

# Sex, drugs, and mating role: testosterone-induced phenotype-switching in Galapagos marine iguanas

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Males of many vertebrate species have flexible reproductive phenotypes and must decide before each mating season whether to adopt sneaker, satellite, or territorial mating tactics. How do males gauge their abilities against others in the population? We tested experimentally whether hormone-behavior feedback loops allow Galapagos marine iguana males to activate their three behavioral phenotypes as predicted by the relative plasticity hypothesis. Territorial males defended small mating areas and had significantly higher plasma testosterone (T) levels ( $75 \pm 11$  ng/ml) than did satellite males that roamed around territories ( $64 \pm 8$  ng/ml) or sneaker males that behaved like females within territories ( $43 \pm 11$  ng/ml). In territorial males, temporary pharmacological blockade of T slowed head-bob patrolling, decreased territory size threefold, and reduced the number of females on territories 20-fold. This supports previous data that females may gauge male attractiveness by using head-bob patrolling, here shown to be a T-dependent trait. Control-treated neighbors reacted to the weakening of T-blocked males by increasing head-bob rate fivefold and territory size 1.6-fold, and female numbers increased 2.5-fold. Unmanipulated or control-injected males remained unchanged. Behavioral effects were partly reversed after 7 days. T injections induced satellite males to establish temporary territories, even at unconventional locations. Some T-boosted satellite males suffered serious fighting injuries. T-injected sneakers left female clusters and behaved like larger satellite males that roam around territories. Thus, territorial and mating tactics are activated by T, but experimental (de-) activation at the wrong ontogenetic stage is costly: manipulated males switched phenotype but thereby lowered their access to females. We hypothesize that T levels of males that are based on early-season behavioral interactions influence a males' subsequent phenotypic role. *Key words:* activation-organization of phenotypes, mating strategies, relative plasticity hypothesis, testosterone. [*Behav Ecol*]

Two of the most important life-history decisions for vertebrates are when to mature sexually and when to switch from one reproductive phenotype into another (Charnov, 1993; Charnov et al., 2001; Ricklefs, 1991; Stearns, 1989). Some organisms make the decision about reproductive phenotypes very early in life and develop into permanently fixed reproductive phenotypes, such as sneaker males in salmon (Metcalf et al., 1995; Metcalf and Monaghan, 2001) or orange or blue morphs in tree lizards (Hews and Moore, 1995; Hews et al., 1994; Moore, 1991; see also Stamps and Krishnan, 1997). However, many other vertebrates keep reproductive phenotypes flexible throughout life and, for example, queue as satellite males to later become hotshot males (East and Hofer, 2001; McDonald 1993). Such flexibility can be particularly important in variable environments when the transition from one reproductive stage to the next often depends on social circumstances such as the availability of large males (Grant, 1990; Tokarz et al., 1998; Warner and Schultz 1992).

However, the proximate regulation of the transition is often less clear. It is generally recognized that territoriality and other aspects of male mating behavior are regulated via androgen hormones such as testosterone (T; Balthazart, et al. 1995; Greenberg et al., 1984; Wingfield and Marler, 1988). The relative plasticity hypothesis (Moore, 1991; Moore et al., 1998)

predicts that hormones such as T that are common to all reproductive phenotypes activate morphological and behavioral changes. Thus, individuals that start queuing should have low plasma levels of the hormone in question, whereas hormone levels should increase when individuals change phenotype (Greenberg and Crews, 1983; Tokarz, 1995). Indeed, it has been suggested that qualitative changes in phenotypes may be induced by mere quantitative changes in hormone levels (Adkins and Schlesinger, 1979; Lovorn et al., 2001). The alternative scenario, that different hormones are controlling different phenotypes, is apparently not realized. Support for hormonally mediated phenotypic transitions is already provided in another context of rapid phenotypic change, namely, seasonal changes in the life history of individuals (Piersma and Lindström, 1997). In temperate-zone birds, a surge in T at the beginning of the breeding season prompts the development of breeding plumage and song (Wingfield, 1984, 1985, 1994). Individuals transition from one finite life-history state (e.g., wintering) into another (e.g., reproduction), with correlated and temporarily irreversible changes in morphology and behavior (the finite state machine analogy; Jacobs and Wingfield, 2000).

Here we tested whether T can induce the transition from one mating role into another in a vertebrate with flexible phenotypes (Crews et al., 1978). T is often secreted during social challenges (Wikelski et al., 1999; Wingfield et al., 1990) and may thus naturally induce a change in aggressiveness or phenotype. Galapagos marine iguana males (*Amblyrhynchus cristatus*, Bell) show three reproductive phenotypes that are

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age- and size-dependent: sneakers, satellites, and territorials (Wikelski and Bäurle, 1996; Wikelski et al., 1996; cf. Taborsky, 1994). However, the age or size when male marine iguanas switch between phenotypes is not predetermined. Instead, males switch phenotype depending on the population structure, that is, depending on how many large males exist in the population and how many territories are available (Wikelski et al., 1996). For example, when an El Niño famine kills off most large territorial males (Laurie, 1989), the satellite males can then move up the queue and gain territories (Wikelski et al., 1996). Because such environmental events are largely unpredictable (Laurie and Brown, 1990), we hypothesized that males should be ready to switch their phenotype during each mating season.

To test whether hormonal changes are sufficient to induce a transition of mating strategy, we first measured unmanipulated T levels and subsequently conducted three experiments: (1) in territorial males, we pharmacologically blocked the actions of T by injecting the androgen receptor antagonist flutamide (Haider and Rai, 1986; Tokarz, 1987) in combination with an aromatase inhibitor 1-4-6 androstatrien-3, 17-dione (ATD). ATD stops the conversion of T into estradiol, which is an indirect pathway by which T can induce physiological and behavioral changes via aromatization (Hau et al., 2000; Schlinger, 1998; Soma et al., 1999; 2000); (2) in satellite males, we injected T to test whether territorial behavior could be activated (Wingfield et al., 1990; Woodley et al., 2000); and (3) sneaker males were also injected with T to test whether they would start behaving like satellite males. Based on the relative plasticity hypothesis (Moore, 1991), we predicted that both territoriality and mating strategy should change in manipulated individuals but remain unchanged in controls that are not adjacent to hormone-manipulated males (Smith and John-Alder, 1999). The relative plasticity hypothesis would be rejected if hormonal manipulation would not alter male phenotype, because then T would not regulate the transition between alternative male morphs. During our experiments we also monitored the stress hormone corticosterone in order to detect potential interactions between T and corticosterone (Romero et al., 1998).

## METHODS

### Background natural history on mating strategies in marine iguanas

Small, young marine iguana males look and behave like females within territories but try to sneak copulations as soon as the territorial male is temporarily out of sight. Territorial males apparently can not distinguish females from sneakers by external morphology or behavior and thus have no choice but to tolerate sneakers within their territories (Wikelski et al., 1996). Once sneakers grow older and larger, they develop longer dorsal spines and broader heads (and color change) and are chased away from territories (Trillmich, 1983; Trillmich and Trillmich, 1984). These males of intermediate size then roam around territorial clusters (satellite males) and try to forcefully grab females that leave territories, for example, en route to foraging (cf. Saether et al., 1999). Territorial males are the largest males. They fight for and establish small display areas (Partecke et al., 2002). They patrol these territories with almost continuous head-bobbing and court females by a gentle head-bob side-walk approach (Carpenter, 1966; Wikelski et al., 2001).

### Study site

We conducted our study on Genovesa Island (89°59' W, 0°19' N), where previous investigations had identified all three alternative male phenotypes (Moore M, Painter D, Woodley S, and Wikelski M, unpublished data; Wikelski and Bäurle, 1996;

Wikelski et al., 1996). The study site consists of several beaches flanked by rocky outcrops, and an intertidal peninsula that is largely used for intertidal foraging (Wikelski and Hau, 1995). Approximately 600 iguanas live at the study site, about 100 of which are currently permanently marked (Wikelski and Trillmich, 1997). Long-term population data for this colony are available (Wikelski et al., 1997). We studied iguanas continuously from 19 December 2000–1 January 2001, during the 3-week-long mating season. Individuals were noosed, measured, weighed, and marked with synthetic paint to ease observation. Paint markings did not change iguana behavior or interactions in previous studies (Audet D and Wikelski M, unpublished data). We marked 48 males (21 territorials, 13 satellites, 14 sneakers).

### Male types

Individuals were categorized as territorial if they occupied a specific area for more than 5 days (Wikelski et al., 1996) and defended this site by head-bobbing against other males. Satellite males were not consistently found at one site and generally did not head-bob. Males were designated sneakers only if they were caught in the act of sneaking copulations within a territory (Dellinger and Von Hegel, 1990).

### Territory size, behavior, and number of females

The entire study site was previously mapped to the nearest 15 cm, and landmarks such as prominent rocks, drift wood logs, or lava cracks allowed us to determine precisely the locations of the iguanas. The accuracy of our location measurements was about  $\pm 20$  cm, and thus much smaller than the differences detected owing to our manipulations (see below). Territorial boundaries were determined from interactions or from male patrolling ranges, and the size of each territory was estimated. The territorial area was calculated as the projected area in a plane. Although this is only a rough measure of territory size, it adequately describes male display areas within the uniform lava boulders of the Genovesa coastline (Partecke et al., 2002; Wikelski et al., 1996).

At the beginning of the observation period, interobserver ( $n = 3$ ) consistency was achieved by conducting behavioral observations together for 2 days (one coauthor was not conducting field work). Interobserver reliability scores were 95% or higher. Subsequently, we observed each male twice daily for 10 min by using focal observations. Repeated 10-min observation bouts appear to adequately describe the behavior of males during the breeding season (Rubenstein and Wikelski, 2003; Wikelski et al., 2001). We considered observation periods to be independent for statistical purposes when they were separated by more than 6 h (more than half a tidal cycle). During each focal observation, we counted the number of females on the territory, as well as head-bobs, number of chases of smaller males, and fights. Because we continuously observed the territories during these observations, it is very unlikely that individual females were counted more than once. We defined one head-bob sequence as a continuous head-bobbing that was not interrupted for more than 2 s. After the experimental manipulations, we also noted whether head-bobs were given during walking or standing or as the male was lying down. The number of females on each territory necessarily includes a low percentage (less than 5%) of sneaker males, which territorial males and researchers cannot distinguish from females (Wikelski et al., 1996).

We quantified the behavior of each male as the average of three focal observations per day before, during (2 days after injection), and 7 days after experimental manipulation. We then compared these averages between phenotypes

and experimental groups. For satellite and sneaker males, we could only use data before and during experimental manipulations for statistical analysis because many of these males were not encountered on day 7. As there were only three observers on the island, we could not conduct double-blind experimental tests. However, we feel that our behavioral response variables were so robust that observer bias can not account for the observed phenomena. Because of observer restrictions, only 17 of the 21 territorial males could be regularly observed; the remaining four individuals were blood sampled only (see below).

### Hormone samples and experimental manipulations

Because corticosterone levels in marine iguanas are influenced by the daily changes in tidal cycle (Woodley et al., 2003), we only took blood samples for corticosterone between 2 and 4 h after low tide. T, on the other hand, only shows a significant peak in the predawn hours (Nelson K, Rubenstein D, and Wikelski M, in preparation) and from 1200–1300 h; thus, we sampled T at any time of day avoiding these two time periods. When we caught males for experimental manipulations (as well as 2 days and 7 days after manipulation), we took a approximately a 200  $\mu$ l blood sample into a heparinized Vacutainer from their caudal vein. Blood was taken within less than 3 min after approaching the animal for capture to ensure that plasma corticosterone levels were at baseline and not affected by approach, chase and/or capture stress (Romero and Wikelski, 2001). Plasma was then separated via centrifugation and stored in 500  $\mu$ l alcohol until transport to Princeton University, where samples were frozen at  $-20^{\circ}\text{C}$  until analysis. This method of plasma storage has been validated against traditional freezing (Nelson K, Rubenstein D, and Wikelski M, in preparation; Tarlow et al., 2003). To receive independent replicates for behavioral manipulations, we selected males such that only one male in each phenotypic category was manipulated per mating aggregation (lek). We injected six randomly selected territorial males intraperitoneally (IP) with 1 ml sesame oil containing ATD and Flutamide (20 mg/ml ATD, 16 mg/ml flutamide). Six randomly chosen territorial males that were neighbors of experimental animals received a 1 ml sesame oil injection (control-injected neighbors), as did four additional territorial males that were not spatially associated with any other experimental male (control-injected nonneighbors). We used the six neighbors as control-injected animals because we wanted to detect any social feedback toward the endocrine control of behavior (see Wingfield, 1985; Wingfield and Marler, 1988). In addition, we used five randomly selected territorial males whose territorial borders were not adjacent to any of the above males as further controls (noninjected nonneighbors). We handled these males like the experimental males except that we did not inject them. We initially observed all males but, because of time limitations, were forced to abandon systematic behavioral observations on the four control-injected nonneighbor males. However, during frequent spot-checks we did not detect any changes in the behavior of these males and are thus confident that the injection procedure itself did not alter behavior.

Seven randomly selected satellite males were IP-injected with 1 mg/ml T in 1 ml sesame oil, whereas six random controls received 1 ml sesame oil only. Six randomly chosen sneaker males, caught in different territories, also received a 1 ml T in sesame oil injection. Again, six randomly selected control sneakers caught in the same territories as the experimental sneakers, received only a 1 ml sesame oil IP injection. We were unable to include unmanipulated control groups for satellite and sneaker males because this would have exceeded our observation capabilities—the Galapagos

National Park strictly limits the number of field workers at any one site. Similarly, we had to sometimes use more than one male of different phenotypic categories from the same mating cluster, which could potentially confound hormonally versus socially induced effects on behavior. However, the magnitude of the experimental change suggests that hormonal manipulations were successful per se. At the same time, we feel confident that our control-injected males represent adequate controls because such males behaved as expected from observations during years of previous research.

### Predictions for successful experimental manipulations

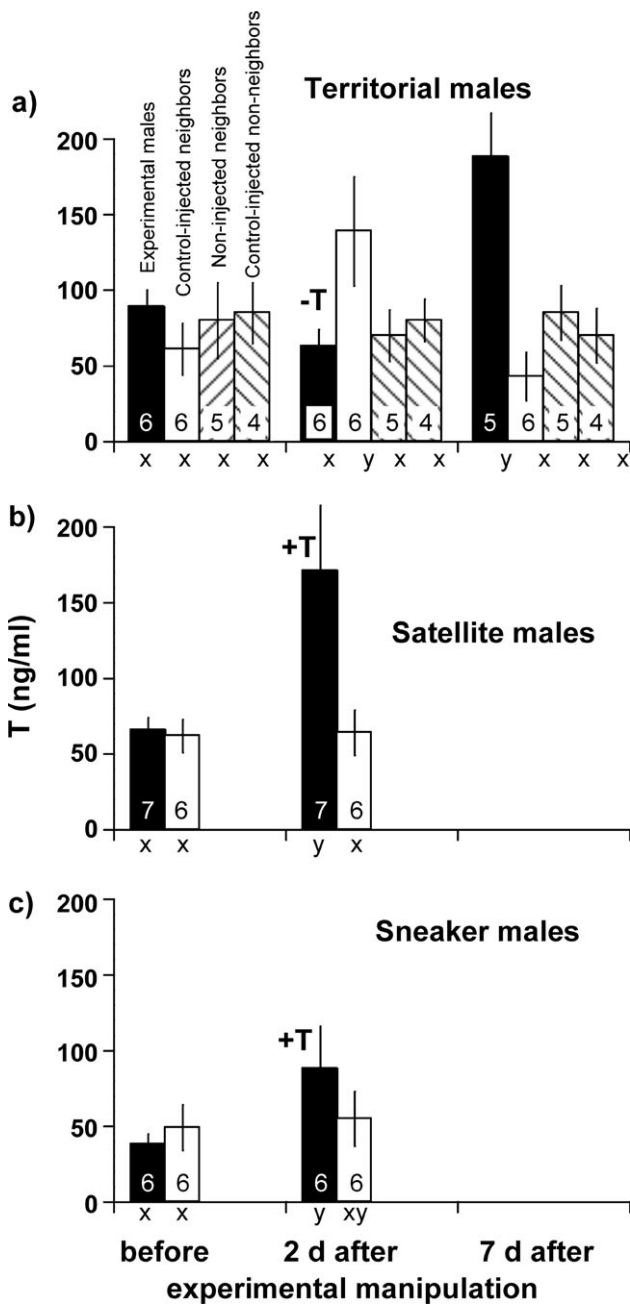
ATD/flutamide injection simultaneously blocks androgen receptors and T conversion into estradiol (Hau et al., 2000; Schlinger, 1998; Soma et al., 1999). We did not have a clear prediction for T levels after androgen receptor/aromatase blockage because the exact mechanisms of T feedback loops are not worked out in iguanids. However, we expected that blockade of T action (via ATD/flutamide) would significantly decrease head-bob rate and the number of females on the territory (Wikelski et al., 1996) and, at the same time, would induce changes in territorial interactions and territory size. We expected T-injected satellite males to start head-bobbing like territorial males and to try to establish territories (i.e., remain at and defend one particular site for several days in a row). For T-injected sneaker males, we predicted that they should leave female clusters and behave like satellite males. Thus, the number of animals within one body length, and within the radius of 1 m, should decrease. Furthermore, we expected that T-induced aggressiveness would increase stress levels of animals that are involved in social interactions, namely, increase plasma levels of corticosterone (Romero and Wikelski, 2001).

### Radioimmunoassays

Plasma levels of T and corticosterone were measured with an indirect radioimmunoassay (Wingfield and Farner, 1975). We initially separated androgens (T, androstendione [ $\text{A}_4$ ], dehydroepiandrosterone [DHEA], and dihydrotestosterone [DHT]) for 20 samples on a chromatography column because the T-antibody cross-reacts with DHT to a low extent. As DHT occurred at very low levels compared with T (5% of T levels), and  $\text{A}_4$  and DHEA also only occurred in insignificant amounts, we subsequently conducted direct radioimmunoassays without column separation on all samples, and we describe those results as plasma T levels (Wikelski et al., 2000). For the radioimmunoassay, trace label (20  $\mu$ l) was added to all alcohol samples to determine recovery values ( $82 \pm 5\%$ ). We treated the alcohol samples as if they were plasma samples and reconstituted the remainder of a methylene chloride extraction with PBS buffer to the original amount of the collected plasma. Then, we used 5  $\mu$ l of this reconstituted plasma for T assays and 50  $\mu$ l for corticosterone assays. Water blanks were taken through the entire assay procedure, and all were below our detection limit. The accuracy of the hormone standards was  $\pm 7.4\%$  for T and  $\pm 8.6\%$  for corticosterone. Intra-assay variation was 3.2% for T and 4.8% for corticosterone. Only one assay was run for each hormone. Assay sensitivity was at  $0.05 \pm 0.06$  ng/ml for T and 1.8 ng/ml for corticosterone (Wikelski et al., 2000). All samples were above the detection limit.

### Statistical analysis

Data were analyzed with SPSS for Windows 10.0, and two-tailed tests were used throughout. We tested the distribution



**Figure 1**  
 Plasma testosterone (T) levels as measured via radioimmunoassay in marine iguana males on Genovesa island. Data show mean  $\pm$  SE for territorial males (a), satellite males (b), and sneaker males (c) before manipulation, during manipulation (2 days after injection), and after manipulation (7 days after injection). Bars depict male classes as indicated. Inset numbers indicate sample sizes. Letters below bars indicate statistically different means (different letters) between the groups in each graph and between times as determined by post hoc tests after using repeated-measures general linear models.

of data before conducting statistical analyses and used repeated-measures general linear models (RM-GLMs) with Scheffe's post-hoc tests (indicated in figures) to determine statistical differences between treatments and/or time periods. We used post-hoc pairwise tests to analyze baseline T levels between male types. We present the power of tests in parentheses after *p* values. When data were skewed (e.g., the number of females per territory) they were log-transformed

for statistical analysis and we present back-transformed means with confidence limits in the figure. Significance was accepted at the  $\alpha = 0.05$  level. Data are reported as mean  $\pm$  SE. Sample sizes for hormone analyses differ between groups as indicated in Figures 1 and 2 because not all males could be recaptured and bled at all times. We substituted the group mean for one such missing data point each in Figures 1 and 2, panels a through c.

**RESULTS**

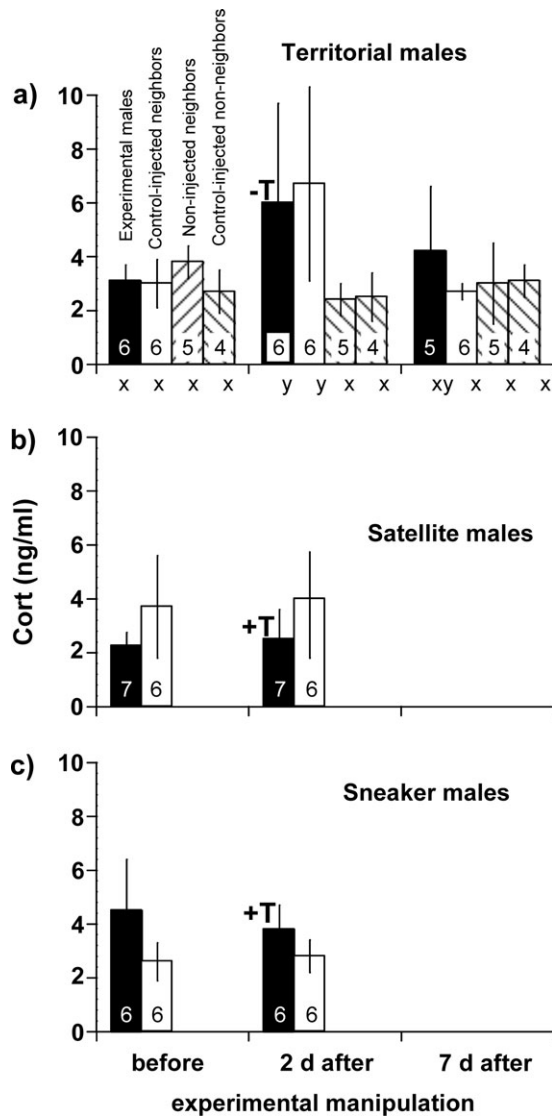
T levels of males at first capture (i.e., before experiments were performed) were highest in territorial males, lower in satellite males, and lowest in sneaker males (ANOVA,  $F_{2,46} = 7.3, p = .002 [0.9]$ ).

**Territorial males**

In territorial males before manipulation, mean plasma T levels were  $75 \pm 11$  ng/ml. T remained unchanged in ATD/flutamide injected males ( $63 \pm 11$  ng/ml) but increased to  $139 \pm 36$  ng/ml in control-injected neighbors 2 days after manipulations (Figure 1a). After 7 days, T increased dramatically in ATD/flutamide-injected males, presumably as a result of reduced negative feedback and the challenge to regain territorial space lost during the experimental manipulation. There was no change in T levels of noninjected or control-injected neighbor or nonneighbor males (RM-GLM for all groups,  $F_{3,20} = 52, p < .001$ ; within groups,  $p = .17 [0.42]$ ; group  $\times$  manipulation,  $p = .02 [0.63]$ ; between groups,  $p = .32 [0.37]$ ).

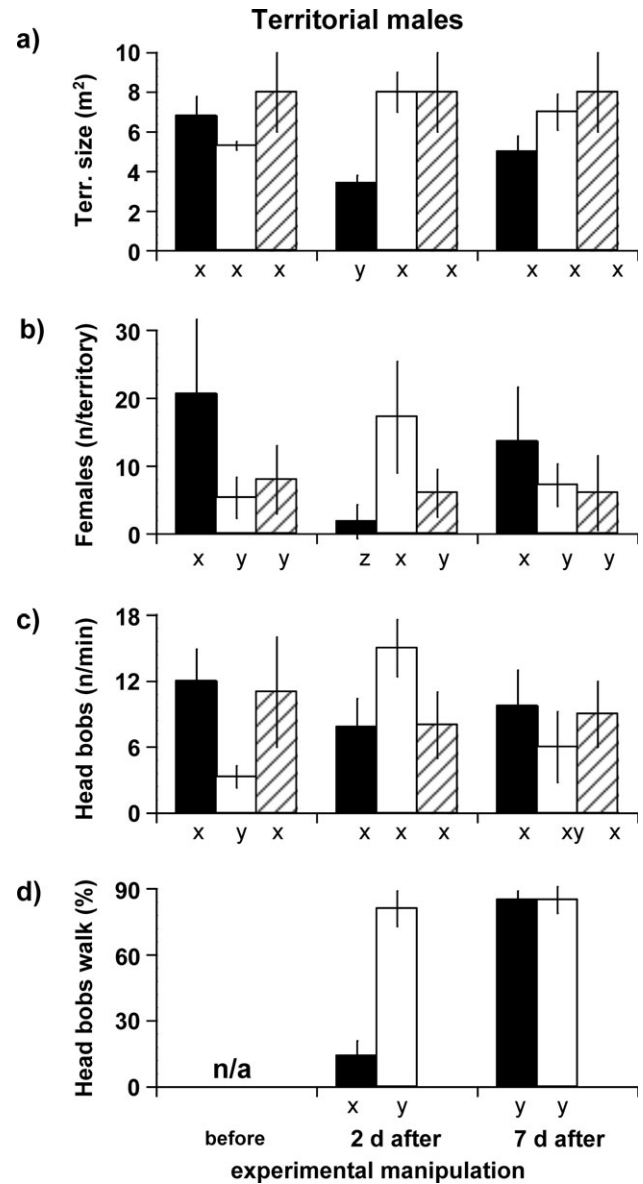
Baseline corticosterone levels were initially low in all individuals (cf. Romero and Wikelski, 2001). Corticosterone levels increased in T-blocked and control-injected neighbor males, but not in non- or control-injected nonneighbors (RM-GLM,  $F_{3,20} = 14, p < .001$ ; within groups,  $p = .04 [0.81]$ ; group  $\times$  manipulation,  $p = .52 [0.58]$ ; between groups,  $p = .26 [0.79]$ ) (Figure 2). Males with high corticosterone levels (during the T manipulation) had more territorial interactions independently of the treatment (ANOVA,  $F_{3,46} = 18, p < .02$ ; experimental period,  $p = .75 [0.07]$ ; group,  $p = .24 [0.21]$ ; period  $\times$  group,  $p = .79 [0.08]$ ; territorial interactions,  $p < .001 [0.95]$ ). An increase in corticosterone could have been caused by the stress of injection and handling alone. However, as no corticosterone increase was observed in satellites and sneakers (see below), we conclude that male-male interactions were the most likely cause of corticosterone increases.

Although a potential physiological indicator of T blockage (i.e., T increase in the plasma as result of negative feedback) was slow or nonexistent (see above), the behavioral effects of T blockage were almost immediate. The territory size decreased by about half 2 days after injection in T-blocked males. At the same time, territory size increased 1.6-fold in control-injected neighbor males, whereas it remained stable in noninjected nonneighbors. Territory sizes returned to premanipulation levels at the end of the experimental period (7 days after injection: RM-GLM,  $F_{2,14} = 281, p < .001$ ; within groups,  $p = .23 [0.22]$ ; group  $\times$  manipulation,  $p < .001 [0.98]$ ; between groups,  $p = .03 [0.71]$ ) (Figure 3a). The number of females showed a similar trend, but the decrease in female numbers in T-blocked males was much steeper (11-fold) than expected from a mere decrease in territory size (twofold). T-blocked males had higher numbers of females and faster rates of head-bobs from the beginning, because by chance we picked more central males for manipulations than often are preferred by females (Wikelski et al., 2001). Although T-blocked males lost females, neighboring control-injected males gained females, and noninjected nonneighbors showed no significant



**Figure 2**  
Plasma corticosterone (CORT) levels as measured via radioimmunoassay in marine iguana males on Genovesa Island. Data presentation as in Figure 1; bars depict male classes as indicated.

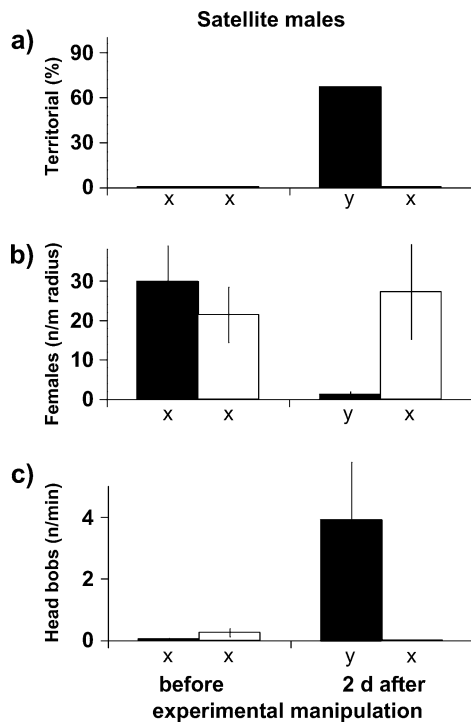
change in female numbers (RM-GLM,  $F_{1,14} = 9$ ,  $p = .01$ ; within groups,  $p = .27$  [0.19]; group  $\times$  manipulation,  $p = .04$  [0.54]; between groups,  $p = .55$  [0.14]) (Figure 3b). The number of head-bobs was initially lower in control-injected neighbor males (Figure 3c), but increased 2 days after injection, possibly because such males won territorial encounters with T-blocked males and gained territories and females. There was no significant change in head-bobs in noninjected control non-neighbor males (RM-GLM,  $F_{3,14} = 96$ ,  $p = .001$ ; within groups,  $p = .8$  [0.06]; group  $\times$  manipulation,  $p = .02$  [0.78]; between groups,  $p = .68$  [0.10]). Although the head-bob rate did not change in T-blocked males, the proportion of time they conducted head-bobs during walking (compared with while sitting or laying) was much lower 2 days after injection, compared with controls. Control-injected neighbor males, on the other hand, increased their head-bob rate fivefold and consistently kept their head-bob walking rate at 85%. The increase in head-bobbing is consistent with an increase in T during the same time. However, we had not realized the importance of head-bob walk versus all head-bobs during the



**Figure 3**  
Change in characteristics of territorial males during the experiment: a) territory size (a), number of females (b), number of head-bobs per minute (c), and percentage of head-bobs (d) conducted during walking versus sitting or laying. Data presentation as in Figure 1, except that noninjected nonneighbors are omitted (see text). Data for head-bob walking before experimental manipulation are not available (n/a).

pre-experimental phase and thus have no data during this time period or on noninjected nonneighbors (RM-GLM,  $F_{1,10} = 260$ ,  $p < .001$ ; within groups,  $p < .001$  [1.0]; group  $\times$  manipulation,  $p < .001$  [1.0]; between groups,  $p = .004$  [0.94]) (Figure 3d).

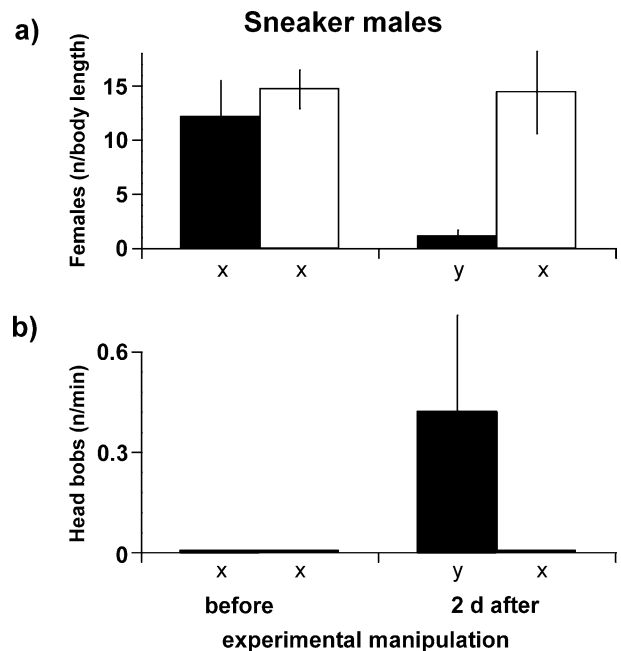
Of the six T-blocked males, three lost their territory temporarily, whereas no control (injected or noninjected) male lost his territory (one control-injected neighbor male temporarily abandoned his territory for several hours). It should be noted that all three manipulated males that lost their territory were forcefully evicted by neighbors, whereas the control-injected male apparently left for several hours on its own. Because the loss of territories was not a completely binary variable, we did not test for significant differences between groups.



**Figure 4**  
Change in characteristics of satellite males during the experiment: number of head-bobs per minute (a), percentage of males that were territorial (b), and number of females within an approximately 1-m radius around the satellite males (c). Data presentation as in Figure 1.

#### Satellite males

Plasma T levels in satellite males before manipulations were at  $64 \pm 8$  ng/ml and remained unchanged in control males, but in T-injected males increased to levels found in territorial males during a social challenge (RM-GLM,  $F_{1,11} = 38$ ,  $p < .001$ ; within groups,  $p = .04$  [0.51]; group  $\times$  manipulation,  $p = .05$  [0.48]; between groups,  $p = .09$  [0.39]) (Figure 1b). Thus, we considered our manipulation successful in increasing plasma T levels to approximately the level of territorial males, which would be the next developmental stage for satellite males. There was no change in corticosterone baseline levels among groups (RM-GLM,  $F_{1,11} = 14$ ,  $p = .005$ ; within groups,  $p = .91$  [0.05]; group  $\times$  manipulation,  $p = .99$  [0.05]; between groups,  $p = .4$  [0.12]) (Figure 2b). Four of six T-injected satellite males established territories (one for 2 days, two for 3 days, and one for 7 days) (Figure 4a). However, these territorial sites were generally not ideal for attracting females: one was among sea lions resting on a beach, another one was in the intertidal zone, and two were on the sandy areas surrounding rocky territorial areas. Consequently, none of these satellite males attracted females to stay within their territories (RM-GLM on average number of females per male,  $F_{1,11} = 117$ ,  $p < .001$ ; within groups,  $p = .07$  [0.46]; group  $\times$  manipulation,  $p = .02$  [0.74]; between groups,  $p = .09$  [0.40]) (Figure 4b). None of the control-injected males defended a territory. All seven T-injected satellites started to head-bob against other males and approached females using a head-bob side-walk courtship, which is otherwise only used by territorial males (RM-GLM,  $F_{1,11} = 5$ ,  $p = .04$ ; within groups,  $p = .08$  [0.43]; group  $\times$  manipulation,  $p = .04$  [0.48]; between groups,  $p = .09$  [0.40]) (Figure 4c). Thus, all T-injected males changed their courtship mode to that performed only by territorial males. One of the control-injected males also



**Figure 5**  
Change in characteristics of sneaker males during the experiment: number of head-bobs per minute (a) and number of females within an approximately 1-m radius around sneaker males (b).

performed several head-bobs, but none showed head-bob walking courtship display.

#### Sneaker males

Premanipulation plasma T levels in sneaker males were  $43 \pm 11$  ng/ml and remained unchanged in control males, but in T-injected males were elevated to levels normally found in satellite males (RM-GLM,  $F_{1,10} = 22$ ,  $p = .001$ ; within groups,  $p = .02$  [0.73]; group  $\times$  manipulation,  $p = .04$  [0.54]; between groups,  $p = .66$  [0.07]) (Figure 1c). Thus, our manipulation successfully increased plasma T levels to approximately the level of satellite males, which would be the next developmental stage for sneaker males. There was no change in corticosterone levels among groups (RM-GLM,  $F_{1,10} = 20$ ,  $p = .001$ ; within groups,  $p = .78$  [0.06]; group  $\times$  manipulation,  $p = .58$  [0.08]; between groups,  $p = .36$  [0.14]) (Figure 2c). T-injected and control-injected sneakers were initially captured while resting in female groups of similar sizes. However, T-injected sneakers left their female clusters, whereas control-injected animals did not leave their group (RM-GLM,  $F_{1,10} = 13$ ,  $p < .001$ ; within groups,  $p = .09$  [0.39]; group  $\times$  manipulation,  $p = .03$  [0.69]; between groups,  $p = .13$  [0.40]) (Figure 5a). Two of five T-injected sneaker males started to head-bob, whereas none of five control sneakers did (Figure 5b).

#### DISCUSSION

T manipulation induced a transition in mating tactics in male marine iguanas. Reproductive phenotypes therefore appear to be flexibly regulated by hormones during adult life. Thus, our data lend experimental support to Moore's (1991; Moore et al., 1998) relative plasticity hypothesis, which states that flexible changes in phenotypes during adult life are regulated by hormones in a way similar to organizational changes during sexual differentiation in early ontogeny.

In marine iguanas, reproductive tactics are largely age-dependent: young males are small and unable to gain territories necessary for access to females and thus try to sneak copulations inside a territory. When sneakers become larger (as they grow older), they are expelled by territorial males and roam around territories as satellites in search for females. Only large males can become territorial and then need to continuously head-bob patrol their territories (Wikelski et al., 2001). The relative plasticity hypothesis predicts that under these conditions territorial males should have the highest levels of T, sneaker males the lowest. We initially confirmed this prediction by measuring hormone levels in all three male types at the start of the present study. We subsequently increased T levels via T injections in sneakers and satellites, aiming to mimic high T levels of territorial males (without, however, accounting for circadian differences in T levels, which could have influenced the results). T injections resulted in a temporary but incomplete switch in phenotype: satellite males became territorial but in unsuitable places, whereas sneaker males head-bobbed but did not become territorial. The small body size of both male types relative to territorial males could ultimately be responsible for an incomplete phenotypic switch. Two physiological reasons why small males did not immediately become territorial males could also be that either levels of hormone binding proteins differ between male types or the number of androgen receptors differ. Binding proteins influence the effect of physiologically active androgen hormone (see Breuner et al., 2003). The observed difference in T levels between satellite and sneaker males after injection of the same amount of T (Figure 1b,c) may be related to differences in hormone binding protein levels. It should also be noted that unmanipulated T levels in satellite males do not differ significantly from those of loser (poor quality) territorial males (Partecke et al., 2002) (Figure 1a,b). Similar T levels are in fact expected for those two groups because the separation into territorial losers and satellites is somewhat arbitrary as some males naturally switch between territorial and satellite tactic or vice versa within a season.

T-blocked territorial males immediately decreased their activities for several days after injection, suggesting that the pharmacological treatment worked (Tokarz, 1987). The exact mechanism of T blockade is still unclear: most likely, flutamide blocked T-receptors whereas ATD inhibited aromatase, thus preventing T-effects via conversion into estrogens (Soma et al., 1999). In any case, as the effect of the T blockade wore off in experimental males (as T-blocked males became active again), their T increased beyond previous levels, perhaps as a response to the social challenge from the control males. This interpretation is supported by the increase in corticosterone levels in territorial males during the manipulation, which could be owing to the immediate stress of fighting against territorial neighbors (Denardo and Sinervo, 1994; Eibl-Eibesfeldt, 1964) (Figure 2a). However, an alternative explanation for enhanced corticosterone levels would be that increased corticosterone was needed to support increased activity. It is interesting to note that corticosterone only increased in territorial males, which in turn are unable to leave their territory. Satellite males, on the other hand, did not show an increase in corticosterone after T injections, possibly because they can leave the area if social interactions become too stressful (Wingfield et al., 1998).

Territorial males may sustain strenuous activities by keeping their T levels high. If T levels and activity pattern drop, males apparently lose territorial space and—more importantly—females (Clutton-Brock et al., 1992; Cooper and Vitt, 1993; Westcott, 1997). Interestingly, females were less inclined to associate with less active T-blocked males (Wikelski et al., 2001),

which is consistent with the hypothesis that female choice may be directly associated with a condition-dependent, intersexual advertisement signal (Bradbury and Gibson, 1983; Bradbury et al., 1985; Edwards and Jones, 2001; McDonald et al., 2001). Our experimental manipulation therefore allowed us to distinguish between apparent phenotypic male quality (such as head-bob rate) and territorial quality (such as temperature; Wikelski et al., 1996; see Carothers, 1984). The fact that females choose male quality by using phenotypic indicators (head-bob patrolling; Decourcy and Jenssen, 1994) confirms earlier suggestions that territorial quality is of minor importance in mating decisions in this species (Wikelski et al., 1996, 2001).

In our experiment, an increase in T levels induced territorial behavior but did not pay off for satellite males because they got into damaging fights (data not shown) and wasted energy on head-bobs and territorial tenure (cf. Klukowski et al., 1998) without obtaining female visits (Marler et al., 1995). Likewise, sneaker males lost access to potential mating partners when they left territories after T injection (see Greenberg and Crews, 1983). Thus, for all male mating types, a manipulation of T levels apparently resulted in decreased mating success—a potentially strong fitness cost of having an inappropriate amount of T for their current social status (Greenberg et al., 1984; Marler and Moore, 1988). In addition, it is conceivable that T inflicts additional costs beyond energetic ones, such as in immune responses during parasite infections (Veiga et al., 1998). We did not test whether increased T levels would boost the reproductive success of territorial males, as it does—at least temporarily—in several animal species (see Beletsky et al., 1989; Ketterson and Nolan, 1992; Sinervo et al., 2000).

At the beginning of each reproductive season, each marine iguana male has to adjust its reproductive phenotype to the current demographic situation. We suggest that the feedback of social interactions into T secretion could be a decisive aspect during such adjustments (Sinervo et al., 2000). Our data allow some preliminary insight: blockade of T in territorial males increased T in neighboring control-injected males. One plausible interpretation for this T increase is a positive feedback from winning the territorial challenge against the neighboring (pharmacologically castrated) male (Wingfield et al., 1990). However, males may not switch immediately and/or completely between phenotypic roles because these transitions also involve morphological and other physiological changes that require time. For example, the activation of a specific phenotypic behavior may involve a longer-term reorganization of neural circuits and changes in muscle physiology (Fusani et al., 2000).

Ultimately, we suggest that reproductive phenotypes remain flexible because the relative numbers and body sizes of males change from year to year, depending on population density and previous environmental perturbations like El Niño (which cause massive mortalities, particularly for large territorial males; Wikelski and Romero, 2003; Wikelski and Trillmich, 1997). In such variable environments, the endocrine system can provide a powerful tool for transferring information about the current social environment into physiological signals that in turn determine phenotypic states of organisms (Ketterson and Nolan, 1992; Oliveira et al., 2001; Wikelski and Ricklefs, 2001).

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