males only; (*f*) CORT for *oo*, *bo*, and *bb* males only; (*g*) delayed-type hypersensitivity test (DTH) response for *yy* males only; (*h*) DTH response for *by* males only; (*i*) DTH response for *oo*, *bo*, and *bb* males only; (*j*) sprint speed for *yy* males only; (*k*) sprint speed for *by* males only; (*l*) sprint speed for *oo*, *bo*, and *bb* males only; (*m*) female courtship behaviors for *yy* males only; (*n*) female courtship behaviors for *yy* males only; and (*o*) female courtship behaviors for *oo* and *bb* males only. Sample sizes for control males and males treated with luteinizing hormone (*LH*), follicle-stimulating hormone (*FSH*), and both, respectively: *a*, *d*, *g*, and *m*, $N = 4, 5, 5,$ and 5; *b*, *e*, *h*, and *n*, $N = 12, 10, 11,$ and 10; *c*, *f*, *i*, and *o*, $N = 4, 4, 3,$ and 2; *j*, $N = 3, 3, 4,$ and 3; *k*, $N = 6, 6, 4,$ and 6; *l*, $N = 11, 10, 11,$ and 10.

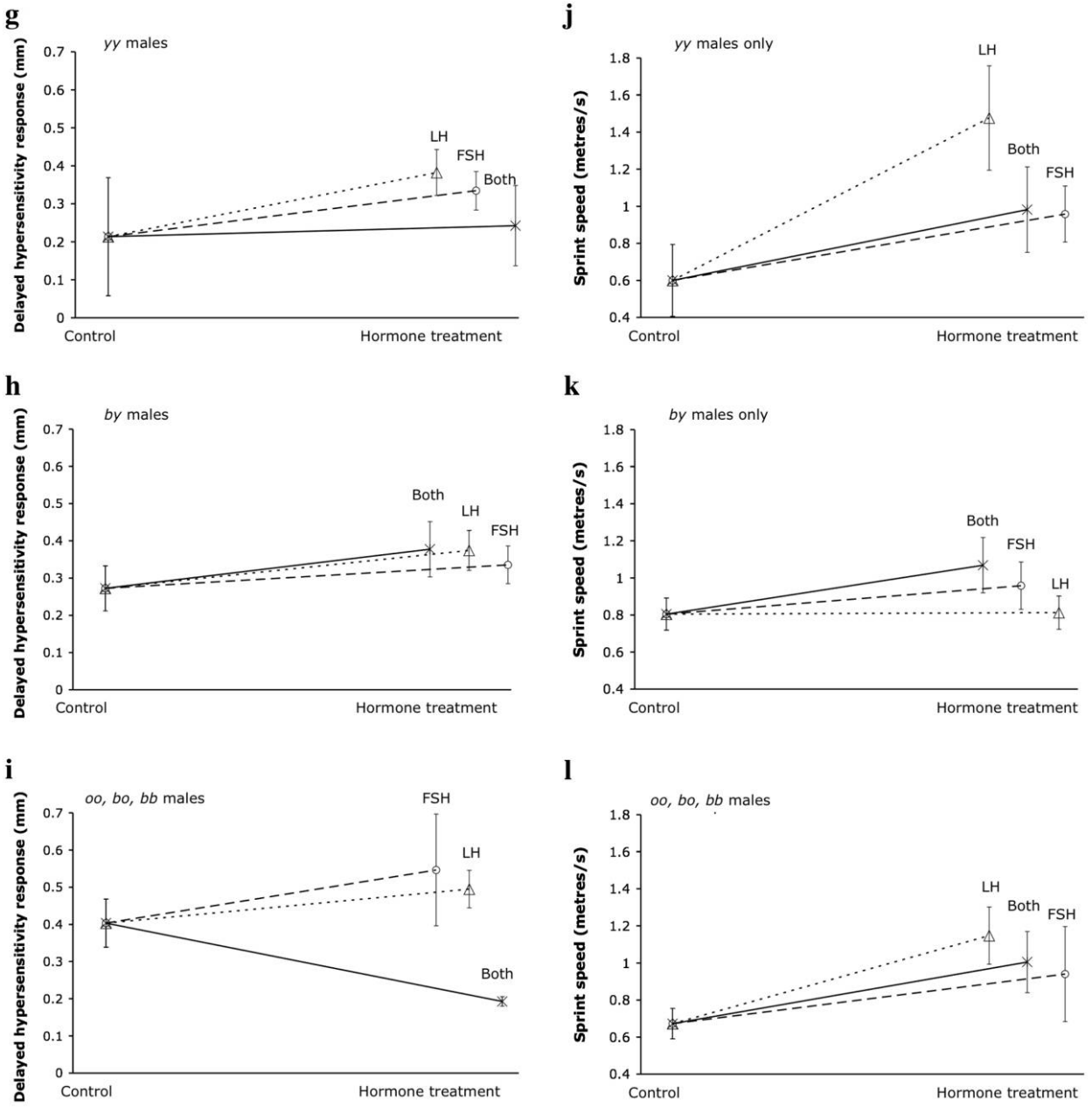

Figure 3 (*Continued*)

measured 8–9 days before hormone treatment and after 8–9 days of daily hormone treatment.

Color

Photographs of lizard throats were taken between 1800 and 2200 hours the day before hormone treatment and after 8 days of daily hormone treatment. Red, blue, and green pixel intensities were measured using Image J 1.35s (http://rsb.info.nih.gov/ij/). Because green was the least variant of the color channels, we normalized for light intensity by dividing red and blue by green intensity.

Female Preference

We carried out female preference experiments after 11–13 days of daily hormone treatment. We assembled males by

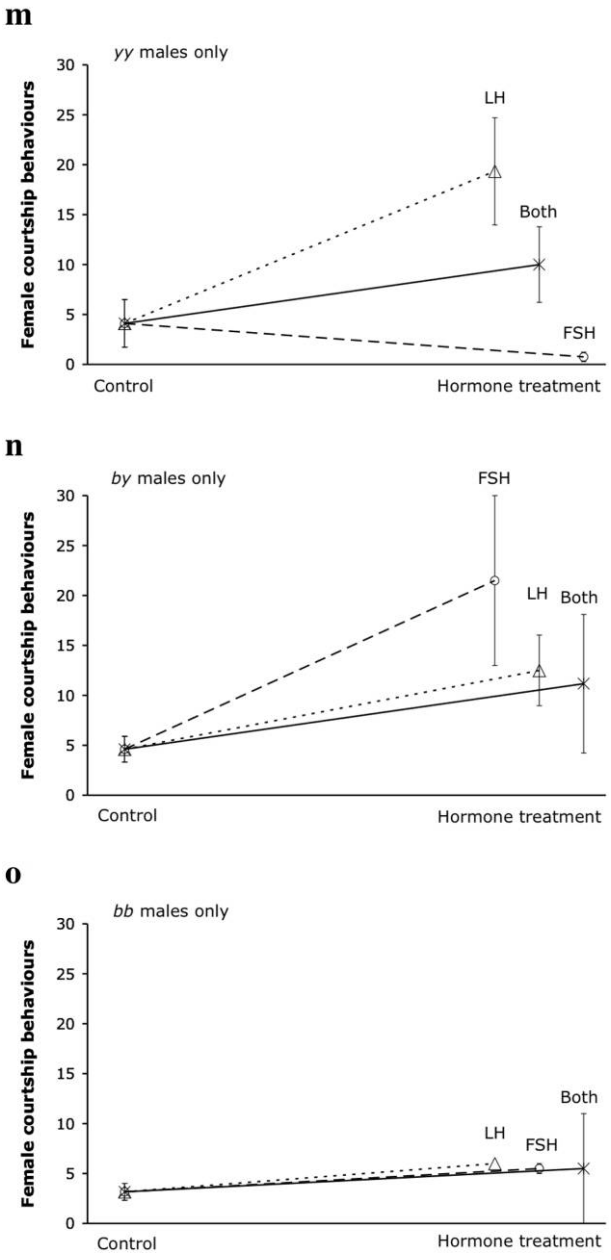

Figure 3 (*Continued*)

throat color morph (*by*, *yy*, and *bb*) and formed preference groups of four individuals each, one from each of the four hormone treatment groups; five *by*, four *yy*, and two *bb* preference groups were formed. We used 33 randomly chosen females that were reproductively receptive (Sinervo and Licht 1991*a*) in the 33 preference trials.

Dyadic female preference experiments (Bleay and Sinervo 2007) consisted of placing two males from a preference group (one control and one hormone treated) in two visually and physically separated circular glass-fronted compartments (30 cm^2). A female was positioned centrally in a larger arena (30 cm × 50 cm × 50 cm) that enabled visual but not physical contact with both males at all times. Behavioral trials were videotaped for 10 min. Three trials were performed per preference group, using three females of different morphs. The control male was used three times with each of the three hormone-treated males (order varied for different groups), with a minimum delay of 24 h.

The number and type of display behaviors performed by both males and each female was determined by video playback. The responses by a female to each male were recorded separately based on the female's position in the terrarium and on body angle. The number of female head bobs minus the number of rejection displays (arching of the body and lateral flattening; Martins 1993, 1994) to each specific male were noted. The resulting number of displays was log transformed to attain normality.

Statistical Analysis

We investigated the effects of experimental gonadotropins on the target response traits using the general linear model routine of SPSS, version 11.0. Throat morphs were included in the models and were treated as a continuous variable with respect to the number of blue and yellow throat color alleles possessed. We developed models that included the main effects of hormone manipulation and throat morph as well as their interactions. We were specifically interested in the interaction among treatments because the potential effects of LH and FSH are likely to be conditional on male genotype. All significance tests were based on Type III sums of squares. We included posttreatment body condition as a covariate in all analyses and pretreatment endurance and throat color as covariates in the analyses of posttreatment endurance and throat color, respectively. Both T and CORT are response variables in our experiment, precluding their use as covariates in our models, since it is inappropriate to include a response variable in an ANOVA model as a covariate. However, we did include T and CORT in the path analyses described below because these analyses use T as an explanatory variable, not a covariate, and thus they are a useful tool for inferring causal relations (Li 1981).

A predicted outcome of gonadotropin manipulation is the increase in T. Previous research has demonstrated that multiple traits are affected by T. Hence, it is necessary to test the effects of gonadotropins on immune function, physiology, and behavior while controlling for the effects of T. Path analysis is an appropriate method for evaluating the direct contribution of a trait on a response variable while controlling for the influence of confounding factors (Li 1981). We favored the approach outlined by Gomez and Zamora (2000) and Svensson et al. (2001*a*) with respect to model choice. The approach compares competing models in a nested, hierarchical fashion. Covariance matrices for each model are compared using a goodness-of-fit test. A statistically significant result indicates a lack of fit of the reduced model. We selected models with the highest P values and lowest χ^2 values. Our first path model considered the effects of gonadotropin, throat genotype, and condition on T. We developed additional path models for immune function, locomotor performance, and behavior. In each analysis we treated hormone treatment as a dichotomous dummy variable (fig. 4*A*). We used PROC Calis (SAS/STAT) to evaluate path models.

Results

Endocrinology

In control individuals, plasma T levels were significantly higher in *by* males (mean ± SE: 16.2 ± 3.4 ng/mL) than in *yy* males (8.4 ± 3.2 ng/mL) or *bb* males (5.8 ± 0.8 ng/mL; ANOVA: *b* alleles, $F = 6.751$, df $= 1, 16$, $P = .019$; *y* alleles, $F = 6.033$, df $= 1, 16$, $P = .026$; $y \times b$ alleles, $F = 3.415$, df $= 1, 16$, $P = .083$). Because of the low number of orange lizards in 2003, we have only one data point for control *oo* males (36.7 ng/mL), representing a T concentration twice as high as that for *by* males.

We found a significant interaction effect of gonadotropin on plasma T level (table 1, "testosterone"). Treatment with either of the gonadotropins or both in combination increased plasma T level compared to that of control males, but the gonadotropins in combination did not have a synergistic effect. The magnitude of increase in T varied among morphs: LH increased T in *yy* males (fig. 3*a*) and FSH increased T in *by* males (fig. 3*b*), whereas there was no difference between the hormones in their effect on T in *oo*, *bo*, and *bb* males (fig. 3*c*). Posttreatment body condition also had a significant positive effect on T level (table 1).

Similarly, path analysis revealed a strong interaction effect between gonadotropins on plasma T (fig. 4*B*–4*E*). Furthermore, path analysis revealed that LH had different effects in the different male morphs (figs. 3*a*–3*c*, 4*B*–4*E*) and that posttreatment body condition had positive effects on plasma T. Treatment with gonadotropin hormones lowered plasma CORT levels compared to controls (fig. 3*d*–3*f*) but not significantly so; no significant effect of gonadotropin treatment or morphotype was found on CORT level (app. B in the online edition of the *American Naturalist*).

Immune Function

We found a significant interaction between LH and FSH on antibody response to diphtheria-tetanus (DT) toxin but no effect of male morphotype (table 2, "antibody response"). While a male's antibody response was increased after treatment with either LH or FSH alone, treatment with both hormones had a synergistic negative effect on antibody production in response to DT toxin (fig. 5*a*). No effects of male morphotype were found on antibody response to DT toxin (table 2, "antibody response").

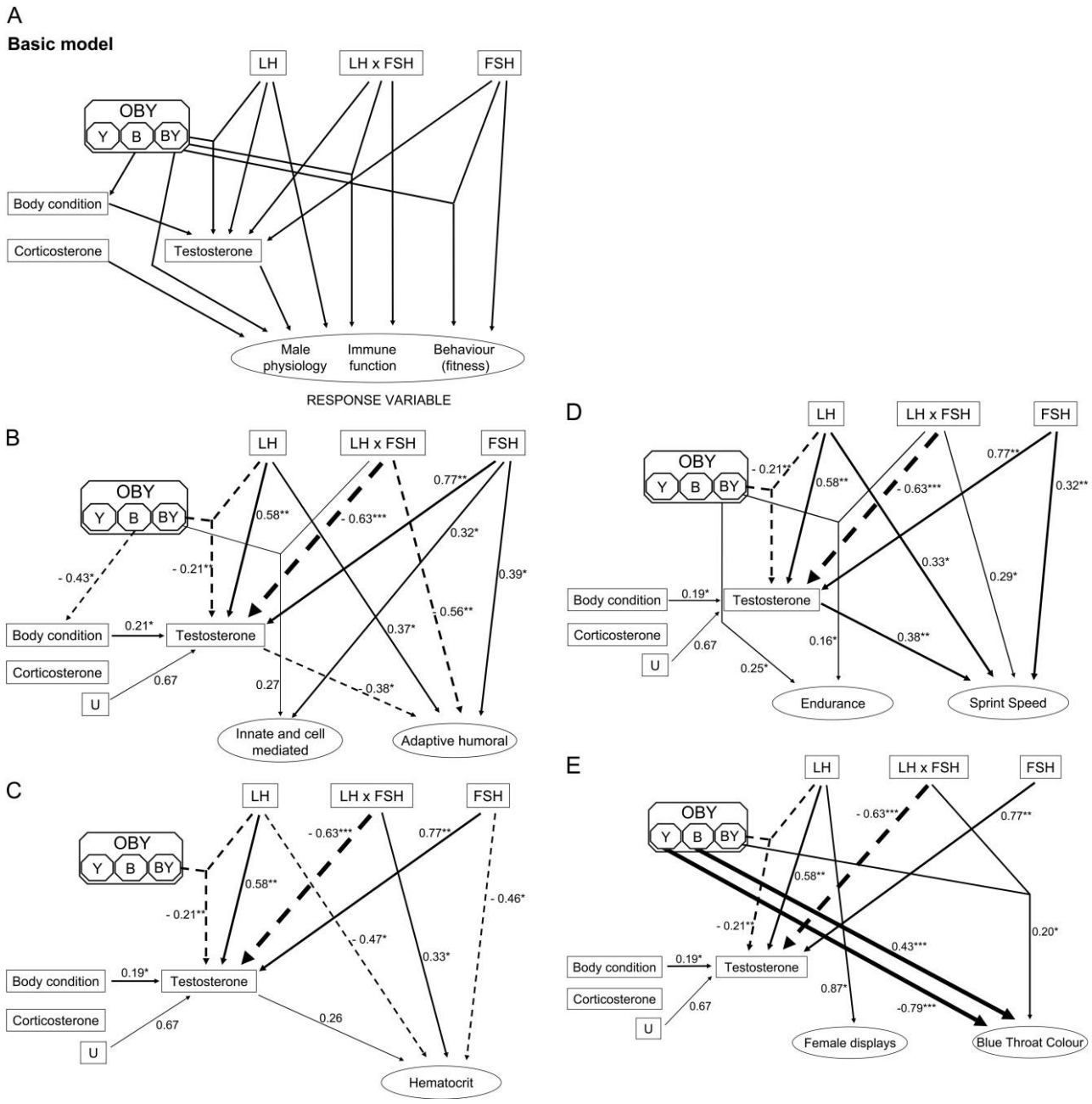

Figure 4: *A*, Basic path model hypothesizing the relationships between gonadotropin treatment, throat genotype, condition, testosterone level, corticosterone level, and the response variables, immune function (innate and cell-mediated and adaptive humoral response), physiology (hematocrit, endurance, sprint speed, and throat color), and behavior (female preference). *B–E*, Complete path models of the response variables. *B*, Immune function (innate and cell-mediated and adaptive humoral response); *C*, physiology (hematocrit); *D*, physiology (endurance and sprint speed); *E*, reproductive behavior (female preference) and blue throat color. Positive paths are shown by solid lines and negative paths by dashed lines. Paths describing interaction terms are depicted by forked arrows leading from the two interaction terms to the downstream dependent variable, and the thickness of each path reflects the magnitude of each path coefficient. *Three asterisks*, $P < .001$; *two asterisks*, $P < .01$; *one asterisk*, $P < .05$. $U =$ unexplained variation. Corticosterone (CORT) did not have a significant effect on the response variable; hence, no paths were needed for CORT. *LH* = luteinizing hormone; *FSH* = follicle-stimulating hormone.

Table 1: Testosterone and delayed hypersensitivity response as a function of treatment with the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), male morphotype (number of *b* and *y* alleles), the interaction terms between and among these variables, and posttreatment body condition

		Testosterone			Delayed hypersensitivity		
Source of variation	df	MS	*F*	*P*	MS	*F*	*P*
LH	1	235.4	1.128	.293	3.04×10^{-4}	.008	.931
FSH	1	16.1	.077	.782	1.50×10^{-3}	.037	.847
Number of *b* alleles	1	138.6	.664	.418	4.42×10^{-3}	.110	.741
Number of *y* alleles	1	132.8	.637	.428	5.45×10^{-4}	.014	.908
LH × FSH	**1**	**3,729.6**	**17.872**	**<.001**	$\mathbf{4.72 \times 10^{-1}}$	**11.769**	**.001**
b alleles × *y* alleles	1	145.7	.698	.407	6.51×10^{-2}	1.622	.208
LH × *b* alleles	1	716.1	3.432	.069	2.17×10^{-2}	.540	.465
LH × *y* alleles	**1**	**962.3**	**4.611**	**.036**	1.23×10^{-2}	.307	.582
FSH × *b* alleles	1	352.5	1.689	.199	8.04×10^{-5}	.002	.964
FSH × *y* alleles	1	166.9	.800	.375	2.07×10^{-2}	.516	.475
LH × *b* alleles × *y* alleles	**1**	**1,344.0**	**6.440**	**.014**	$\mathbf{2.32 \times 10^{-1}}$	**5.781**	**.019**
FSH × *b* alleles × *y* alleles	1	109.9	.527	.471	5.14×10^{-4}	.013	.910
FSH × LH × *b* alleles × *y* alleles	1	402.3	1.928	.170	$\mathbf{2.38 \times 10^{-1}}$	**5.927**	**.018**
Posttreatment body condition	**1**	**1,835.9**	**8.797**	**.004**	3.82×10^{-3}	.095	.759
Residual	59	208.7			4.01×10^{-2}		

Note: Values in boldface are significant. Number of *b* alleles is counted as follows: *bb* = 2; *by*, *ob* = 1; *yy*, *oo* = 0; number of *y* alleles: *yy* = 2; *by* = 1; *oo*, *bb* = 0.

Path analysis also revealed positive effects of both LH and FSH alone but a negative effect of both gonadotropins together on antibody production in response to DT toxin, while no effect of male morphotype was found (fig. 4*B*). In addition, path analysis revealed a significant negative effect of T on antibody production in response to DT toxin (fig. 4*B*), revealing that gonadotropins affect humoral immune response independent of T as well as indirectly via T. Since levels of plasma T increased after treatment with gonadotropin hormones in combination, the strong effect of both gonadotropins on antibody production is consistent with the direct immunosuppressive effects of T. Plasma T level and antibody production in response to DT toxin were strongly negatively correlated in control males (fig. 5*b*).

The interaction between LH and FSH on combined innate and adaptive immune response was significant, as was the interaction with male morphotype (table 1, "delayed hypersensitivity"). Combined immune response to phytohemagglutinin was increased by either LH or FSH alone (fig. 3*g*–3*i*), however, males receiving both hormones together showed no change (*yy* males; fig. 3*g*), an increase (*by* males; fig. 3*h*), or a significant decrease (*oo, bo, bb* males; fig. 3*i*) in their combined innate and adaptive response compared to control males (table 1, "delayed hypersensitivity").

Path analysis revealed a significant positive effect of FSH on combined innate and adaptive response (fig. 4*B*), but unlike in the GLM results, no significant interaction between male morphotype and gonadotropin treatment was detected (fig. 4*B*). T level did not affect combined innate and adaptive response (fig. 4*B*); therefore, FSH affects combined immune response independent of T. There was no effect of CORT on either antibody production or combined immune response.

Physiology and Performance

Hematocrit. We found a significant interaction between LH and FSH on hematocrit but no effect of male morphotype or posttreatment body condition (table 3, "hematocrit"). Treatment with either hormone decreased the percentage of red blood cells in a male's blood (fig. 6); in contrast, treatment with both hormones reduced hematocrit less than treatment with LH or FSH alone (fig. 6).

Similarly, path analysis revealed negative effects of LH and FSH alone but positive effects after treatment with both LH and FSH (fig. 4*C*), and no effects of either male morphotype or T were found (fig. 4*C*). Therefore, gonadotropins affect hematocrit independent of T. There was no effect of CORT on hematocrit.

Sprint Speed. The interaction between gonadotropins and male morphotype had a significant effect on male sprint speed, as did body condition (table 3, "sprint speed"). Gonadotropin treatment had the greatest effect on sprint speed in *yy* males (fig. 3*j*), with smaller effects in *oo*, *bo*, and *bb* males (fig. 3*l*) and in *by* males (fig. 3*k*).

Path analysis also revealed significant positive effects of gonadotropins alone (in particular, FSH) and in combi-

Table 2: Antibody response and female courtship displays toward males as a function of treatment with the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), male morphotype (number of *b* alleles), the interaction terms between and among these variables, and posttreatment body condition

		Antibody response			Female courtship behavior		
Source of variation	df	MS	*F*	*P*	MS	*F*	*P*
LH	1	.322	1.891	.175	**1.447**	**7.214**	**.011**
FSH	1	.001	.003	.956	.243	1.213	.279
Number of *b* alleles	1	.001	.004	.951	.001	.001	.973
LH × FSH	**1**	**.744**	**4.367**	**.041**	.001	.004	.947
LH × *b* alleles	1	.377	2.213	.142	**.912**	**4.547**	**.040**
FSH × *b* alleles	1	.051	.302	.585	.202	1.007	.323
FSH × LH × *b* alleles	1	.185	1.083	.302	.155	.772	.386
Posttreatment body condition	1	.000	.000	.999	.023	.114	.738
Residual	56/33	.170			.201		

Note: Values in boldface are significant. Number of *b* alleles is counted as follows: *bb* = 2; *by*, *ob* = 1; *yy*, *oo* = 0. Antibody response was not obtained for any *oo* males receiving FSH; therefore, in order to prevent empty cells in the model, the number of *y* alleles was not included. The number of *y* alleles was not included in the model for female courtship displays because the number of *b* alleles covers all genotypes used in experiments on female preference.

nation on sprint speed, but no interaction with male morphotype was detected (fig. 4*D*). In addition, a large effect of T on sprint speed was found (fig. 4*D*). We also found a significant correlation between plasma T and sprint speed (fig. 7); therefore, gonadotropins affect sprint speed indirectly via T and independent of T. There was no effect of CORT on sprint speed.

Endurance. Male morphotype had a significant effect on male endurance (table 4, "endurance"). The lowest endurance was shown by *yy* males (mean ± SE: 46.9 ± 11 s), and *by* males showed the highest endurance (202.9 ± 12 s), followed by *oo* (181.5 ± 45 s) and *bb* males (172.9 ± 16 s). We also found a significant interaction effect between FSH and male morphotype on endurance (table 4, "endurance"). Treatment with FSH increased endurance only in males with two *y* alleles (*yy*).

Path analysis also found a significant effect of male morphotype on endurance as well as a significant interaction between gonadotropin treatment and male morphotype (fig. 4*D*). Because no significant effect of T was found (fig. 4*D*), we can conclude that gonadotropins affect endurance independent of T. There was no effect of CORT on endurance.

Color

As expected, male morphotype was correlated with blue throat color, even at the end of the experiment (table 4, "blue throat color"); the greater the number of *b* alleles (or the smaller the number of *y* alleles) that a male possesses, the greater the intensity of blue throat color (fig. 8). Furthermore, FSH had different effects on blue throat color in the different male morphs (table 4, "blue throat color"). While FSH treatment enhanced blue throat color in *by* and *yy* males, FSH attenuated blue throat color in *oo* and *bb* males.

Path analysis also revealed highly significant effects of male morphotype on blue throat color and an interaction effect between gonadotropin treatment and male morphotype (fig. 4*E*). Because no significant effect of T was found (fig. 4*E*), we can conclude that gonadotropins affect blue throat color independent of T. There was no effect of CORT on throat color.

Female Preference

We found a significant interaction between LH and male morphotype on female courtship displays (table 2, "female courtship behavior"). Females showed more courtship displays toward LH-treated *yy* males than toward the control males (fig. 3*m*) and more displays toward FSH-treated *by* males than toward the controls (fig. 3*n*). Hormone treatment had no significant effect on female courtship displays toward *bb* males compared to controls (fig. 3*o*).

Path analysis found a significant effect of LH on female courtship behaviors (fig. 4*E*). No significant interaction of gonadotropin and morphotype was found, nor was an effect of T; therefore, gonadotropins affect female preference independent of T. There was no effect of CORT on female preference.

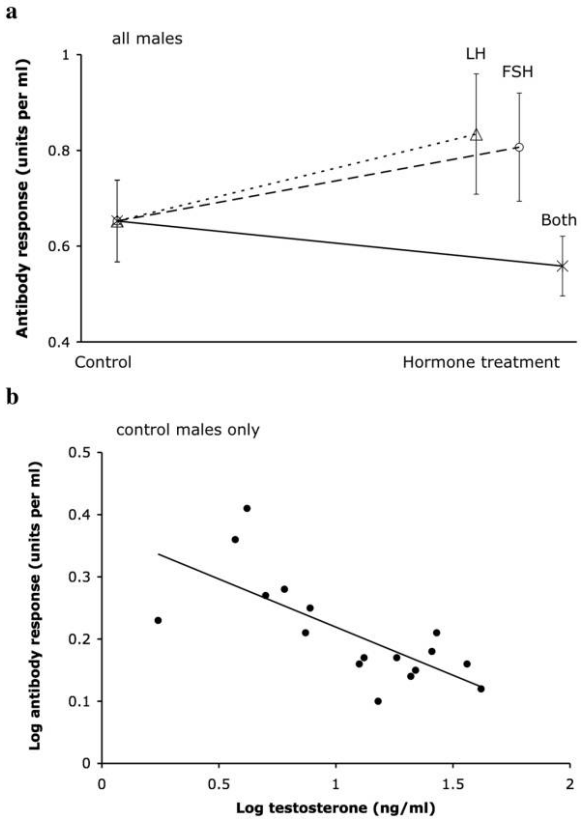

Figure 5: *a*, Mean (±SE) results of gonadotropin treatment on antibody response ($N = 17$, 17, 16, and 15 for control males and males treated with luteinizing hormone [*LH*], follicle-stimulating hormone [*FSH*], and both, respectively). *b*, Regression of log antibody production on log plasma testosterone for control males ($y = 0.373 - 0.154x$; $R^2 = 0.508$, $P = .001$, $N = 16$).

Discussion

We assessed the activational role of the gonadotropins FSH and LH on production of T and CORT. Our goal was to characterize the cascading synergistic endocrine interactions on the expression of alternative male physiological, morphological, and behavioral traits in the throat color polymorphism of the side-blotched lizard. Path analyses emphasized the importance of endocrine function upstream of T: gonadotropins directly regulate plasma T and regulate combined innate and adaptive immune function, hematocrit level, endurance, blue throat color, and mate attraction independent of T. In addition, gonadotropins indirectly regulate humoral immune response to DT toxin and sprint speed, both independently and via T. Thus, our study highlights the importance of the hypothalamus-pituitary-gonadal axis, in particular FSH and LH, for the regulation and maintenance of male physiological traits. These male traits span multiple functional modules, including endocrinology, immune function, male physiology, and throat color, as well as female preference, and have important consequences for survivorship and reproductive success (fig. 1). Our study also highlights the fact that plasma T, combined immune response, sprint speed, endurance, blue throat color, and mate attraction are also modulated by the OBY locus, which demonstrates the interaction between hormone treatment and genotype on physiological and morphological traits

Trade-Offs

Treatment with either one or both gonadotropins increased plasma T levels (fig. 3*a*–3*c*), with concomitant positive effects on sprint speed (figs. 4*D*, 7). However, T is also considered to have a direct suppressive effect on immune function (Folstad and Karter 1992; Wedekind and Folstad 1994), and T-implanted male lizards *Psammodromus algirus* had higher tick loads and higher mortality than control males (Salvador et al. 1996). Our study revealed that immune function, including humoral and combined innate and adaptive responses, was enhanced by the gonadotropins independently, but when gonadotropins were administered in combination, the result was a cascading negative effect on immune function (figs. 3*i*, 5*a*). Although humoral immune function to DT toxin was affected by the hypothalamic-pituitary-gonadal axis via T, our path analysis demonstrates that this effect ($P = .02$; fig. 4*B*) was not as large as the effects of gonadotropins independent of T ($P = .01$; fig. 4*B*). Despite the evidence indicating that T has direct immunosuppressive effects in many animals (Saino et al. 1995; Verhulst et al. 1999; Mougeot et al. 2004; but see Peters 2000; Roberts et al. 2004), our data suggest that it is actually the synergistic effect of hormones produced upstream of T, as well as of T itself, that suppresses immune function. Therefore, previous immunosuppression studies may have found more significant effects if they had examined gonadotropins rather than T because they would have revealed the immunosuppressive effects of gonadotropins that act independent of T as well as those acting via T. Elevated levels of the stress hormone CORT may also depress immune function (Sapolsky et al. 2000); however, we found no evidence that gonadotropins affected plasma CORT levels (fig. 3*d*–3*f*) or any evidence for an effect of CORT on humoral adaptive immune response to DT toxin. This is not surprising since the immunosuppressive effects of CORT are likely to arise after defeat in agonistic encounters when CORT levels are raised, whereas the lizards in our study were kept in isolation.

Treatment with LH, FSH, and the two in combination decreased male hematocrit (figs. 4*C*, 6). Hematocrit contributes to the efficiency of oxygen uptake; consequently,

our results suggest that gonadotropin secretion places additional physiological stress on lizards, which may ultimately affect their ability to avoid predators and hence their survival. Plasma T level was positively affected by body condition, yet path analyses revealed that the positive effects of body condition on sprint speed and blue throat were acting via T. Therefore, T and body condition are intimately linked in their effects on male physiology.

Although treatment of males with gonadotropins in isolation or in tandem increased locomotor performance, blue throat color, and a female's preference for males, trade-offs with immune function and hematocrit were also apparent. Furthermore, we demonstrate that these trade-offs act via endocrine factors (FSH and LH) not only indirectly via T, as is commonly assumed, but also independent of T (fig. 4*C*, 4*D*). Therefore, while selection should act indirectly on endocrine factors via male physiological, morphological, and behavioral traits, the response to selection will be constrained by trade-offs involving immune response and hematocrit.

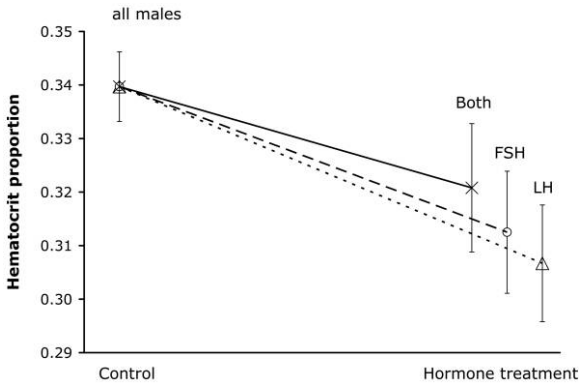

Figure 6: Mean (±SE) results of gonadotropin treatment on hematocrit for all males; $N = 20$, 19, 19, and 16 for control males and males treated with luteinizing hormone (*LH*), follicle-stimulating hormone (*FSH*), and both, respectively.

Male Polymorphism

Our results revealed an interesting interplay between the OBY locus and the hypothalamic-pituitary-gonadal axis. In agreement with previous work on side-blotched lizards (Sinervo et al. 2000*b*), we found that *yy* males showed the lowest endurance, sprint speed, and plasma T level, whereas *by* males showed the highest, and values for *oo* and *bb* males were lower but not significantly different from those of *by* males. Both endurance, a stringent measure of whole-organism aerobic capacity (Bennett 1978; Sinervo and Huey 1990; Garland and Losos 1994), and sprint speed are important in contests to maintain territories, gain access to females, and escape predators (Robson and Miles 2000; Brandt 2003). Our results show that endocrinological and physiological differences between individuals characterize the different morphs of the side-blotched lizard. Male territorial behavior is absent from the strategy of *yy* males; it is highest in *oo* and *bb* males; and *by* males are intermediate in this regard (Sinervo et al. 2000*b*).

Table 3: Hematocrit and sprint speed as a function of treatment with the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), male morphotype (number of *b* and *y* alleles), their interaction terms, and posttreatment body condition

		Hematocrit			Sprint speed		
Source of variation	df	MS	*F*	*P*	MS	*F*	*P*
LH	1	1.56×10^{-3}	.830	.366	1.66×10^{-4}	.001	.973
FSH	1	8.14×10^{-6}	.004	.948	2.24×10^{-1}	1.564	.216
Number of *b* alleles	1	3.24×10^{-4}	.172	.680	7.93×10^{-2}	.554	.460
Number of *y* alleles	1	1.12×10^{-5}	.006	.939	8.37×10^{-2}	.585	.448
LH × FSH	**1**	$\mathbf{9.81 \times 10^{-3}}$	**5.216**	**.026**	$\mathbf{1.12 \times 10^{-1}}$	**7.805**	**.007**
b alleles × *y* alleles	1	7.91×10^{-4}	.421	.519	4.89×10^{-3}	.034	.854
LH × *b* alleles	1	1.94×10^{-3}	1.031	.314	8.49×10^{-4}	.006	.939
LH × *y* alleles	1	5.55×10^{-4}	.295	.589	7.24×10^{-2}	.506	.480
FSH × *y* alleles	1	2.59×10^{-6}	.001	.971	4.13×10^{-1}	2.887	.095
FSH × *y* alleles	1	7.84×10^{-5}	.042	.839	2.02×10^{-1}	1.411	.240
LH × *b* alleles × *y* alleles	1	2.08×10^{-3}	1.105	.297	3.30×10^{-1}	2.309	.134
FSH × *b* alleles × *y* alleles	1	1.22×10^{-3}	.650	.423	3.24×10^{-2}	.226	.636
FSH × LH × *b* alleles × *y* alleles	1	1.16×10^{-3}	.681	.435	6.27×10^{-1}	**4.385**	**.041**
Posttreatment body condition	1	1.02×10^{-3}	.544	.464	6.85×10^{-1}	**4.785**	**.033**
Residual	59	1.88×10^{-3}			1.43×10^{-1}		

Note: Values in boldface are significant. Number of *b* alleles is counted as follows: *bb* = 2; *by*, *ob* = 1; *yy*, *oo* = 0; number of *y* alleles: *yy* = 2; *by* = 1; *oo*, *bb* = 0..

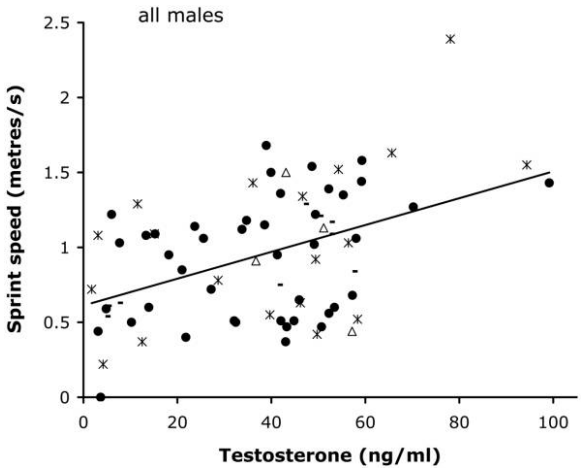

Figure 7: Regression of sprint speed on plasma testosterone for all experimental males ($y = 0.637 + 0.008x$; $R^2 = 0.19$, $P < .001$, $N = 73$). Symbols indicate male morphotype: *circles* = *by*, *asterisks* = *yy*, *dashes* = *bb*, and *triangles* = *oo*.

Experimental manipulation of the polymorphic side-blotched lizards enabled us to determine which morphs are at or near their maximum in terms of physiological and behavioral traits. Neither LH nor FSH significantly increased endurance or sprint speed in *by*, *oo*, or *bb* males, suggesting that these males were already at their physiological maximum for performance and territorial behavior. The situation is different in *yy* males, where LH increased sprint speed (fig. 3*j*) and FSH increased endurance, suggesting that these males were below their physiological maximum. These measures of performance are costly in terms of survival (Sinervo et al. 2000*b*), and high survival is essential for the success of this sneaker male strategy (*yy* morph), which obtains highest fitness from late-season clutches (Zamudio and Sinervo 2000). Therefore, selection is likely to maintain performance traits below the physiological maximum in *yy* morphs and result in the absence of territoriality.

Successful male-male interactions and female acquisition are dependent not only on territoriality but also on throat color. Gonadotropin treatment decreased blue throat color in *oo* and *bb* males and did not affect female reproductive behavior toward *bb* males (fig. 3*o*). Yet in *yy* and *by* males, at least one gonadotropin increased throat color and female reproductive behaviors (fig. 3*n*, 3*o*). Therefore, the natural levels of gonadotropin hormones are likely to maintain *oo* and *bb* males near the maxima of not only their physiological but also their morphological and behavioral capacities for reproductive success. In *yy* males, LH increased plasma T level (fig. 3*a*), sprint speed (fig. 3*j*), and mate acquisition behavior (fig. 3*m*), suggesting that LH is maintained at a low level in *yy* males. Previous studies in vertebrates that have linked LH to territorial aggression (Meddle et al. 2002; Chastel et al. 2005) lend support to this hypothesis; *yy* males lack territoriality and would thus be expected to have low LH levels. In *by* males, FSH increased plasma T level (fig. 3*b*), sprint speed (fig. 3*k*), and mate acquisition behavior (fig. 3*n*), suggesting that FSH is maintained at a low level in *by* males. In accordance, the natural low levels of LH and FSH structure the difference between the morphs and maintain *yy* and *by* males, respectively, below their maximum physiological, morphological, and behavioral capacities.

We found that gonadotropin hormones have multifaceted effects; however, their mode of action was conditional on male genotype, demonstrating the important pleiotropic effects of OBY locus that govern the maintenance of alternative behaviors and physiology of male side-blotched lizards. Treatment with gonadotropins revealed that physiological, morphological, and behavioral traits could be increased in *by* and, in particular, *yy* morphs. However, the trade-offs between these traits and either immune response or hematocrit found in this study may lead to lower survival in the wild (S. C. Mills, A. Grapputo, I. Jokinen, E. Koskela, T. Mappes, and T. Puttonen, unpublished manuscript), as reported for endurance and sprint speed (Sinervo et al. 2000*b*). The functional trade-offs between these traits important for reproductive success versus survival are likely to be selecting for the differences in physiological, morphological, and behavioral traits of the alternative behaviors linked to the OBY locus expressed by the side-blotched lizard.

Morph Plasticity

It is known that some morphs may undergo a transformation under certain conditions, but the importance of the interaction between the OBY locus and the hypothalamic-pituitary-gonadal axis is not known. Late in the season, some nonterritorial yellow males with a *by* genotype transform to a blue morphology and exhibit territoriality and higher reproductive success (Sinervo et al. 2000*b*). Here we extend these observations to the *yy* genotype, suggesting that *y* alleles harbor important components of plasticity.

In terms of performance, we have shown that *yy* males appear to remain below their physiological capacity for sprint speed and endurance and below their reproductive maximum because behavior, endurance, and throat color, traits that are increased by gonadotropin treatment, are considered the important factors affecting male mate acquisition and those that ultimately lead to reproductive success in side-blotched lizards. Gonadotropins regulate

male performance; consequently, stimulation with LH and FSH, increasing sprint speed and endurance, respectively, could enable *yy* males to initiate dominance behaviors, such as territory defense, which under certain density and frequency conditions, such as a very high frequency of Y, would increase their reproductive success. LH also increases mate attraction of *yy* males (fig. 3*m*), and the blue throat color of *yy* males darkened after treatment with both gonadotropins. In 2003, we observed the highest *yy* male frequency, and an unprecedented number had darker throats late in the season, compared to earlier in the season or any other year of observations (B. Sinervo, unpublished data). This dark color appears to be melanic in origin, unlike the blue color expressed by males with blue genotypes, and may be linked to higher gonadotropin levels. Therefore, gonadotropins, in particular, LH, upregulate *yy* male traits as territorial vacancies arise, suggesting that *yy* males may adopt a season-dependent strategy with plasticity from nonterritorial to territorial strategies.

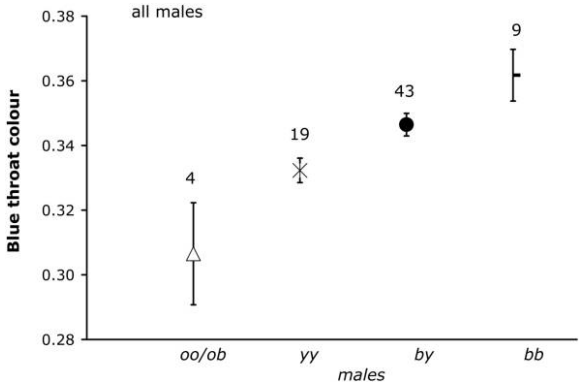

Figure 8: Mean (±SE) post–hormone treatment blue throat color as a function of male morphotype (numbers above bars refer to sample size). Blue color was normalized for light intensity by dividing by the least variant color, green intensity.

The transformation of males with a *by* genotype to a blue morphology is known to occur in conjunction with an elevation in plasma T level and endurance (Sinervo et al. 2000*b*). However, although gonadotropins, alone and in combination, increased plasma T level (fig. 3*b*), FSH increased mate attraction (fig. 3*n*), and both gonadotropins in combination increased blue color in *by* males, hormone treatment did not increase endurance of *by* males. Thus, although gonadotropins may play a role, the proximate trigger for morph transformation of *by* males remains to be determined. Alternatively, these *by* males may have been induced to undergo transformation by our laboratory conditions. The absence of O males is thought to be the inducing cue that transforms a *by* genotype from Y to B phenotype (see below). Because they lack O neighbors in laboratory terraria, we would expect them to transform and adopt a territorial strategy (Sinervo et al. 2000*b*; Sinervo 2001).

What is the stimulus triggering release of gonadotropins from the anterior pituitary that results in morph trans-

Table 4: Endurance and blue throat color as a function of treatment with the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), male morphotype (number of *b* and *y* alleles), the interaction terms between and among these variables, pretreatment covariate, and posttreatment body condition

		Endurance			Blue throat color		
Source of variation	df	MS	*F*	*P*	MS	*F*	*P*
LH	1	14,186.2	2.825	.098	1.11×10^{-6}	.007	.933
FSH	1	19,398.1	3.863	.054	7.34×10^{-5}	.476	.493
Number of *b* alleles	1	5,986.8	1.192	.279	$\mathbf{1.34 \times 10^{-3}}$	**8.667**	**.005**
Number of *y* alleles	1	1,685.2	.336	.565	$\mathbf{1.29 \times 10^{-3}}$	**8.369**	**.005**
LH × FSH	1	2,213.8	.441	.509	4.38×10^{-6}	.028	.867
b alleles × *y* alleles	**1**	**30,611.7**	**6.096**	**.017**	8.59×10^{-5}	.558	.485
LH × *b* alleles	1	10,898.2	2.170	.146	2.70×10^{-6}	.018	.895
LH × *y* alleles	1	9,662.3	1.924	.171	1.23×10^{-6}	.008	.929
FSH × *b* alleles	1	16,384.5	3.263	.076	1.37×10^{-6}	.009	.925
FSH × *y* alleles	**1**	**23,920.4**	**4.763**	**.033**	7.71×10^{-5}	.500	.483
LH × *b* alleles × *y* alleles	1	38.3	.008	.931	3.69×10^{-6}	.239	.627
FSH × *b* alleles × *y* alleles	1	140.1	.028	.868	$\mathbf{9.71 \times 10^{-4}}$	**6.299**	**.015**
FSH × LH × *b* alleles × *y* alleles	1	5,032.2	1.002	.321	1.38×10^{-4}	.893	.349
Pretreatment covariate	1	1,390.4	.277	.601	$\mathbf{1.66 \times 10^{-2}}$	**107.867**	**<.001**
Posttreatment body condition	1	3,788.0	.754	.389	$\mathbf{6.38 \times 10^{-4}}$	**4.135**	**.047**
Residual	58	5,021.6			1.54×10^{-4}		

Note: Values in boldface are significant. Number of *b* alleles is counted as follows: *bb* = 2; *by*, *ob* = 1; *yy*, *oo* = 0; number of *y* alleles: *yy* = 2; *by* = 1; *oo*, *bb* = 0.

formation and subsequent increase in reproductive success? Social interactions affect plasma CORT level in female side-blotched lizards (Comendant et al. 2003), and we hypothesize that social interactions in males, such as the disappearance of a territorial male (B. Sinervo, unpublished data), which causes a reduction in CORT level, may be the proximate cue triggering male morph change. Because social interactions were absent from our laboratory study, it is not surprising that gonadotropin treatment did not regulate plasma CORT production. We are currently assessing the fitness consequences of CORT, T, and gonadotropin (FSH and LH) interactions on male morphs in a separate series of laboratory and field experiments.

Evolutionary Implications Arising from the Proximate Control of Male Behavior

Although natural selection acts on the differences in survival and reproduction resulting from male behaviors, physiology, and morphology, an evolutionary response would in fact be manifest on the hypothalamus-pituitary axis controlling gonadotropin release. Our results suggest that there is little scope for an evolutionary response in the activational role of gonadotropins on the *oo* and *bb* throat color morphs. However, considerable flexibility in male behaviors and physiology has been observed in *by* and especially in *yy* throat color morphs. Even so, this flexibility appears in response to environmental stimuli, such as the social environment (neighboring orange males and females) and the trade-offs with immune function and hematocrit.

Our study highlights the importance of gonadotropin hormones in regulating male physiological, morphological, and behavioral traits, suggesting that future investigations into the mechanisms maintaining alternative reproductive strategies or their trade-offs should concentrate not only on T but also on gonadotropin hormones, the upstream triggers of these traits. The individual roles of these gonadotropin hormones are not as clear-cut as previously considered (e.g., Saez 1994; Simoni et al. 1997; Habert et al. 2001). Not only LH but also FSH stimulated T secretion in this study, and both hormones had effects on multiple physiological, morphological, and behavioral traits that are independent of the proposed role of FSH in sperm production. Furthermore, the roles of these gonadotropins differ with throat morph, so FSH and LH are likely to have and share multiple roles in the testes. Finally, gonadotropins increase T secretion but decrease combined innate and adaptive immunity as well as humoral immune response to DT toxins. Gonadotropins may therefore play a role, both via T and independent of T, in mediating the immunosuppression costs of sexual signals according to the immunocompetence handicap hypothesis (Folstad and Karter 1992). For this reason, gonadotropin hormones present an interesting area of future research.

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