



Testosterone, Aggression, and Territoriality in Male Western Screech-owls (*Otus kennicottii*): Results from Preliminary Experiments

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Abstract.—Using a hormone implant protocol, we created treatment groups in which circulating levels of testosterone (T) were increased, decreased, or maintained at normal levels (controls) in male Western Screech-owls (*Otus kennicottii*). Owls were exposed to tape-recorded vocalizations of a conspecific, to which territory holders responded with aggression. Several measures of the intensity of aggression (e.g., approach and vocal behavior) provided the basis for comparisons among treatment groups. Exogenous T elicited increased aggression during the breeding and nonbreeding seasons. Although male owls remained sensitive to exogenous T during the nonbreeding season, aggression also occurred in the absence of circulating T at this time.

Aggressive defense of territories by birds during the breeding season is often associated with circulating testosterone (T) levels (Beletsky *et al.* 1990, Harding and Follet 1979, Ramenofsky 1984, Wingfield *et al.* 1987, Wingfield and Wada 1989). Elevated T allows males to compete with other males for territories and females, and to respond effectively to territorial intruders (Wingfield and Farner 1978). However, maintenance of elevated T levels is costly. For example, individuals with high T spend significantly more time in territorial defense and less time tending offspring (Hegner and Wingfield 1987, Ketterson and Nolan 1992, Silver 1977), and high T increases basal metabolism and reduces survival (Dufty 1989). Therefore, it is advantageous for birds to maintain high T only in periods when the potential for male-male interactions is greatest, such as during the breeding season, periods of territory establishment, and other periods during which instability might occur (Wingfield *et al.* 1987, 1990). Testosterone should be reduced during periods of relative social stability, i.e., late in the breeding season and during the nonbreeding season. The testes are the major source of androgens in male birds,

but they regress during the nonbreeding season; consequently T levels are low at this time of the year. However, some avian species maintain and aggressively defend territories throughout the year despite the regression of testes during the nonbreeding season (Logan and Wingfield 1990). The role of T in mediating territorial defense during the nonbreeding season is less well understood (but see Logan and Wingfield 1990).

Our study was designed to understand the role of T in territorial aggression in nonmigratory species of birds that defend territories throughout the year. To understand this relationship we used the Western Screech-owl (*Otus kennicottii*) as our model species. This is an appropriate species in which to examine this relationship because:

1. male screech-owls appear to defend territories throughout the year,
2. males respond aggressively to tape-recorded conspecific vocalizations,
3. owls are easily captured using both mist nets and artificial roosting boxes which allows for radio-marking and administering of the hormone/drug treatments, and
4. a population of marked owls exists in our study area in southwestern Idaho.

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We employed a hormone/drug implant protocol that experimentally increased (T_+) or decreases (T_-) testosterone in relation to control birds (T_0).

Using approach and vocal responses to playback of tape-recorded conspecific vocalizations as indices of aggression, we examined the role of T in mediating aggression during the breeding and nonbreeding seasons.

METHODS

From January to early March, and October to December 1996, we monitored two Western Screech-owls populations in southwest Idaho. One population was located in the Snake River Birds of Prey National Conservation Area (Owyhee and Elmore Counties) and the other inhabited riparian woodlands and suburban areas along the Boise River between Boise (Ada County) and Middleton (Canyon County). During both the breeding and non-breeding seasons, male owls were captured from artificial nest/roost boxes or at night using mist nets. Following capture, each individual received a subcutaneous implant containing testosterone, antiandrogenic compounds, or a placebo. During the breeding season, four males received exogenous T (one 20 mm implant of silastic tubing packed with crystalline testosterone, sealed at both ends); four males received the antiandrogenic drug flutamide (20 mm) and a T aromatization inhibitor, ADT (28 mm); and five males received placebo implants (20 mm). During the nonbreeding season, three males received exogenous T, two received T-inhibiting drugs, and three received placebo implants. Each male owl in both seasons were outfitted with a 5 g backpack mounted radio transmitter so that their movements could be monitored (see Belthoff and Ritchison 1989, 1990 for details on radio-transmitters and attachment). One to 2 weeks after owls received the implants, we performed the playback experiment, which consisted of three 10 minute test periods. The first period (preplayback period) involved two observers radiotracking the focal bird by taking a compass bearing of its position each minute during the experiment. The second 10 minutes, the playback period, was similar to the first, except that during this period we broadcast tape-recorded vocalizations of a conspecific (i.e., simulated territorial intruder; one of three different tapes was played during each experiment) from a speaker placed within the territory (100 m from the nest or focal roosting area) of each radio-tagged focal owl. Again, compass bearings of the owl's location were recorded every minute. The third 10 minutes, the postplayback period, was identical

to the preplayback period. Compass bearings were later plotted to determine the owl's distance from the speaker during each minute of the 30 minute playback experiment. During each playback experiment we determined distance (m) of the focal owl from the playback speaker during each minute, the overall closest approach (m) to the playback speaker by the focal owl during any of the three test periods (in all cases this occurred during the playback period), the number of vocalizations uttered by the focal owl during each of the test periods (any vocal responses by the focal owls were tape-recorded during the experiment using a directional microphone and later counted), and the number of minutes that the focal owl continued to call into the 10 minute postplayback period (maximum of 10 minutes/ referred to as duration of calling). We assumed that owls that called more and approached the playback speaker more closely were the most aggressive.

To control for variation in T related to breeding stage, all experiments during the breeding season were performed while the focal owl's mate was incubating eggs, a time period during which circulating levels of T are typically high (B. Herting, J. Belthoff, and A. Dufty, unpubl. data). During the nonbreeding season, all experiments were performed during November and December, a time period when T levels are very low (B. Herting, J. Belthoff, and A. Dufty, unpubl. data). All data were analyzed using single way or multiway analysis of variance (ANOVA), and repeated measures analyses were conducted where appropriate. To examine effects of treatment, season, and period on distance, the 10 locations of each focal owl (obtained from compass bearings) were averaged for each period of the playback experiment, and these averages were entered into the analysis. Post hoc means comparisons were performed using pairwise t-tests.

RESULTS AND DISCUSSION

Effects of Playback Protocol on Aggression by Focal Males

Our first objective was to determine whether the tape-recorded vocalizations elicited an aggressive response by focal owls. We compared two indices of aggression, vocalizations and distance from speaker, among test periods. When the playback stimulus was applied, we expected resident males to approach the



Table 1.—Mean ($\pm$ SE) distance from the playback stimulus and mean ($\pm$ SE) number of vocalizations uttered by each focal male Western Screech-owl during the three playback periods (see text for description of playback periods) during 21 playback experiments in southwestern Idaho during 1996. Values are averaged across seasons and treatment groups.

Variable	Playback period			Period effect
	Preplayback	Playback	Postplayback	
Distance from playback speaker (m) ¹	96.0 $\pm$ 13.9	64.7 $\pm$ 13.9	63.7 $\pm$ 13.9	F _{2,30} = 1.73 P = 0.19
Number of vocalizations uttered	1.7 $\pm$ 3.4	25.7 $\pm$ 3.4	13.7 $\pm$ 3.4	F _{2,30} = 12.24 P = 0.0001

¹ Average of 10 locations obtained during each playback period for each focal owl were entered into analysis for this variable.

speaker and call in response to the simulated territorial intrusion by another male screech-owl. In line with this expectation, focal owls vocalized significantly more often during the playback and postplayback periods than during the preplayback period (table 1). Moreover, the average distance between the focal owl and the playback speaker was lower during the playback and postplayback periods (table 1), although differences among test periods were not significant ($P = 0.19$). The lack of significance associated with the lower average distance during the playback period may have resulted from large variation in the focal owl's initial distance from the playback speaker during the preplayback period. For example, at the beginning of the experiment some owls were very close to the playback speaker and moved relatively closer, while other owls were initially very far away and approached when the playback began. There was no significant effect of season (breeding vs. nonbreeding) on average distance from playback speaker ($F_{1,15} = 1.89$, $P = 0.189$) or total vocalizations uttered during the 30 minute experiment ($F_{1,15} = 1.90$, $P = 0.187$). These latter results indicate that the aggressive response of focal males toward the playback stimulus was similar between seasons, and they are consistent with the hypothesis that these screech-owls are territorial throughout the year.

Effects of Hormone/Drug Treatments on Aggression by Focal Males

Approach Behavior Toward Playback Stimulus

There was a significant effect of treatment on average distance from the playback stimulus (table 2); owls treated with exogenous T were significantly closer to the playback speaker than owls whose T production and uptake were blocked. This result suggests that the drug/hormone implants were efficacious, that birds treated with exogenous T were significantly more aggressive, and that owls treated with T-blocking drugs showed decreased aggression. In addition, owls treated with T during the nonbreeding season exhibited aggressive responses comparable to owls that received exogenous T during the breeding season (table 2). This result indicates that, even though circulating T levels are normally very low during the nonbreeding season, male Western Screech-owls continued to be sensitive to the presence of this steroid hormone throughout the year. Finally, owls treated with T approached the speaker most closely (e.g., closest approach to the playback speaker during the entire 30 minute experiment) when compared to owls in either of the other two treatment groups, although the difference among groups was significant only at the $P = 0.10$ level (table 2).

Table 2.—Aggression indices (mean $\pm$ SE) by season for each treatment group of Western Screech-owls in southwestern Idaho. Numbers in brackets represent sample sizes. T_+ refers to subjects whose testosterone levels were experimentally increased, T_- individuals had testosterone levels lowered experimentally, and testosterone levels in T_0 were allowed to fluctuate naturally. There were no significant season effects (P values for seasons ranged from 0.08 to 0.18 for each variable below) or interactions with treatment, F - and P -values are shown for treatment effects only.

Aggression indices	T_+		T_-		T_0		Treatment effect
	Breeding {4}	Nonbreeding {3}	Breeding {4}	Nonbreeding {2}	Breeding {5}	Nonbreeding {3}	
Mean distance from speaker (m) ¹	28.7 $\pm$ 30.6	17.6 $\pm$ 35.4	162.1 $\pm$ 30.6	96.2 $\pm$ 43.3	41.1 $\pm$ 27.4	42.5 $\pm$ 35.4	$F_{2,15} = 3.95$ $P = 0.04$
Overall closest approach to speaker (m)	4.9 $\pm$ 5.4	5.4 $\pm$ 6.3	8.7 $\pm$ 5.4	27.0 $\pm$ 7.7	13.0 $\pm$ 4.8	18.6 $\pm$ 6.3	$F_{2,15} = 2.57$ $P = 0.10$
Total number of vocalizations	72.0 $\pm$ 16.6	84.0 $\pm$ 19.2	50.5 $\pm$ 16.6	0.0 $\pm$ 23.5	49.8 $\pm$ 14.9	4.0 $\pm$ 19.2	$F_{2,15} = 5.43$ $P = 0.02$
Duration of vocalization (min.)	8.5 $\pm$ 1.6	9.3 $\pm$ 1.8	3.3 $\pm$ 1.6	0.0 $\pm$ 2.2	5.0 $\pm$ 1.4	0.0 $\pm$ 1.8	$F_{2,15} = 10.4$ $P = 0.001$

¹ Average of 10 locations during each playback period for each focal owl were entered into analysis for this variable.

Vocal Response to Playback Stimulus

Owls treated with T uttered significantly more vocalizations and continued to vocalize longer into the postplayback period following removal of the playback stimulus (table 2) than owls in the other two groups. We detected no differences in the total number or duration of vocalizations between T -blocked and control birds, particularly during the nonbreeding season. This suggests that although exogenous T increases calling during both seasons, owls treated with T -blocking drugs are just as likely as control owls to vocalize in response to the playback stimulus.

CONCLUSIONS

Our results suggest that Western Screech-owls express aggressive behavior during both the breeding and nonbreeding seasons, which is consistent with the observations that these owls are territorial throughout the year. Our study also suggests that T is an important mediator of aggression in male screech-owls during the breeding season; owls with high T were most aggressive and when T was blocked aggression was reduced. Although they remained sensitive to T during the nonbreeding season (a time period when circulating T levels

are normally low), male Western Screech-owls exhibited aggressive behavior comparable to that observed during the breeding season. This suggests that some other neuroendocrine mechanism may regulate aggression in these owls during this time. Our goal is to perform additional playback experiments during both the breeding and nonbreeding seasons in 1997 to clarify the relationships among testosterone, territoriality, and aggression in male Western Screech-owls.

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