

## Testosterone Mediates Mating Success in Greater Prairie-Chickens

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**Abstract.** Testosterone plays a key role in influencing behaviors that enhance male breeding success, but elevated testosterone levels can also reduce immunocompetence and survival. In socially monogamous species, males with higher levels of circulating testosterone experience advantages during both female choice and male–male competition activities. The role of testosterone influencing male mating success in lek-mating systems is largely unknown. Over three years, we quantified natural and experimental levels of testosterone among Greater Prairie-Chickens (*Tympanuchus cupido*) at five leks. Our project had three objectives: (1) to quantify natural variation in testosterone in blood plasma and to determine whether testosterone levels are correlated with male traits and mating success; (2) to experimentally elevate testosterone levels to determine causal relationships between testosterone levels, male traits, and mating success; and (3) to analyze return rates to determine if increased levels of circulating testosterone are costly in terms of annual survival. Using a before–after control-impact (BACI) experimental design, changes in aggression, display behavior, territory size and location, mating success, and survival were compared between males with testosterone

implants (15 males) and sham implanted controls (13 males) over two years. Natural level of testosterone was a strong predictor of male mating success, but testosterone level in unmanipulated males was not related to any morphological, behavioral, or territorial traits considered. Comb area, tarsus length, and distance to the center of the lek were also significant predictors of male mating success. Similar results were also found in our field experiment: Testosterone-implanted males tended to gain more copulations than sham-implanted males, although the difference was not significant. In addition, the T- and sham-implanted males did not vary with respect to their behavior or territory attributes. Neither naturally nor experimentally elevated T levels were related to annual return rates of males. Our project is one of the first manipulations of testosterone levels in a lek-mating bird. Future work should search for links between testosterone and male attributes in lek-mating systems because females are unlikely to be able to assess testosterone levels directly.

**Key Words:** Galliformes, grouse, lek-mating system, mate choice, mating success, multinomial discrete choice models, physiological causes, sexual selection.

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Life history trade-offs are ubiquitous in organismal biology, but the proximate mechanisms driving variation in life history trade-offs are poorly understood (Ricklefs and Wikelski 2002). According to the immunocompetence handicap hypothesis, testosterone (T) enhances male sexual traits but has immunosuppressive effects (Folstad and Karter 1992, Mougeot et al. 2004), leading to a negative correlation between circulating T and annual survival (Ketterson and Nolan 1999, Reed et al. 2006). Nevertheless, if T increases reproductive success but decreases survival, natural selection should confer an advantage to individuals who can balance these two fitness components.

Trade-offs between mating and parental effort create a continuum among avian mating systems ranging from social monogamy, to facultative polygyny, to promiscuity. In socially monogamous species of temperate songbirds, circulating T in males peaks during pair formation and drops during brood rearing (Ketterson et al. 1992, Ketterson and Nolan 1999). In facultatively polygynous species, males have high T levels during pair formation and brood-rearing, leading to increased mating effort and reduced parental effort (Wingfield 1984). Lek mating is a type of promiscuous mating system where males perform aggregated displays at a lek site, which females visit to obtain copulations (Bradbury 1981). Only females provide parental care, so T is predicted to remain high in lek-mating males during periods of territory establishment and female visitation (Wingfield et al. 1990). However, the available data from lek-mating grouse and manakins suggest that T is elevated only during the short period of peak female visitation to the lek during the mating season (Alatalo et al. 1996, Wikelski et al. 2003). Compared to the abundance of research in monogamous and polygamous mating systems (Beletsky et al. 1989, 1990; Ketterson and Nolan 1999; Westneat et al. 2003), the role of T in lek-mating systems is largely unknown.

T may influence sexual selection through female choice or male–male competition. Because T peaks during periods of high mating activity, T-mediated traits may be used by females to select a mate. Female choice for T-dependent traits could arise via indirect benefits, such as good genes for her offspring (Andersson 1994). Studies in galliformes have found T-dependent

traits are often associated with female choice, such as large fleshy head ornaments (Zuk et al. 1995a, 1995b), high-quality territories (Moss et al. 1994), and more vigorous courtship displays (Mateos and Carranza 1999). Red Jungle Fowl (*Gallus gallus*) with larger combs were more likely to attract multiple mates (Parker and Ligon 2003). T could also affect male fitness through male–male aggression, and T-mediated male ornaments or behaviors often predict the winner of paired tests (Ligon et al. 1990, Hagelin 2002).

T-implants have been used to determine experimentally how sex hormones influence male attributes, female choice, and male–male competition. T-implanted males displayed more frequently and attracted more females than sham-implanted control males (Wingfield 1984, Ketterson et al. 1992, Hill et al. 1999). T-implanted male Ring-necked Pheasants (*Phasianus colchicus*), with harem-defense polygyny, were more aggressive, had higher social rank, and increased frequency of male–male interactions (Briganti et al. 1999). T-implanted males of monogamous Red Grouse (*Lagopus lagopus scoticus*) had higher display rates and territory sizes compared to control males (Moss et al. 1994; Mougeot et al. 2003a, 2003b).

Most T-implant studies have been conducted on monogamous, territorial songbirds where T can simultaneously affect mating effort and parental care of young. Only two studies have been conducted on lek-mating males to determine the effect of T on mating effort and success while avoiding the potentially confounding effects of T on parental care. T-implanted lek-mating Golden-collared Manakins (*Manacus vitellinus*) increased rates of several display behaviors compared to sham-implanted controls during the non-breeding season (Day et al. 2006). Aggressive, display, and territory attributes of T-implanted males of the lek-mating Sharp-tailed Grouse (*Tympanuchus phasianellus*) remained unchanged relative to unmanipulated control males, but lek attendance was higher for T-implanted males (Trobec and Oring 1972).

In a three-year study, we examined how male traits, mating success, and survival vary with natural and experimentally manipulated levels of T in male Greater Prairie-Chickens (*T. cupido*), a lek-mating bird. Our objectives were: (1) to quantify natural variation in circulating

T levels during the breeding season and determine whether T levels correlate with male traits and mating success; (2) to experimentally manipulate T to determine causal relationships between T and male traits and mating success; and (3) to analyze annual return rates to determine if high levels of T are costly. Our project is one of the first experimental field studies of the interacting effects of T, male attributes, mating success, and survival in a lek-mating bird. We predicted that naturally and experimentally elevated T would have a large positive impact on male aggression, display, and mating success in prairie chickens. We expected comb size to be positively correlated with T, similar to previous studies on Red Jungle Fowl (Zuk et al. 1995a, 1995b). We did not expect T to affect other aspects of male plumage, because male plumage ornamentation is a primitive character in Galliformes, with the cryptic female plumage being a derived estrogen-dependent character (Kimball and Ligon 1999). Males in the lek center tend to obtain more copulations than those on the periphery (Hamerstrom and Hamerstrom 1973, Schroeder and Robb 1993), and we expected high T to be negatively correlated with territory distance to the center of the lek. Finally, we expected T to be negatively correlated with annual return rates according to the immunocompetence handicap hypothesis (Folstad and Karter 1992).

## METHODS

We observed Greater Prairie-Chickens at lek sites during the breeding season between mid-March and mid-May, 2004–2007. All leks were located on cattle-grazed pastures in Riley and Geary Counties in northeastern Kansas (39°05' N, 96°34' W). Four leks were observed in 2004–2005, and we added a fifth lek in 2006. All leks were surveyed in 2007 to determine annual return rates from 2006 to 2007.

### Trapping and Morphometrics

We used walk-in funnel traps and drop nets to trap males and females at lek sites during the breeding season (Hamerstrom and Hamerstrom 1973, Silvy et al. 1990, Schroeder and Braun 1991). Males were given a unique combination of colored leg bands and tail markings

using nontoxic permanent markers to aid in individual identification. Seven morphometric measurements were recorded during handling: body mass ( $\pm 1$  g), three linear measurements of body size (lengths of tarsus plus the longest toe, wing, tail;  $\pm 1$  mm for all), and two sexually dimorphic ornaments (comb area, measured as length  $\times$  height,  $\pm 1$  mm<sup>2</sup>; length of pinnae feathers behind head erected during display,  $\pm 1$  mm). We determined age-class as second-year (SY) or after-second-year (ASY) from the shape, coloration, and wear of the outermost two primaries (numbers 9 and 10; Schroeder and Robb 1993).

### Behavioral Observations

At lek sites, male Greater Prairie-Chickens perform ritualized courtship displays and territorial behaviors (Schroeder and Robb 1993, Nooker and Sandercock 2008). A low “boom” vocalization and short “flutter-jump” flights are associated with courtship displays. Territorial behaviors include facing adjacent males and physical combat. The behavior of individual males was recorded with continuous 10-min focal observations from blinds placed  $\sim 6$  m from the edge of the lek. Time spent in each of three main behavior categories (display, fighting, other) was calculated. Tallies were taken of the number of fights, males approached, boom vocalizations, and flutter-jump displays. Female presence or absence on the lek during the observation period was recorded for every focal observation. Opportunistically throughout the morning, position of males performing courtship displays or engaged in territorial disputes was recorded relative to grid stakes placed at 6-m intervals. Copulations were also recorded as they occurred and were deemed “successful” if the females shook their wings vigorously and departed the lek shortly after copulation (Schroeder and Robb 1993). To ensure repeatability among observers, pairs of observers conducted independent behavioral observations of the same male at the same time. These behavioral observations were highly correlated (PC1:  $r^2 = 0.85$ ,  $F_{1,54} = 315.8$ ,  $P < 0.001$ ; PC2:  $r^2 = 0.79$ ,  $F_{1,54} = 201.4$ ,  $P < 0.001$ ; see Statistical Analyses section for description of PC scores), so we did not make any adjustments for observer bias. Nevertheless, observers were rotated among the leks and males, and all

observations were conducted within three hours of sunrise on days without rainfall.

### Testosterone Sampling

Because handling stress can affect circulating T, leks were monitored from a blind to determine how long each male or female was in a trap. Birds were not left in the trap for more than 30 min. We collected a blood sample immediately following the bird's removal from the trap or drop net and placed the sample on wet ice. Within three hours, the blood sample was centrifuged at 14,000 rpm for 5 min to separate the blood plasma from the red blood cells. Blood plasma was collected with a pipette, transferred to a new tube, and frozen at  $-20^{\circ}\text{C}$  until it could be transferred to a  $-70^{\circ}\text{C}$  freezer.

We measured plasma T concentrations in each blood sample using the Salimetrics protocol and commercially available kit for salivary T enzyme immunoassay (EIA; Cat. #1-2402, Salimetrics LLC, State College, PA; Washburn et al. 2007). All plasma samples (15  $\mu\text{L}$ ) were diluted 1:10 with assay dilutant (135  $\mu\text{L}$ ). We conducted standard assay validations, including assessment of parallelism, recovery of exogenous T, intra- and inter-assay precision, and assay sensitivity (Jeffcoate 1981, Grotjan and Keel 1996, O'Fegan 2000) to confirm that T concentrations in prairie chicken plasma were measured accurately and precisely. Serial dilutions (1:1, 1:2, 1:4, 1:8, and 1:16) of two pooled plasma samples (low and high, where each pooled sample consisted of plasma from three individuals) yielded a displacement curve that was parallel (test of equal slopes,  $P > 0.6$ ) to the standard T curve, which indicated linearity under dilution (Jeffcoate 1981). Mean recovery of exogenous T (range 38.4–240 pg/mL; levels chosen to correspond with expected plasma T levels from actual samples) was added to low ( $109.9 \pm 1.1\% \pm \text{SE}$ ,  $n = 6$ ) and high pooled plasma samples ( $108.4 \pm 0.7\% \pm \text{SE}$ ,  $n = 6$ ). Acceptable recovery of exogenous T (within 90–110%) verified accurate measurement throughout the working range of the assay, and demonstration of parallelism suggested no sample matrix effects (Jeffcoate 1981, Grotjan and Keel 1996, O'Fegan 2000). We used the low and high controls from the kit and analyzed them in each of the assays. Inter-assay variation was calculated from these two controls by averaging the coefficient of variation (CV) of

replicate wells from 20 randomly chosen samples. Inter-assay variation for six assays was 9.1% and average intra-assay variation was 4.7%. The sensitivity of this assay is 1.0 pg/mL. Estimates of plasma T concentration were  $\log_{10}$ -transformed to normalize the data.

### Testosterone Implant Experiment

To test for a link between T and male behavior, we used a Before–After–Control–Impact design (BACI; Conquest 2000). Behavior, territory attributes, and mating success were compared before and after application of subcutaneous implants (35-mm lengths of silastic tubing; Dow Corning, inner diam = 1.47 mm, outer diam = 1.95 mm; sealed with silastic glue) filled with T (30 mg of testosterone propionate; Sigma-Aldrich, St. Louis, MO) or left empty (sham implants). Similar implants last up to 5–6 weeks in Red Grouse (Trobec and Oring 1972; Moss et al. 1994; Mougeot et al. 2003b, 2004; F. Mougeot, pers. comm.). The incision was closed with “liquid bandage” to prevent infection (Band-Aid brand).

The breeding season began in mid-March, when males start regularly attending the lek site. Female visitation to lek sites commenced around 25 March and peaked at 10 April, with a second, smaller peak around 5 May, presumably corresponding to reneating attempts (McNew et al., this volume, chapter 19 unpubl. data). We divided the breeding season into three periods: before the peak of female visitation (mid-March–3 April); peak female visitation and target dates for T implants (4–7 April); and post-peak female visitation (18 April–mid-May).

Only unsuccessful males received T or sham implants. Banding and behavioral observations beginning in mid-March were used to determine which males were successful and which were unsuccessful. Males were considered “successful” if they held territories that were completely surrounded by other males, fought often with many adjacent males, and had obtained copulations previously in that season or in a previous year. Males were considered “unsuccessful” if they held territories at the edge of the lek (at least 25% of territory not shared with another male), engaged in few fights, and obtained no copulations prior to implant that season or in a previous year. Unsuccessful males were captured using drop nets between 4 and 17 April and alternately assigned

to the T or the sham treatment. The effects of the treatment were monitored by comparing blood serum analyses, behavioral observations, and territory size and location of T- and sham-implanted males before and after treatment. In addition, a subset of T-implanted males were recaptured 2–3 weeks after implantation, their implants were removed, dried in a desiccator at room temperature for 24 h, and weighed to determine the amount of T remaining.

### Statistical Analyses

Statistical analyses were conducted in JMP IN (ver. 4.0.4, SAS Institute, 2001), except where otherwise noted. Sample sizes varied among analyses because it was not possible to measure every attribute for all males. Descriptive statistics are presented as  $0 \pm 1$  SD unless otherwise indicated.

Eleven of 15 pairwise comparisons among the six behavioral variables (% time displaying, % time fighting, and number of boom vocalizations, flutter-jump displays, fights, and males approached) were correlated ( $|r| > 0.44$ ;  $P < 0.01$ ). Thus, we used principal components analysis (PCA) to obtain two principal components of behavior that were statistically independent of each other (Table 14.1), and retained two principal components (eigenvalues  $\geq 1$ ) for use in our analysis (Kaiser 1960). Female presence at the lek has a large effect on male behavior in lek-mating grouse (Höglund et al. 1997, Nooner and Sandercock

2008). To control for female presence, principal component scores were averaged separately for each male for observations when females were either present or absent from the lek.

Territorial positions of males were plotted in ArcView (ver. 3.3; Environmental Systems Research Institute, Inc., St. Charles, MO). Using the Animal Movement extension (Hooge and Eichenlaub 2000), we obtained 95% kernel estimates of territory size from positions of each male and lek size from the pooled positions of all males ( $\pm 0.1$  m<sup>2</sup>). Centroids of the 95% kernel estimates were determined using the XTools extension (Delaune 2003). Distance to lek center was defined as the distance between the centroid of a male's territory to the centroid of the entire lek ( $\pm 0.1$  m).

Annual survival was estimated from return rates of male prairie chickens to lek sites. Return rates are the product of multiple probabilities, including true survival ( $S$ ), site fidelity ( $F$ ), and encounter rate ( $p$ ; Sandercock 2006). Auxiliary data indicate that  $F$  and  $p$  are close to unity in our study population (Nooner and Sandercock 2008), and we interpret return rates as estimates of true survival for male Greater Prairie-Chickens.

Changes in behavior, territory attributes, mating success, and survival were compared before and after treatment or mean date of implant among four groups: T-implanted males, sham-implanted males, unmanipulated unsuccessful males, and unmanipulated successful males.

TABLE 14.1  
*Eigenvectors of a principal component analysis of reproductive behaviors from 1,332 10-min focal observations of 129 unmanipulated male Greater Prairie-Chickens at 4–5 leks/yr in northeast Kansas, 2004–2006.*

Behavior	PC1 (Display) <sup>a</sup>	PC2 (Aggression) <sup>a</sup>
Percent time displaying	0.53	0.26
Percent time fighting	−0.50	0.08
Number of boom vocalizations	0.49	0.31
Number of flutter jump displays	0.38	0.02
Number of fights	−0.21	0.65
Number of males engaged	−0.19	0.64
Eigenvalue	2.79	1.69
Percent of variance explained	46.4	28.2

<sup>a</sup>Principal component loadings  $>0.4$  are in italics.

Mean PC score for behavior, SD, and number of observations were calculated for each male before and after treatment. Effect sizes were calculated in MetaWin version 2.0 (Rosenberg et al. 2000). Differences before and after mean implant date among T-implanted, sham-implanted, unmanipulated unsuccessful, and unmanipulated successful males were analyzed using  $Q_B$  statistics from a categorical fixed effects model in MetaWin (Gurevitch and Hedges 1993). Changes in territory size and distance to lek center were analyzed using a matched pairs analysis. Mating success and return rates were analyzed using a Fisher's Exact test. Finally, the odds ratio of mating success was calculated using Proc Freq in SAS (ver. 9.1, SAS Institute 2003).

#### Multinomial Discrete Choice Model

We used multinomial discrete choice (MDC) models to determine the characteristics of successful males by modeling how females chose mates at unmanipulated leks (Nooker and Sandercock 2008). A limited number of copulations were observed among implanted males, and we did not model female choice at manipulated leks. Correlates of female choice were analyzed using a conditional logit multinomial discrete choice model (Proc MDC, SAS ver. 9.1). The utility of a choice ( $U$ ) in benefiting the individual is modeled as:

$$U_{ij} = x_{1,ij}\beta_1 + x_{2,ij}\beta_2 + x_{n,ij}\beta_n + \varepsilon_j$$

where female  $i$  chooses among males  $j$  using characteristics of the choice  $j$ ,  $\beta_n$  is the slope coefficient for explanatory variables, and  $\varepsilon$  is the error term. In this study, each lek represents a different set of choices (males) from which the females choose, which are represented in the model by different sets of values for the characteristics,  $x_1, \dots, x_n$ .

Only copulations observed on unmanipulated leks were used in the MDC model. Each successful copulation was considered an independent choice of a female among the males at a lek. Studies of other lek-mating grouse indicate that females may copy choices of prior-mating females (Gibson et al. 1991; Gibson 1996; but see Spurrier et al. 1994), but we found no evidence of mate choice copying in Greater Prairie-Chickens (Nooker and Sandercock 2008).

In the global discrete choice model, male characteristics hypothesized to be linked to

female choice included four behavioral indices (PC scores of display and aggression with and without females), four measurements of body size (mass, tarsus, wing, and tail), two ornaments used in display (comb and pinnae), age-class (SY or ASY, coded as 0 and 1), and two territory measurements (size and distance to lek center). Morphometric and territory measurements were standardized to z-scores before analysis (mean = 0, SD = 1) so slope coefficients would be directly comparable with our PC scores for components of behavior. Backward elimination was used to simplify the model, and the resulting models were evaluated using an information-theoretic approach (Burnham and Anderson 2002). Additionally, we considered the minAIC model from Nooker and Sandercock (2008) and a minAIC model including  $\text{Log}_{10}T$ . In all, 12 models were analyzed. Akaike weights ( $w_i$ ), model-averaged estimates ( $\hat{\theta}_a$ ), and weighted unconditional standard error [SE ( $\hat{\theta}_a$ )] were calculated using formulae of Burnham and Anderson (2002, equations 4.1 and 4.9).

## RESULTS

### Natural Variation in Testosterone

Over three years, 164 blood samples were collected from 100 individuals at five leks (Table 14.2). T levels could not be quantified for six samples (3.7%) due to sample levels being below ( $n = 2$ ) or above the detection limits ( $n = 4$ ). An average of  $1.6 \pm 0.9$  samples were collected from 87 males and 10 females.  $\text{Log}_{10}T$  differed by sex ( $F = 23.38$ ,  $P < 0.001$ ) and varied by the amount of time the bird was in the trap ( $F = 4.92$ ,  $P = 0.03$ ), but was not affected by lek site ( $F = 1.12$ ,  $P = 0.11$ ), time of day relative to sunrise ( $F = 0.97$ ,  $P = 0.34$ ), or either linear or quadratic seasonal trend ( $F < 0.28$ ,  $P > 0.60$ ; overall model  $F_{10,137} = 4.01$ ,  $P < 0.001$ ). T levels were four times higher in males ( $1.15 \pm 0.91$  ng/mL,  $n = 82$ ) than females ( $0.29 \pm 0.18$  ng/mL,  $n = 10$ ). Time in trap was negatively correlated with  $\text{Log}_{10}T$  levels ( $F_{1,146} = 4.88$ ,  $P = 0.03$ ), but the coefficient of determination was low ( $r^2 = 0.03$ ). Because this correlation explained 3% of the variation, we did not control for time in trap in analyses examining associations between T, male traits, mating success, and survival.

TABLE 14.2  
*Number of birds captured and uncaptured (in parentheses) per year at  
 Greater Prairie-Chicken leks in northeast Kansas, 2004–2006.*

Year	Lek	No of unmanipulated successful males <sup>a</sup>	No of unmanipulated unsuccessful males <sup>b</sup>	No of sham-implanted males <sup>c</sup>	No of T-implanted males <sup>d</sup>
2004	KM	4 (1)	5 (4)	0	0
	KP	3	6 (4)	0	0
	PL	1	4 (5)	0	0
	RRN	2 (3)	3 (4)	0	0
2005	KM	2 (2)	4 (5)	0	0
	KP	(1)	2	3	3
	PL	1 (1)	7 (3)	0	0
	RRN	3 (1)	4 (2)	3	3
2006	KM	0	4 (1)	0	1
	KP	(2)	1 (2)	0	0
	PL	3	1 (4)	3	2
	RRN	4 (1)	1	1	2
	HESS	0	2 (1)	3	4
Total		23 (12)	44 (35)	13	15

<sup>a</sup>Unmanipulated males observed receiving at least one copulation.

<sup>b</sup>Unmanipulated males that were not observed to receive any copulations.

<sup>c</sup>Implanted with an empty silastic tube.

<sup>d</sup>Implanted with a silastic tube containing 30 mg testosterone.

A lack of seasonal trend allowed us to average  $\text{Log}_{10}\text{T}$  levels for each male each year, which were compared with male traits and mating success. A subset of males were not observed at the leks after they were captured. Non-territorial males had lower  $\text{Log}_{10}\text{T}$  levels ( $0.86 \pm 0.85$  ng/mL,  $n = 14$ ) than territorial males ( $1.28 \pm 0.96$  ng/mL,  $n = 81$ ;  $F_{1,93} = 9.72$ ,  $P = 0.002$ ).

No male behavioral or morphological traits were correlated with natural variation in T ( $\text{Log}_{10}\text{T}$ ) among territorial males at unmanipulated leks. Neither male territory size nor the territory's distance from lek center were correlated with  $\text{Log}_{10}\text{T}$  (size:  $F_{1,43} = 0.001$ ,  $P = 0.97$ ; distance:  $F_{1,43} = 0.001$ ,  $P = 0.97$ ).  $\text{Log}_{10}\text{T}$  did not correlate with display or aggressive behavior when females were present (display:  $F_{1,40} = 0.06$ ,  $P = 0.81$ ; aggression:  $F_{1,40} = 0.16$ ,  $P = 0.69$ ) or when females were absent (display:  $F_{1,44} = 1.22$ ,  $P = 0.28$ ; aggression:  $F_{1,44} = 0.12$ ,  $P = 0.73$ ). Of the five morphological traits considered (mass,

length of tarsus plus longest toe, keel, wing, and tail), none were correlated to T levels ( $F_{1,43} < 3.10$ ,  $P > 0.09$ ). Age did not affect T levels ( $F_{1,43} < 0.01$ ,  $P = 0.91$ ). Two sexually dimorphic traits were not associated with  $\text{Log}_{10}\text{T}$  (pinnae:  $F_{1,41} < 0.01$ ,  $P = 0.99$ ; comb area:  $F_{1,42} = 2.39$ ,  $P = 0.13$ ).

The MDC model was based on 23 copulations recorded for 12 successful and 26 unsuccessful males at five unmanipulated Greater Prairie-Chicken leks over three years. Of the 15 models considered, two had  $\Delta\text{AIC}$  values  $< 2$  (Table 14.3). Mating success was correlated with natural variation in  $\text{Log}_{10}\text{T}$ , two morphological traits and one territorial trait as indicated by the minAIC model (Table 14.3).  $\text{Log}_{10}\text{T}$  had the largest slope coefficient, indicating that it was the strongest predictor of male mating success (Fig. 14.1).  $\text{Log}_{10}\text{T}$  was positively correlated with mating success, such that males with higher natural levels of T obtained more copulations ( $t = 2.87$ ,  $P = 0.004$ ). One sexually selected trait and one measurement

TABLE 14.3

Comparison of multinomial discrete choice models examining the effects of male testosterone, morphology, behavior, and territory attributes on mating success of 23 copulations observed among 12 successful and 23 unsuccessful males at five unmanipulated Greater Prairie-Chicken leks in northeast Kansas, 2004–2006.

Model	Testosterone <sup>a</sup>	Morphology <sup>b</sup>	Behavior <sup>c</sup>	Territory <sup>d</sup>	K <sup>e</sup>	Dev <sup>f</sup>	AIC <sup>g</sup>	ΔAIC <sup>h</sup>	w <sub>i</sub> <sup>i</sup>
1	T	rc	—	l	4	60.6	68.62	0.00	0.47
2	T	rc	d	l	5	60.1	70.07	1.45	0.23
3	T	rcp	d	l	6	59.2	71.17	2.56	0.13
4	T	rcp	d	ls	7	58.5	72.48	3.86	0.07
5	T	wrcp	d	ls	8	57.5	73.50	4.89	0.04
6	T	wrcpg	d	ls	9	56.4	74.43	5.81	0.03
7	T	twrcpg	d	ls	10	55.7	75.74	7.13	0.01
8	T	mrc	DAda	—	8	59.9	75.89	7.28	0.01
9	T	twrcpg	Ad	ls	11	54.9	76.89	8.27	0.01
10	—	mrc	DAda	—	7	62.9	76.92	8.30	0.01
11	T	twrcpmg	Ad	ls	12	54.4	78.37	9.75	0.00
12	T	twrcpmg	DAd	ls	13	54.1	80.13	11.51	0.00
13	T	twrcpmg	DAda	ls	14	54.0	82.00	13.38	0.00
14	—	twrcpmg	DAda	ls	13	59.7	85.71	17.09	0.00
15	T	—	—	—	1	92.3	94.33	25.71	0.00

NOTE: Model 10 is the min AIC model from Nooker and Sandercock (2008); Model 8 is model 10 + T; Model 14 is the global model without T; Model 13 is model 14 + T.

<sup>a</sup>Testosterone variables (T = log<sub>10</sub>-transformed testosterone levels, — = not included).

<sup>b</sup>Morphological variables (c = comb area; p = pinnae; r = tarsus; m = mass at capture; w = wing; t = tail; g = age class, — = none included).

<sup>c</sup>Behavioral variables (D = display with females; A = aggression with females; d = display without females; a = aggression without females, — = none included).

<sup>d</sup>Territory variables (l = distance to center of lek, s = territory size, — = none included).

<sup>e</sup>K = number of parameters.

<sup>f</sup>Dev = deviance.

<sup>g</sup>AIC = Akaike's Information Criterion.

<sup>h</sup>ΔAIC = difference of the AIC value in the given model compared to the minimum AIC model.

<sup>i</sup>w<sub>i</sub> = Akaike weights.

of body size were positively correlated with higher mating success (comb area:  $t = 2.43$ ,  $P = 0.02$ ; length of tarsus plus longest toe:  $t = 3.24$ ,  $P = 0.001$ ). Neither display nor aggressive behavior was correlated with mating success since it was not included in the minAIC model (Table 14.3). There was weak support ( $\Delta\text{AIC} = 1.45$ ) for a model that included a negative, but nonsignificant, correlation between display behavior when females were absent and mating success during the breeding season ( $t = -0.72$ ,  $P = 0.47$ ; Table 14.3). Distance to lek center was negatively correlated with male mating success, so that males closer to the center of the lek obtained more copulations ( $t = -2.26$ ,  $P = 0.02$ ).

Annual survival was 55.2% ( $n = 67$ ) among unmanipulated males and did not vary with traits that affected male mating success. Survival did not vary with natural variation in  $\text{Log}_{10}\text{T}$  ( $\chi^2 < 0.03$ ,  $P = 0.86$ ,  $n = 45$ ), comb area ( $\chi^2 = 0.23$ ,  $P = 0.63$ ,  $n = 50$ ), tarsus length ( $\chi^2 = 0.06$ ,  $P = 0.80$ ,  $n = 50$ ), or distance from the center of the lek ( $\chi^2 = 0.01$ ,  $P = 0.93$ ,  $n = 66$ ). Males that displayed less when females were not present had higher return rates ( $\chi^2 = 6.52$ ,  $P = 0.01$ ,  $n = 67$ ), even after Bonferroni correction ( $P < 0.01$ ). The odds of returning were 2.1 times higher (95% CI: 0.73–5.91) among males that did not receive a single copulation than among males that received one or more copulations (Fisher's Exact test:  $P = 0.19$ ).

## Experimental Implants of Testosterone

In 2005 and 2006, 15 T-implants and 13 sham implants were conducted, for a total of 28 males at five leks (Table 14.2). Male behavior before implanting did not differ between the two treatment groups (display with females:  $F_{1,22} = 0.69$ ,  $P = 0.41$ ; aggression with females:  $F_{1,22} = 0.68$ ,  $P = 0.42$ ; display without females:  $F_{1,23} = 0.01$ ,  $P = 0.94$ ; aggression without females:  $F_{1,23} = 3.21$ ,  $P = 0.09$ ). Neither territory size nor distance to lek center varied by treatment before the experiment (size:  $F_{1,26} = 1.02$ ,  $P = 0.17$ ; distance:  $F_{1,22} = 0.20$ ,  $P = 0.66$ ). Comb size, pinnae length, tarsus length, mass, wing length, and tail length did not differ between T- and sham-implanted males (all  $F_{1,25} < 1.37$ ,  $P > 0.25$ ). Males in the two treatments had the same proportion of yearlings and adults (T: 27% SY,  $n = 15$ ; S: 46% SY,  $n = 13$ ; Fisher's Exact  $P = 0.43$ ). There was no difference in  $\text{Log}_{10}\text{T}$  levels at the time of implant between T- and sham-implanted males (natural T levels of T-males:  $1.33 \pm 1.24$  ng/mL,  $n = 14$ ; sham-males:  $1.84 \pm 1.48$  ng/mL,  $n = 13$ ;  $F_{1,25} = 0.94$ ,  $P = 0.34$ ) or the date which the implant occurred (T-males: 10 April  $\pm 4$ ,  $n = 15$ ; sham-males: 12 April  $\pm 5$ ,  $n = 13$ ;  $F_{1,26} = 1.67$ ,  $P = 0.21$ ). After implant, the odds of T levels increasing were 2.0 times higher for of T-males than sham-implanted males (95% CI: 0.08–51.6). Three days following implantation, the T level of a single sham-implanted male decreased by 2.25 ng/mL, whereas the T level of a single T-implanted male increased by 2.36 ng/mL. Between 22 and 35 days following implant, T-implanted males ( $1.58 \pm 0.93$  ng/mL,  $n = 6$ ) had  $\text{Log}_{10}\text{T}$  levels similar to sham-implanted males ( $0.71 \pm 0.33$  ng/mL,  $n = 2$ ;  $F_{1,6} = 2.82$ ,  $P = 0.14$ ), but T-implanted males had higher  $\text{Log}_{10}\text{T}$  levels than successful, unmanipulated males ( $0.59 \pm 0.34$  ng/mL,  $n = 6$ ;  $F_{2,11} = 5.29$ ,  $P = 0.02$ ). The T levels of sham-implanted males were intermediate between T-implanted males and unmanipulated successful males. T-implants removed from two experimental males 26 and 35 days after implant were completely empty of T. Because T-levels between sham- and T-implanted males 22 days following implant was not significant, behavioral observations  $>21$  days post-implant were excluded from the behavioral analyses.

Experimental T treatments did not affect male morphometrics, behavior, or territory attributes.

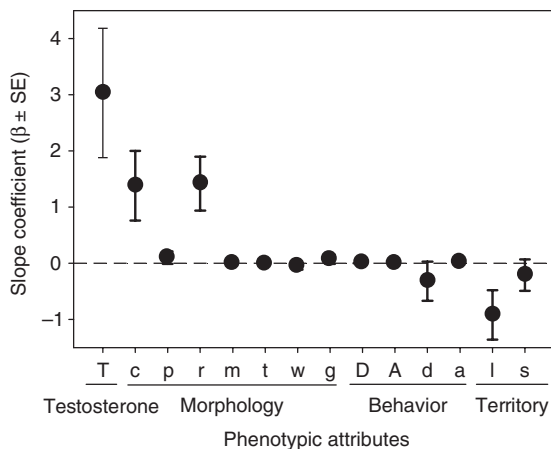


Figure 14.1. Model-averaged estimates of the slope coefficients from a multinomial discrete choice model estimating the effect of male attributes on male mating success, based on 23 copulations among 12 successful and 26 unsuccessful males at five Greater Prairie-Chicken leks in northeast Kansas, 2004–2006. Explanatory variables were z-transformed so slope estimates are directly comparable. Estimates of SE are unconditional and include uncertainty due to model selection, and confidence intervals that do not overlap zero are significant. Abbreviations for explanatory variables include: testosterone (T = log-transformed testosterone levels), morphological variables (c = comb area; p = pinnae; r = tarsus; m = mass at capture; t = tail; w = wing; g = age class); behavioral variables (D/d = display with and without females present; A/a = aggression with and without females present); and territory variables (l = distance to center of lek, s = territory size).

Comb size increased during the breeding season in both T-implanted and unmanipulated males (T-males:  $+25.4 \pm 69.0$  mm<sup>2</sup>,  $n = 5$ ; unmanipulated:  $+21.5 \pm 17.7$  mm<sup>2</sup>,  $n = 4$ ; matched pairs test  $F = 0.01$ ,  $df = 8$ ,  $P = 0.92$ ). Sham-implanted males and unsuccessful, unmanipulated males did not differ in behavior standardized for female presence (meta-analysis; display:  $Q_B = 0.19$ ,  $P = 0.66$ ; aggression:  $Q_B = 2.36$ ,  $P = 0.12$ ;  $n = 23$  unsuccessful and 8 sham-implanted males) or territory attributes (matched pairs test; size:  $F$  ratio = 0.20,  $P = 0.66$ ; distance to center:  $F$  ratio = 0.49,  $P = 0.49$ ;  $n = 15$  unsuccessful and 11 sham males,  $df = 25$ ), so they were pooled in subsequent analyses and referred to as “unsuccessful” males. Changes in aggressive and display behaviors did not differ between T-implanted males, unsuccessful males, and successful males following treatment (display:  $Q_B = 3.76$ ,  $P = 0.15$ ; aggression:  $Q_B = 5.44$ ,

TABLE 14.4

Comparison of effect sizes of changes in display and aggressive behavior following a testosterone implant experiment with Greater Prairie-Chickens at five leks in northeast Kansas, 2005–2006.

	Unsuccessful <sup>a</sup>			T-Implants			Successful		
	E+	95% CI	<i>n</i>	E+	95% CI	<i>n</i>	E+	95% CI	<i>n</i>
Display <sup>b</sup>	0.22	−0.02 to 0.47	31	0.54	0.16 to 0.92	13	0.13	−0.11 to 0.38	24
Aggression <sup>b</sup>	0.01	−0.24 to 0.25	31	−0.20	−0.57 to 0.16	13	0.26	0.01 to 0.51	24

<sup>a</sup>Sham-implanted males and unsuccessful unmanipulated males combined.

<sup>b</sup>Display and aggressive behavior was quantified using a principal component analysis of behavior observed during 10-min focal observations and standardized for female presence (Table 14.1).

$P = 0.07$ ; Table 14.4). Using a matched pairs analysis comparing territory size and distance to lek center before and after treatment, no differences were detected among T-implanted, unsuccessful and successful males (matched pairs test; size:  $F$  ratio = 1.39,  $P = 0.26$ ; distance to center:  $F$  ratio = 1.11,  $P = 0.34$ ;  $n = 14$  T-implanted, 26 unsuccessful, 16 successful males).

T-implanted and sham-implanted males had similar mating success and survival. Four of 15 T-implanted males (14.3%) received six copulations, whereas only one of 13 sham-implanted males (3.6%) received a single copulation, but the difference was not significant (Fisher's Exact test,  $P = 0.33$ ). The odds of returning were 4.1 times (95% CI: 0.63–26.1) higher for sham-implanted (5 of 13 returned) than T-implanted males (2 of 15 returned), but the probability of returning did not differ with respect to treatment (Fisher's Exact test,  $P = 0.20$ ).

## DISCUSSION

Although much work has investigated the role of T in monogamous and facultatively polygamous mating systems, our project is one of the few studies to demonstrate a direct link between natural variation in T and mating success in a lek-mating species. We improve on earlier studies of the natural variation of T in a lek-mating species by analyzing T levels from a larger sample of males ( $n = 87$ , this study;  $n = 23$  males, Alatalo et al. 1996;  $n = 15$ –27 per species, Wikelski et al. 2003). Our experimental manipulations of T in wild birds suggest that there may be a causal link between T and mating success, but low sample sizes and high variability among T-implanted

individuals limited our power for stronger inferences. Surprisingly, natural or experimental variation in T did not correspond with any male traits examined, including aggression. In addition, we did not find support for two of the predictions of the immunocompetence handicap hypothesis because natural or experimentally elevated T did not correlate with male morphological, behavioral, or territorial traits, nor did elevated T decrease annual survival of males.

T was related to male mating success, but not via any of the behavioral, morphological, or territorial traits considered. Territory size, distance from lek center, and morphology did not vary with T. A previous study found an increase in display behavior following the administration of T implants during the non-breeding season (Day et al. 2006), but we did not find any relationship between display behavior and natural or experimentally elevated T. Perhaps we did not find a relationship because our study was conducted during the breeding season, and males were already displaying at maximal levels of effort. We also did not detect a positive correlation between T and comb size or aggressive behavior, as found in previous studies (Briganti et al. 1999, Parker et al. 2002, Mougeot et al. 2005a).

A lack of a relationship between T and male behavioral, morphological, or territorial traits was surprising because females are unlikely to assess T levels directly. However, T may affect mating success via cumulative effects, unmeasured male traits, or during territory establishment in fall. First, T may have small, statistically insignificant, effects on multiple male traits that, when taken together, influence male mating success (Ligon et al. 1998), but we were unable to test for such

cumulative effects. Second, T could also be related to male traits that were not measured, such as parasite load, UV reflectance, color of air sacs and combs, or attributes of vocalizations (Gibson et al. 1991; Mougeot et al. 2005b, 2006; Blas et al. 2006). Last, male grouse visit lek sites in both spring and autumn (Baines 1996, Rintamäki et al. 1999, Salter and Robel 2000, pers. obs.), but the role of lek attendance in autumn is poorly understood. Juvenile males may visit multiple leks to prospect for territories during the non-breeding season, and adult males may maintain or compete for better territories. T could enhance a male's aggressiveness outside of the breeding season and be a proximate mechanism that mediates territory establishment (Mougeot et al. 2005a).

Contrary to a prediction of the immunocompetence handicap hypothesis, T increased male mating success, but did not decrease survival. Similar to an autumn study of Red Grouse (Redpath et al. 2006), we found a nonsignificant trend for males with higher T to have decreased survival. The immunocompetence handicap hypothesis assumes that the birds live long enough that cumulative detrimental effects become apparent. The short average lifespan of Greater Prairie-Chickens (1.6 yr; Robel and Ballard 1974), and high rates of annual turnover among males at leks (Nooker 2007, Nooker and Sandercock 2008) may indicate that natural selection is a stronger determinant of male traits than sexual selection (Bleiweiss 1997, Drovetski et al. 2006). Therefore, longer-lived species of lek-mating birds, such as manakins, may be better study species for testing the immunocompetence handicap hypothesis.

The experimental T implants did not increase aggressive behavior as predicted. Changes in aggression among T-implanted males may have been difficult to detect if sham-implanted males increased their aggressive levels in response to repeated intrusions of T-implanted males (i.e., the challenge hypothesis; Wingfield et al. 1990). Alternatively, our T implants may not have had the intended physiological effects. T can be converted to estradiol in the skin of domestic chickens, producing female-like feathers on males (George et al. 1981). We were unable to examine this possibility in our field study, but estradiol also plays a role in aggression and territory defense in males (Soma et al. 2000). To separate the effects of the two sex steroids, future field studies could potentially manipulate conversion rates of T to

estradiol by using implants of fadrozole, an aromatase inhibitor (Soma et al. 2000, Mougeot et al. 2005a).

The inclusion of T in the MDC model reduced the relative importance of the effects of behavior on male mating success. Model 10 in Table 14.3 included similar variables as the minAIC model presented in Nooker and Sandercock (2008; aggressive and display behavior with and without females, mass, tail or tarsus, comb). The slope coefficients of these two models were similar in magnitude and direction (data not presented), indicating that the results presented here are due to the inclusion of T in the model and not due to smaller sample sizes in this study. However, inclusion of distance to lek center in the analysis of male mating success suggested that certain specific aggressive behaviors that determine territory location, but that were not quantified in our PCA of behavior, may be important in mediating male mating success. We also found a link between comb size and mating success similar to previous studies (Rintamäki et al. 2000). Our research showed the potential importance of examining a suite of male traits since multiple traits were used by females to select a mate.

In conclusion, we found that both natural and elevated T was related to mating success of prairie chickens, but we were unable to detect any individual or suites of male traits correlated with T levels. To obtain a better understanding of the seasonal changes in natural levels of T, multiple samples from individuals throughout the breeding and non-breeding seasons are needed (Mougeot et al. 2005a, Redpath et al. 2006, Kempnaers et al. 2008). Future work should also examine parasites, plumage coloration, and vocalizations as possible intermediary steps linking T to mating success.

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