

Context-specific territorial behavior in urban birds: No evidence for involvement of testosterone or corticosterone

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ABSTRACT

Testosterone produced by the gonads is a primary mediator of seasonal patterns of territoriality and may directly facilitate territorial behavior during an encounter with a potential intruder. Costs and benefits associated with territoriality can vary as a function of habitat, for example through differences in resource distribution between areas occupied by different individuals. We investigated behaviors in response to simulated territorial intrusions (*hereafter* territorial behaviors) in urban (Phoenix, Arizona) and nearby desert populations of two Sonoran Desert birds (Curve-billed Thrasher and Abert's Towhee). We also examined the degree to which these behaviors are mediated by testosterone (T) and the adrenal steroid, corticosterone (CORT), which can interact with T in territorial contexts. In both species, urban birds displayed more territorial behaviors than their desert conspecifics, but this difference was not associated with variation in either plasma total or in plasma free (i.e., unbound to binding globulins) T or CORT. In addition, neither plasma T nor plasma CORT changed as a function of duration of the simulated territorial intrusion. Urban Abert's Towhees displayed more territorial behaviors in areas where their population densities were high than in areas of low population densities. Urban Curve-billed Thrashers displayed more territorial behaviors in areas with a high proportion of desert-type vegetation, particularly in areas that differed in vegetation composition from nearby randomly sampled areas, than in areas with a high proportion of exotic or non-desert type vegetation. Associations between territorial behavior and habitat characteristics were not related to plasma T or CORT. Understanding the hormonal processes underlying these associations between behavior and habitat may provide insight into how free-ranging animals assess territorial quality and alter their defensive behavior accordingly.

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Introduction

In many bird species, the onset of breeding is marked by the male establishment of territories and its subsequent defense from conspecific intrusion (Beletsky and Orians, 1987a, 1987b; Wingfield, 1984). The advantage that birds gain from defending a territory presumably is species-specific (Maher and Lott, 1995) and may include nest protection (Burger and Beer, 1975; Larsen et al., 1996), mate-guarding (Bodily and Neudorf, 2004; Komdeur et al., 2007; Moore, 1984), or monopolization of spatially limited resources (Gorrell et al., 2005; Sasvari, 1991). Available resources (e.g., food and nest sites) may vary between habitats and may in turn influence the necessity to engage in territorial behaviors. In environments where resources are clumped, such as remnant woodlands or natural habitats in urban areas, selection may favor increased territorial behavior and vigilance during agonistic encounters as compared to the situation where resources are uniformly distributed. This may be the case particularly during the

energetically costly reproductive period (reviewed in Harshman and Zera, 2006).

The conversion of native to urbanized landscapes can result in a mosaic of habitat types that differ in their suitability for use by native birds (Brennan and Schnell, 2005; Hodgson et al., 2007; Palomino and Carrascal, 2007; Turner, 2006). This habitat “patchwork” can alter territorial behavior that is driven by the need for resource defense. Studies on the impacts of urbanization found increased population densities in urban compared to adjacent wild lands (Chace and Walsh, 2006; Green and Baker, 2003; McKinney, 2006; Van Turnhout et al., 2007). Increased population densities may be associated with a parallel increase in vigilance and territorial behavior resulting from more frequent intrusion by conspecifics (Sasvari, 1991). In addition, predation pressure is often lower in urban than native environments due either to decreased predator numbers or to lower predator hunting success in urban areas (reviewed in Chace and Walsh, 2006; Anderies et al., 2007). This difference may reflect a decreased fitness cost of engaging in territorial behavior, which may involve making an individual more conspicuous to ward off territorial intruders.

Antagonistic encounters between males involve the expression of behaviors that are often regulated by testosterone (T), particularly

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during reproductive periods (Farner and Wingfield, 1980; Jawor, 2007; Wingfield, 1984; Wingfield et al., 1987). Furthermore, persistent or frequent territorial intrusion can elevate plasma T, which is thought to in turn facilitate the persistence of territorial behavior (“challenge hypothesis”: Ramos-Fernandez et al., 2000; Ros et al., 2002; Wikelski et al., 1999; Wingfield et al., 1990; Goymann et al., 2007; Goymann, 2009). In many monogamous species, plasma T is elevated in males during territory establishment and decreases with progression towards nestling and fledging stages (Wingfield et al., 1990; Wingfield, 1984; Van Duyse et al., 2003). In contrast, in species exhibiting frequent extra-pair copulations, plasma T often remains elevated throughout the breeding season (Wingfield et al., 1990; Wingfield, 1984).

Many species, including those inhabiting hot deserts and tropical regions, show year-round territorial behavior. In these species, territoriality outside the breeding season may be independent of circulating T (Moore et al., 2004; Hau, 2001; Hau et al., 2000, 2004). Several hypotheses have been proposed to explain this seasonal uncoupling of territorial behavior and plasma T (Hau et al., 2004; Wacker et al., 2008). One hypothesis is that fluctuations in secretion of glucocorticoids, such as corticosterone (CORT), in response to the stress of territorial intrusion influence T levels, with CORT generally suppressing T secretion (reviewed in Wingfield and Sapolsky, 2003) by acting directly on the hypothalamus, pituitary gland, and/or gonads (Dobson and Smith, 2000; Wingfield and Sapolsky, 2003). In avian plasma, most T and CORT are thought to bind competitively to “corticosterone binding globulins” (CBG), resulting in only a small fraction (free hormone) of these hormones circulating unbound (Breuner et al., 2006; Deviche et al., 2001). According to the “free hormone” hypothesis, only unbound steroids can diffuse into cells and influence intracellular receptors or be cleared from circulation (Rosner et al., 1991). An increase in plasma CORT presumably displaces a portion of globulin-bound T and as a result, increases plasma free T even though T secretion may not be affected (Deviche et al., 2001). Although increased CORT occupancy of CBG may elevate free T levels during territorial intrusion in the short-term, this increased circulating free T may also induce a negative feedback effect of T on the hypothalamus and pituitary gland, resulting in decreased overall T secretion in the long term. Thus increased CORT occupancy can conceivably increase or decrease overall T in circulation depending on time-course, however these hypotheses remain untested. Indeed, few studies have focused on how the interaction of T and CORT with CBG can influence territorial behavior. However, no differences in total or free plasma T, CORT or CBG were observed in male Mountain White-crowned Sparrows (*Zonotrichia leucophrys oriantha*) exposed to simulated territorial intrusion relative to passively captured controls (Lynn et al., 2007). Similarly, in Blue Tits (*Cyanistes caeruleus*) increases in occupancy of CBG by CORT during challenge, was not accompanied by significant increases in free T, thus suggesting this mechanism is not responsible for changes in circulating T levels (Landys et al., 2007). However there is still considerable debate concerning the interrelationships of CBG, CORT, and T in free-living birds and particularly in the context of behavioral regulation.

We investigated differences in territorial behavior in two Sonoran Desert bird species, the Curve-billed Thrasher (*Toxostoma curvirostre*) and the Abert's Towhee (*Melospiza aberti*), inhabiting the city of Phoenix, Arizona and adjacent native habitat. These species are sedentary and exhibit year-round territorial behavior towards conspecific intrusion (Finch, 1984; Fokidis et al., 2008; Vleck, 1984) unlike both the Mountain White-crowned Sparrows and Blue Tits. The present study tested the hypothesis that urban birds are more responsive to simulated territorial intrusions (i.e., display more territorial behaviors) than desert birds and that this difference in responsiveness is mediated by interactions between plasma T, CORT, and CBG. We also hypothesized that urban–desert differences in behavior are related to the need to defend spatially clumped

resources, particularly native vegetation that these birds use as nest sites, which can be limited in an urban environment (Dunford and Freemark, 2005). Urban thrashers and towhees show less seasonal variation in body condition than their desert counterparts suggesting urban habitats may provide stable food resources for individuals capable of maintaining territories in the city (Fokidis et al., 2008). In this study we determined whether: (1) urban birds displayed more territorial behaviors than desert conspecifics; (2) territorial behaviors were related to plasma total or free T and CORT; (3) plasma T or CORT increased in response to duration of simulated territorial intrusion (i.e. song playback); and (4) territorial behaviors were related to differences in population density or suitable nesting sites. We predicted that compared to desert birds, urban birds would display more territorial behaviors during a territorial intrusion and would have higher total T levels (i.e. more T secretion) and/or higher levels of free T (i.e. increased CORT occupancy of CBG). We also predicted that the levels of free or total T would increase more in urban than desert birds in response to prolonged territorial intrusion, particularly in areas of high bird density or more vegetation for nesting than in areas of low bird density or low nesting site availability.

Materials and methods

Study sites and populations

The study was conducted at three desert and three urban sites. Desert sites are largely unpopulated but differ with regard to their proximity to urban housing developments. The Sierra Estrella Wilderness Area (14,400 acres) is located at the base of the Sierra Estrella Mountain Range about 26 km southwest of Phoenix. The McDowell Mountain Sonoran Preserve and Regional Park (940 acres and 21,099 acres, respectively) is located at the northern periphery of Phoenix, 2 km from the fringe suburban developments of Troon, Fountain Hills, Ancala, and Pinnacle Peaks. The vegetation at both desert sites is typical of upland Sonoran Desert and includes columnar cacti, cholla (*Opuntia* spp.), ocotillo (*Fouquieria splendens*), mesquite (*Prosopis* spp.), and palo verde (*Prosopis* spp.). A third desert site was the Robbin's Butte Wildlife area (1,511 acres) located 48 km west of Phoenix. This area borders the Gila River and vegetation primarily consists of mesquite, saltbush (*Atriplex* spp.), creosote (*Larrea tridentata*), bursage (*Ambrosia* spp.), and willows (*Salix* spp.). This area also contains dense thickets of invasive salt cedar (*Tamarix* spp.) and retired agricultural lands. Urban sites included high- and low-income residential housing tracts, commercial areas, business districts, and city parks of varying sizes in Phoenix, Scottsdale, and Tempe. Most urban sampling occurred in three areas: South-Central Phoenix, East Phoenix and Tempe, and North Scottsdale and North Phoenix. Each site was sampled weekly between 15 March and 2 June 2007, which coincides with the early (i.e., nest-building and territory establishment) and mid-reproductive periods (i.e., incubation and nestling stages) of the two study species. Birds were caught between 0500 and 1100 h.

Simulated territorial intrusions and bird sampling

To simulate territorial intrusions, we used recordings of songs and calls of thrashers and towhees compiled from independent recordings of several individual birds from sites in and around Phoenix, Arizona. Studies involving simulated territorial intrusions often couple song playback recordings from a speaker with the presence of either a stuffed or live conspecific decoy. Although this technique provides a more natural simulation of a territorial intrusion, logistic and permitting issues prevented our use of live decoys or our obtaining new taxidermy specimens. However use of a taxidermy specimen of an Abert's Towhee did not reveal significant differences in territorial behavior between the presence of a stuffed decoy coupled to a speaker

and a speaker alone in this species (all $p > 0.26$; $n = 9$). In addition, responses by individuals of both species tended to be rapid and focused on the area around the speaker. Thus the presence of the speaker alone we felt was sufficient to elicit representative territorial responses in these two bird species, and this method has been successfully used in other passerine species Antbirds (*Hylophylax naevioides*; Wikelski et al., 1999), and Great Tits (*Parus major*; Franco and Slabbekoorn, 2009). Recordings were played on a Sony MD Walkman minidisk player through a handheld speaker at constant volume (74 dB at 2 m). Birds in urban areas were sampled during times of minimal human disturbance (early mornings and weekends). All males were exposed to the same compilation of songs and calls for their respective species. Upon locating a male bird visually without use of song playback, a mist net was erected and the bird was exposed to the recording with the observer (HBF) located 40–80 m away and having an unobstructed view of the mist net and surrounding area. Additional birds (4 thrashers and 3 towhees) were captured by passive netting (no playback) near known nest sites.

The observer, dressed in camouflage and remaining quiet and still, recorded the occurrence of several stereotyped behaviors associated with responses to playback: latency to first approach to playback speaker; number of approaches from the air and ground; number of times the bird flew over the net; number of times the bird sang and/or called; number of calls made in direct response to calls from the speaker (match calls); number of times the bird wiped its beak on vegetation; number of times the bird raised its wings (wing flash) and/or spread its tail feathers (tail fan); and number of times the bird was observed feeding on the ground. In addition to these behaviors, for the towhees we also recorded the number of times a squeal duet was heard. This vocalization is produced by birds of a mated pair (Tweitt and Finch, 1994) and was frequently heard in response to playback.

Birds under study were naïve and, in some cases, were lost sight of during experimental trials. The period during which a focal bird was not in view was recorded, thus enabling us to correct behavioral observations as a function of the actual time the bird was observed (*hereafter* number of behaviors per minute observed). The behavioral response of two birds seen at the same time was also noted, as was the presence of another individual, which likely indicated a mated pair, as one individual would sing in response to playback (presumably the male), but would be closely accompanied by a non-singing adult bird (likely the female). In such cases, male birds that were captured were identified either via observations of singing behavior (as in thrashers) or by the persistent presence of the bird around the net until it was captured and identified by hand. All birds were observed displaying these behaviors just before capture, thus allowing us to identify them in hand.

During preliminary research, Brown-headed Cowbirds (*Molothrus ater*) were observed during 20–30% of the playback trials in both urban and desert settings. Brown-headed Cowbirds are brood parasites and are known to use Abert's Towhees (Finch, 1983) and thrasher species as hosts (Finch, 1982; Haas and Haas, 1998). Their presence was noted as it may alter the behavior of nesting parents wanting to deflect attention from a nest site. The amount of time that a bird was exposed to the playback recording before capture was also recorded. Behavioral data for birds that we were unable to capture ($n = 4$ towhees, 5 thrashers) were used to supplement the behavioral data.

Upon capture, birds were removed from the net and about 200 μ l of blood was collected from the right jugular vein using a heparinized 0.3 cc syringe with a 29.5 gauge needle within 3 min for measuring baseline plasma CORT and T. An acute stress response was then induced by holding the bird in a cloth bag for 30 min as previously described (Wingfield et al., 1994a, 1994b). Thirty minutes later, a second 200 μ l blood sample was collected to measure stress-associated changes in plasma CORT and T. Blood samples were kept on ice until plasma was separated by centrifugation and then stored at -80 °C until the assays

were run. Adult males were identified by the presence of a developed cloacal protuberance (CP). Body mass (± 0.1 g), tarsus and culmen length (± 1 mm), and wing chord (± 1 mm) were also measured. All birds received a uniquely numbered aluminum US Geological Survey leg band and released at the site of capture. All procedures followed guidelines approved by the Arizona State University Institutional Animal Care and Use Committee and were done under scientific collecting permits from the US Fish and Wildlife Service and Arizona Game and Fish Department.

CORT and T assays

Total plasma T and CORT concentrations were measured using validated commercial competitive enzyme-linked immunoassays (ELISA; Assay Designs Inc. Ann Arbor, Michigan, USA) as described by Fokidis et al. (2009). Additional validations included the successful recovery of known T and CORT amounts added to samples ($n = 3$ for each steroid) before assay (96% and 93% recovery, respectively). Overnight incubation of plasma with dextran-coated charcoal resulted in undetectable plasma T or CORT. Plasma samples from the two study species were assayed independently. The sensitivity of the CORT assay ranged from 7.3 to 18.1 pg/ml depending on the plate and the mean intra-assay coefficient of variation was 9.19% ($n = 4$ plates; 156 samples assayed in duplicate). The sensitivity of the T assay ranged from 4.93 to 9.65 pg/ml depending on the plate and the mean intra-assay coefficient of variation was 7.14% ($n = 4$ plates; 156 samples in duplicate).

CBG Assay and estimation of free hormone concentration

Radioligand binding assays to determine plasma CBG binding capacity were performed using ^3H -CORT according to Breuner and Orchinik (2001) and Orchinik et al. (2000) with minor modifications specified in Fokidis et al. (2009). To determine CBG binding capacity (B_{max}) we incubated samples with a saturating concentration of ^3H -CORT, which was equivalent to 9 times the equilibrium dissociation constant (K_d) for each species. The K_d values for CORT binding by CBG in the two study species have been previously described (Abert's Towhee: $K_d = 5.97 \pm 0.89$ nM; Curve-billed Thrasher: $K_d = 6.13 \pm 0.78$ nM; Fokidis et al., 2009). We also used previously calculated inhibition constants for T (K_i ; Fokidis et al., 2009), determined from IC_{50} values using the Cheng-Prusoff equation (Cheng and Prusoff, 1973). The K_i values for T in the Abert's Towhee and Curve-billed Thrasher were 22.88 ± 1.07 nM and 15.97 ± 1.05 nM, respectively (Fokidis et al., 2009). The B_{max} and K_i values were used to estimate plasma concentrations of free and CBG-bound CORT and T for each sample using the equation of Barsano and Baumann (1989). For this, we assumed that T and CORT bind to CBG competitively and first estimated the amount of plasma CBG occupied by empirically determined basal and stress-induced CORT concentrations. We then estimated how much T binds to the remaining (free or unsaturated) portion of CBG, thus providing an estimate of both free and bound T concentrations (see Deviche et al., 2001 for details).

Estimating population density and nest site availability

To estimate population densities, we surveyed birds along 800 m transects where the midpoint was located at the site of a captured bird. Transects in urban areas were oriented east–west to coincide with main roads and alleyways. At one desert site (Sierra Estrella wilderness area), transects were oriented in a north–south direction to avoid sampling steep mountain slopes with different plant communities. Transects were surveyed twice, once in September 2007 and once in October 2007, and only birds found within 50 m of either side of a transect were counted. We were interested only in sampling densities for the species captured at the midpoint site, and

thus the survey was conducted by playing species-specific song every 50 m along transects and tallying the number of individuals that sang in response to playback (indicating an adult male bird). When two or more individuals appeared to respond together (likely a breeding pair and fledglings) they were counted as one individual. The survey was conducted in fall for logistic reasons. As adult Curve-billed Thrashers and Abert's Towhees are sedentary and philopatric (Tweit, 1996; Tweit and Finch, 1994), we assumed that estimated fall densities of both species were similar to densities during the breeding season earlier in the year.

Assessing nest site availability involved conducting vegetation surveys where the site of capture represented the mid-point of north-south and east-west oriented transects that were 200 m in length. This length was chosen as representative of the vegetation present in the average home range of a Curve-billed Thrasher or Abert's Towhee (Tweit, 1996; Tweit and Finch, 1994). We compared vegetation data collected from the native desert habitat with those from urban sites using a protocol modified from Daniels and Kirkpatrick (2006). Specifically, we recorded the number of tree types, cacti, succulents, and artificial perching structures (e.g., electric wires, roof edges, TV antennas, fences, and electric poles) as well as the height of ground-covering vegetation, and the number of natural shrubs and hedges, the latter being defined as shrubs that have been actively pruned (Table 1). For each vegetation category, we recorded the number of each plant type with thorns. We only recorded vegetation within 10 m of the transect on either side. At urban sites this method only measured vegetation located in the front yards of residential homes. Urban mid-points were for the most part located near street intersections. We also assessed vegetation structure at sites where birds were captured and relative to the surrounding landscape. To do this we conducted transect surveys at three randomly selected points 1 km from each site of capture (hereafter random sites). In urban areas, these points were also selected to coincide with street intersections and were conducted in a north-south and east-west direction.

Table 1
Habitat variables for assessing habitat structure in urban and desert bird populations.

Habitat categories	Abbreviations	Inclusion criteria
Trees		
Broad-leaved Evergreen	BE	<i>Citrus</i> spp.
Thin-leaved Evergreen (no thorns)	TEnt	<i>Prosopis</i> spp. no thorns
Thin-leaved Evergreen (thorns)	TEt	<i>Prosopis</i> spp. with thorns
Coniferous	C	<i>Pinus</i> , <i>Abies</i> , <i>Picea</i> spp.
Monocot	M	Arecaceae or Palmae
Deciduous	D	Seasonal shedding of leaves
Shrubs	Sh	unmanaged
Hedges	Hg	actively pruned
Cacti		
Saguaro	Sag	<i>Carnegiea gigantea</i>
Teddy Bear Cholla	TbC	<i>Cylindropuntia bigelovii</i>
Chain-Fruit Cholla	ChC	<i>Cylindropuntia fulgida</i>
Buckhorn Cholla	BuC	<i>Cylindropuntia acanthocarpa</i>
Prickly Pear	PP	<i>Opuntia</i> spp.
Barrel Cacti	BC	<i>Ferocactus</i> spp.
Desert shrubs and succulents		
Yucca	Yuc	<i>Yucca</i> spp.
Agave	Ag	<i>Agave</i> spp.
Ocotillo	Oco	<i>Fouquieria splendens</i>
Creosote	Cr	<i>Larrea tridentata</i>
Bamboo or Canebrake	Cb	Poaceae, or <i>Saccharum</i> spp.
Washes	W	Wash crosses transect
Artificial perching structures		
Telephone Poles	TP	
Telephone Wires	TW	
Transformers	T	
Fences, Walls	Fen	
Television Antennae	TV	
Street Signs	Sig	
Street Lights	Lig	

Statistical analyses

Behavioral variables were corrected for the amount of time a bird was observed by determining the number of behaviors performed per minute that the bird was directly observed (see above). This time-corrected data were then reduced using principal components analysis (PCA) to generate a single score, with mean standardized to zero, which represented territorial responses to playback. PCA analyses included all birds exposed to playback, captured and not captured (thrashers: $n = 44$, towhees: $n = 25$). We assume such individuals were males because in both species, males displayed more territorial behaviors in response to playback than females and the latter rarely respond alone (Fokidis unpublished data). Most behavioral variables associated with territorial displays (e.g., approaches towards speaker) loaded on the first component (PC1) in a unidirectional manner (Table 2) and thus the larger the score's value, the more responsive the individual was to playback. Other non-territorial behaviors (e.g., feeding, bill-wiping) loaded primarily on the second component (PC2), but directionality differed between variables (Table 2). This statistical technique has been used in similar studies to reduce behavioral variables to single score values representing most of the variation in behavior (see Landys et al., 2010). However univariate approaches were also used to compare the most common behaviors between urban and desert birds to determine whether individual behaviors were different between habitats.

We used two-tailed Student's t -tests to compare latencies to first response (i.e. approaches to the speaker) and individual component behaviors between urban and desert birds, using data from all the birds for which these data were available. Student's t -tests were also used to compare PC scores between birds that were captured and those that were not captured to ensure that our study sample was not biased towards more territorial individuals. Further analyses focused only on captured birds. We used Student's paired t -tests to compare plasma hormone levels in samples collected 3 vs. 30 min after capture. Student's t -tests were used to compare hormone levels of passively captured birds with those of birds captured using song playback. Differences in behaviors were then assessed using analysis of covariance (ANCOVA) with PC1 and PC2 as the dependent variables, site (urban or desert) as the fixed independent variable, and baseline total T and CORT, capture date, time (minutes after sunrise), cowbird presence, and body mass as covariates. This analysis was repeated, substituting baseline free plasma T and CORT for total plasma hormone concentrations. Separate analyses were necessary because these variables were correlated and not doing so would violate statistical assumptions of data independence. To assess differences in hormone concentrations between urban and desert birds, we used multivariate analysis of covariance (MANCOVA), with plasma

Table 2
Territorial behaviors (calculated as number of observations/min the bird was observed) and associated principal component (PC) scores, in response to simulated territorial intrusions in Curve-billed Thrashers ($n = 44$) and Abert's Towhees ($n = 25$).

Component behaviors	Abert's Towhee			Curve-billed Thrasher		
	PC1	PC2	PC3	PC1	PC2	PC3
Air approach	0.775	-0.225	-0.680	0.857	-0.141	-0.166
Ground approach	0.662	-0.243	0.200	0.807	0.103	-0.030
Fly over	0.718	-0.273	0.146	0.776	0.213	0.033
Song	-0.345	0.232	-0.960	-0.576	-0.039	-0.376
Calls	0.595	-0.064	0.024	0.571	-0.301	0.008
Match calls	0.083	-0.140	0.038	-0.065	-0.129	-0.006
Squeal duet	-0.027	0.587	0.076	-	-	-
Bill wiping	-0.358	0.629	-0.041	-0.329	0.745	-0.402
Feeding	0.012	0.015	0.157	-0.342	0.569	-0.269
Tail fan	-0.082	-0.820	0.004	0.100	0.275	0.582
Wing flash	0.172	0.374	-0.401	0.283	0.129	0.039
% Cumulative variance						
Explained	48.11	58.53	67.15	60.89	72.34	81.77

hormone concentrations as the dependent variables, site as fixed factor, and date, time of day, playback duration, wing chord length and body mass as covariates. We used linear regression to determine whether duration of playback exposure elevated plasma T or CORT.

Regression analysis was used to determine if mean population density was associated with territoriality. To identify the habitat variables that significantly explained variation ($p \leq 0.05$ —see below) in territorial behavior, we used canonical correspondence analysis (CCA; XLSTAT 4.03 software) with a stepwise forward selection model and a Monte Carlo permutation test (500 random permutations; ter Braak and Schaffers, 2004). An analysis was performed separately for both urban and desert sites and for each species, and was repeated with the inclusion of capture sites as well as random sites to generate scores for habitat structure for each site. This approach was used to assess habitat similarity between capture and random sites by calculating the Euclidean distance between sites. The mean from these comparisons for each site provided a general index of similarity in vegetation structure, with a smaller value indicating greater similarity between sites. We eliminated sites, such as parking lots, where vegetation was completely lacking ($n = 3$). These “zero” data points did, therefore, not contribute to the overall similarity index. Relationships between habitat variables and similarities with individual behaviors (see above) were assessed using Pearson correlation coefficients.

Where necessary data were tested for the presence of outliers using Chauvenet's criterion outlier test (at $p < 0.05$) which identifies data points beyond two standard deviations from the mean, which were then excluded from subsequent analysis. All data are presented as means + standard errors (s.e.) and the critical alpha level was set at $p \leq 0.05$. For the sake of brevity only the p -values are presented for results that were not statistically significant.

Results

Behavioral differences between urban and desert birds

There was no difference in sampling between urban and desert birds in terms of capture date (thrashers: $p = 0.672$; towhees: $p = 0.490$) or time of capture (thrashers: $p = 0.781$; towhees: $p = 0.901$). There were also no significant differences in the proportion of time that birds were observed before being captured between sites (Table 3). Urban birds responded to song playbacks more quickly (thrashers: $t_{38} = 2.007$, $p = 0.032$; towhees: $t_{21} = 3.501$, $p = 0.019$; Fig. 1); with a greater number of approaches to the speaker, and by making more calls (Table 3) than desert conspecifics. Confirming this difference, urban birds displayed more territorial behaviors (i.e., greater PC1 scores) than desert conspecifics (thrashers: $F_{1,38} = 8.510$, $p = 0.007$; towhees: $F_{1,21} = 13.177$, $p = 0.003$; Fig. 2). In contrast PC2 scores, consisting of behaviors not usually associated with territoriality (see Table 2), did not differ between urban and desert birds of either species (both $p \geq 0.083$; Fig. 2).

Thrasher behavioral scores were not influenced by date ($p \geq 0.081$), time ($p \geq 0.357$) or the presence of cowbirds ($p \geq 0.230$). In towhees, territorial behavior (PC1) was not influenced by date ($p = 0.148$), time after sunrise ($p = 0.380$), or cowbird presence ($p = 0.412$). However, non-territorial behavior (PC2) in this species was influenced by date ($F_{1,21} = 3.547$, $p = 0.034$), but not time after sunrise ($p = 0.147$) or presence of cowbirds ($p = 0.110$). Body mass and wing chord was not associated with behavior in either thrashers (both $p \geq 0.401$) or towhees (both $p \geq 0.104$).

Is territorial behavior related to plasma T or CORT?

Thrashers

Urban and desert thrashers did not differ with respect to their plasma total baseline T or CORT concentrations (Table 4). The CBG binding capacity did not differ between urban and desert birds.

Table 3

Behavioral responses (here as occurrences per minute of direct observation) to simulated territorial intrusion in two Sonoran Desert songbird species inhabiting both urban and desert habitats. The amount of time that birds were directly observed is expressed as “proportion of time observed”, and all data are means + s.e. *indicates significant differences between urban and desert birds at $p \leq 0.05$.

	Urban	Desert	<i>t</i>	<i>p</i>
<i>Abert's Towhee</i> ($n = 10$ Urban, $n = 11$ Desert)				
Proportion of time observed	0.88 ± 0.15	0.82 ± 0.23	0.613	0.520
Air approach	4.27 ± 0.742	1.75 ± 0.629	6.14	< 0.001*
Ground approach	7.24 ± 0.633	1.91 ± 0.679	7.29	< 0.001*
Fly over	4.01 ± 0.568	2.08 ± 0.519	2.17	0.016*
Song	0.11 ± 0.116	0.08 ± 0.083	0.410	0.180
Calls	7.72 ± 0.925	2.91 ± 1.395	2.30	0.026*
Match calls	0.33 ± 0.213	–	–	–
Squeal duet	3.91 ± 0.795	3.08 ± 0.668	0.073	0.314
Bill wiping	3.57 ± 1.157	5.75 ± 1.508	0.137	0.714
Feeding	0.31 ± 0.213	0.58 ± 0.287	0.638	0.202
Tail fan	1.12 ± 0.822	0.83 ± 0.505	1.001	0.079
Wing flash	4.86 ± 0.853	4.83 ± 1.429	0.456	0.090
<i>Curve-billed Thrasher</i> ($n = 18$ Urban, $n = 16$ Desert)				
Proportion of time observed	0.83 ± 0.23	0.91 ± 0.11	0.382	0.711
Air approach	7.56 ± 0.549	2.31 ± 0.497	6.83	< 0.001*
Ground approach	5.63 ± 0.530	1.56 ± 0.67	3.01	< 0.001*
Fly over	6.38 ± 0.555	1.19 ± 1.17	1.99	0.035*
Song	3.53 ± 0.444	5.25 ± 0.392	0.044	0.384
Calls	6.11 ± 1.137	3.62 ± 0.789	1.98	0.041*
Match calls	0.81 ± 0.301	0.75 ± 0.381	0.059	0.414
Bill wiping	0.40 ± 0.831	1.43 ± 0.24	1.720	0.039*
Feeding	0.44 ± 0.258	0.18 ± 0.135	0.177	0.679
Wing flash	1.28 ± 0.348	0.56 ± 0.24	1.51	0.046*

Additionally, neither baseline total nor free plasma levels of either hormone were associated with capture date, suggesting that these levels did not fluctuate during the thrasher reproductive period sampled (all $p \geq 0.681$). Estimated free CORT concentrations were higher in urban birds than desert birds, but free T concentrations were similar (Table 4, Fig. 3). We estimated that, at baseline levels, $98.1 \pm 0.1\%$ of CORT and $94.9 \pm 0.2\%$ of T were bound to CBG. Birds caught without or with the assistance of song playback had comparable baseline levels of T and CORT ($p \geq 0.992$), but firm conclusions on this topic are limited by the small number of birds captured (urban: $n = 2$; desert: $n = 2$). Total and free baseline T or CORT were not associated either with territorial or with non-territorial behavior (PC1 or PC2, respectively (all $p \geq 0.216$).

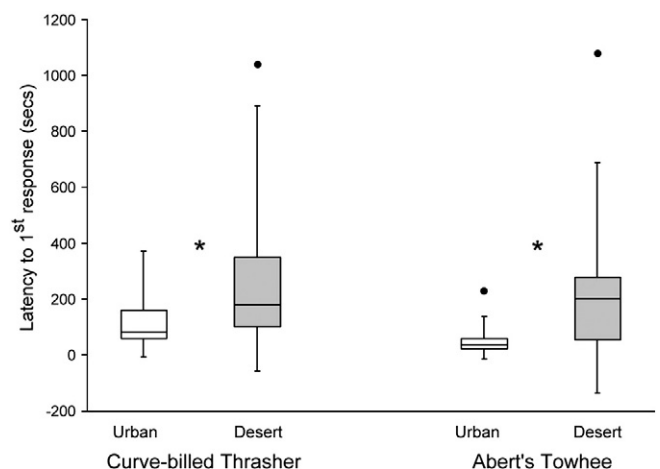


Fig. 1. Differences in latency to response to simulated territorial intrusion between urban and desert populations of Curve-billed Thrasher and Abert's Towhee. Boxplot illustrates median, 10th and 90th percentiles, bars indicate range, and circles indicate outliers. Sample sizes: Urban thrashers $n = 19$, desert thrashers $n = 20$, urban towhees $n = 12$, desert towhees $n = 13$.

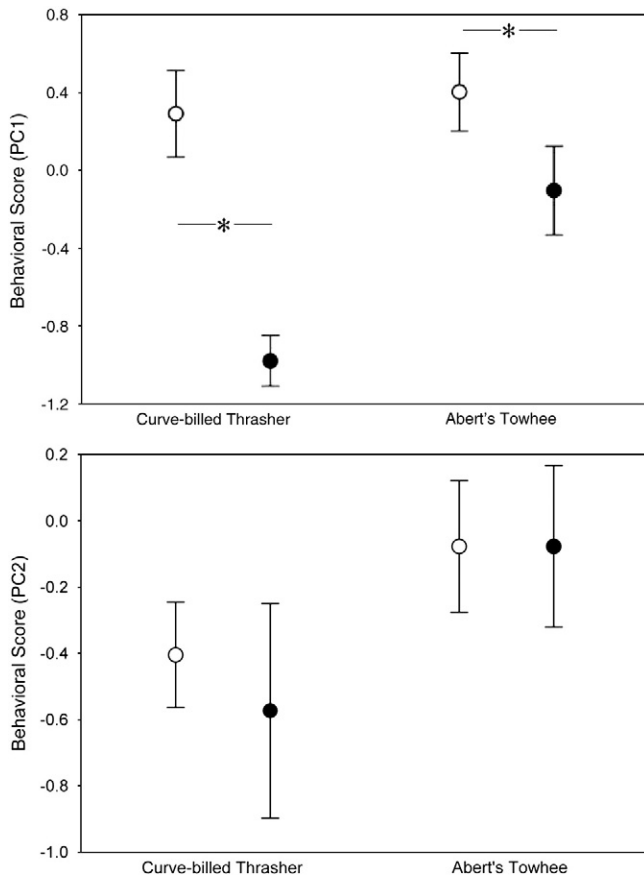


Fig. 2. Mean principal component (PC) scores based on behavioral responses to simulated territorial intrusions in urban (unfilled) and desert (filled) populations of Curve-billed Thrashers and Abert's Towhees. See methods for explanations of PC analysis and Table 2 for component behaviors. Bars indicate standard errors of the mean. Sample sizes: Urban thrashers $n = 19$, desert thrashers $n = 20$, urban towhees $n = 12$, desert towhees $n = 13$. * indicates significant differences ($P \leq 0.05$) between populations.

Table 4

Baseline and stress-induced (30 min) concentrations (mean \pm s.e.) of total testosterone (T), total corticosterone (CORT), corticosterone-binding globulin (CBG), and free (unbound to CBG) fractions of T and CORT in both urban and desert populations of two Sonoran Desert bird species. Hormone (T and CORT) data are presented in ng/ml of plasma, and CBG data are shown in nM. * indicates significant differences ($P \leq 0.05$) between populations based on analysis of covariance.

	Urban	Desert	<i>F</i>	<i>df</i>	<i>p</i>
Curve-billed Thrasher					
Total Baseline T	5.54 ± 1.284	4.49 ± 1.404	0.263	1, 39	0.612
Total Stress T	3.11 ± 0.730	3.76 ± 0.799	0.310	1, 39	0.581
Total Baseline CORT	9.57 ± 2.632	15.00 ± 2.878	1.680	1, 39	0.084
Total Stress CORT	48.09 ± 6.882	36.23 ± 7.523	2.170	1, 39	0.007*
CBG	368.28 ± 19.632	377.48 ± 21.463	0.870	1, 39	0.770
Free Baseline T	0.31 ± 0.094	0.26 ± 0.103	0.137	1, 39	0.714
Free Stress T	0.35 ± 0.102	0.21 ± 0.112	0.721	1, 39	0.402
Free Baseline CORT	0.19 ± 0.068	0.33 ± 0.074	1.084	1, 39	0.179
Free Stress CORT	4.18 ± 2.000	1.28 ± 2.186	1.830	1, 39	0.039*
Abert's Towhee					
Total Baseline T	7.48 ± 3.604	5.82 ± 1.651	0.180	1, 22	0.677
Total Stress T	3.77 ± 0.924	2.08 ± 0.272	2.860	1, 22	0.109
Total Baseline CORT	10.37 ± 3.711	10.02 ± 3.572	0.005	1, 22	0.947
Total Stress CORT	38.27 ± 5.253	40.23 ± 7.031	0.033	1, 22	0.858
CBG	486.18 ± 31.523	435.41 ± 72.392	0.311	1, 22	0.584
Free Baseline T	0.42 ± 0.126	0.31 ± 0.092	0.177	1, 22	0.679
Free Stress T	0.32 ± 0.125	0.14 ± 0.116	1.101	1, 22	0.309
Free Baseline CORT	0.13 ± 0.081	0.14 ± 0.048	0.016	1, 22	0.900
Free Stress CORT	0.81 ± 0.104	0.73 ± 0.253	0.045	1, 22	0.835

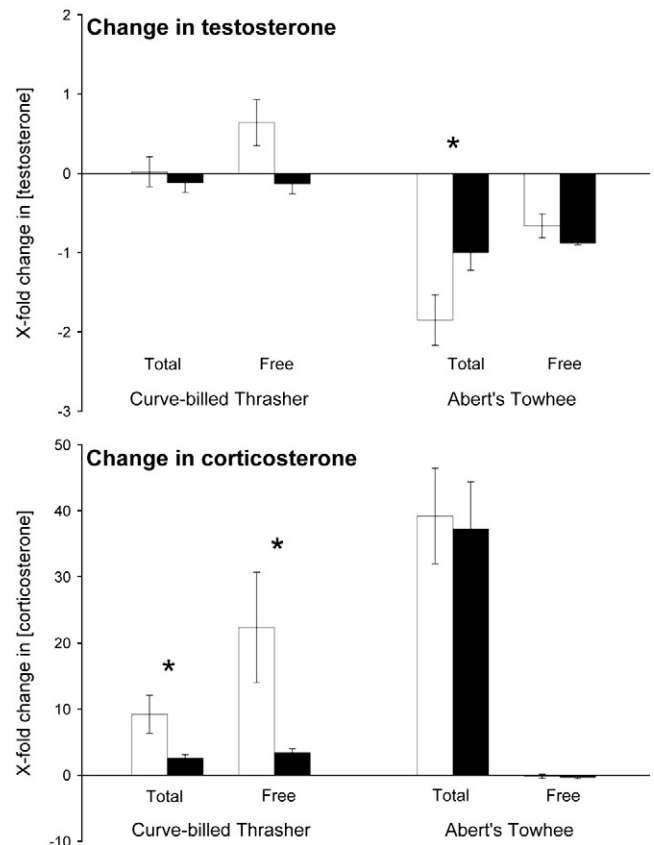


Fig. 3. Effect of capture and handling on steroid hormone levels via X-fold changes relative to baseline levels in both total and free (unbound to binding globulins) testosterone and corticosterone with 30 min of capture and handling in two bird species: Curve-billed Thrasher (urban: $n = 18$; desert: $n = 16$) and Abert's Towhee (urban: $n = 10$; desert: $n = 11$). White and black bars indicate urban and desert populations, respectively. * indicates significant differences ($P \leq 0.05$) between populations based on analysis of covariance.

Plasma total CORT and free CORT ($t = 2.833$, $p = 0.036$) increased in response to 30 min of capture and restraint ($t = 7.408$, $p < 0.0001$ and $t = 2.833$, $p = 0.036$, respectively). After 30 min of capture and restraint, plasma total CORT was higher in urban than desert birds; however there was no difference in plasma total T between sites (Table 4, Fig. 3). We estimated that, after 30 min of restraint, $96.2 \pm 0.9\%$ and $91.6 \pm 1.9\%$ of CORT and T were bound to CBG, respectively. Plasma total T decreased after 30 min of capture and restraint ($t = -2.264$, $p = 0.029$) but plasma free T did not change significantly ($p = 0.957$). Urban and desert thrashers had similar plasma T in response to restraint (Fig. 3).

Towhees

Baseline plasma hormone (free and total) and CBG concentrations did not differ between urban and desert birds (Table 4). Similarly, hormone levels did not differ between urban and desert towhees after 30 min of capture and restraint (Table 4, Fig. 3). Capture date was not associated either with baseline total or with free levels of CORT or T, indicating that hormone levels did not change during the study period (all $p \geq 0.443$). Plasma baseline T and CORT were also similar in passively captured towhees and in birds captured using playback recordings (desert: $n = 3$; both hormones $p = 0.981$). We estimated that at baseline levels, $98.7 \pm 0.1\%$ and $95.0 \pm 0.2\%$ of plasma CORT and T were bound to CBG, respectively.

As in thrashers, plasma total T decreased after 30 min of capture and restraint ($t = -2.426$, $p = 0.024$), and this decrease was more pronounced in urban than desert towhees (Fig. 3), but plasma free T did not change ($p = 0.222$). After 30 min of capture and handling, 98.3

±0.1% and 93.6±0.6% of plasma CORT and T were bound to CBG, respectively. Plasma total CORT ($t = 6.709, p < 0.0001$), but not plasma free CORT ($p = 0.064$), increased following capture and restraint (Fig. 3). As for thrashers, neither behavioral score (PC1 nor PC2) was associated with plasma total or free baseline T or CORT (all $p \geq 0.136$).

Does playback exposure elevate plasma T or CORT?

There was no statistical association between the duration of exposure to playback (from start of playback until bird capture; 8.3 ± 19.1 min: range 13 s to 34 min) and plasma total or free baseline total plasma T or CORT (all $p \geq 0.272$) in thrashers. Likewise, playback duration (4.6 ± 11.3 min: range: 27 s to 26 min) did not alter plasma total or free baseline total T or CORT (all $p \geq 0.073$) concentrations in towhees.

Is territorial behavior related to population density or nest site availability?

In thrashers, population density (2.3 ± 1.4 and 3.4 ± 1.1 birds/transect for urban and desert, respectively) was not associated with behavior (PC1 or PC2 scores) either in urban or in desert birds (site × population density: both $p \geq 0.452$). In towhees, territorial behavior (PC1) was positively associated with population density (3.6 ± 0.9 and 1.8 ± 0.5 birds/transect for urban and desert, respectively) in urban (site × population density: $F_{3, 12} = 7.787, p = 0.020$), but not desert areas. Towhees in high density urban areas showed more territorial behavior to playback ($p = 0.009$) than birds in low density urban areas. In contrast, non-territorial behavior (PC2) was not associated with population density in urban or desert areas (site × population density: both $p = 0.499$). There were no associations between population density and any hormonal parameter measured in either species (all $p \geq 0.064$).

Variation in territorial behavior was linearly related to habitat composition in both urban ($F = 4.219; p = 0.014$) and desert ($F = 2.361; p = 0.043$) thrashers according to CCA analysis with 500 random permutations. For urban thrashers, thin-leaved evergreens containing thorns, shrubs, prickly pear, teddy bear cholla, and canebrake were significant predictors of variation in territorial behavior (Table 5). However, some desert-type vegetation (TET, PP, TbC) were associated primarily with territorial behaviors (PC1), and non-territorial behavior (PC2) was associated with non-desert (Sh, Cb) vegetation (Fig. 4).

Habitat explained less variation in behavior in desert than urban birds. The best predictor of behavior in desert thrashers was the presence of teddy bear cholla, but other predictors included other cacti, creosote, and thorny thin-leaved evergreens (Table 5). All significant habitat predictors were associated with territorial behavior (Fig. 4). Thrashers captured at urban sites with larger Euclidean distances (i.e., distances between two points) than nearby random sites were also more territorial (i.e., higher PC1 scores: $r = 0.73; F = 2.383; p = 0.007$) but this was not the case in desert thrashers ($r = 0.36; F = 0.042; p = 0.64; Fig. 5$).

Habitat variables were linearly related to territorial behavior in towhees sampled in both urban and desert areas (urban: $F = 1.145; p = 0.010$, desert: $F = 1.012; p = 0.026$). Over 50% of the variation in territorial behavior in urban towhees was explained by the presence of shrubs, but the presence of various tree types were also related to territorial behavior (Table 5). By contrast, territorial behavior in desert towhees was primarily associated with creosote and thin-leaved evergreens (Table 5). Unlike in thrashers, there were no distinct associations between behavior and vegetation associated either with non-desert or with desert-type vegetation in either urban or desert towhees (Fig. 6). Territorial behavior in both urban and desert towhees did not show associations between the Euclidean distance between sites of capture and nearby random sites (both $p \geq 0.10; Fig. 5$).

Table 5

Percentage of variance in territorial behaviors explained by habitat variables in urban and desert populations of two Sonoran Desert bird species. **Bold** indicates significant ($P \leq 0.05$) association with behavioral data. See Table 1 for definitions of variable abbreviations.

Variable	Curve-billed Thrasher		Abert's Towhee	
	Urban	Desert	Urban	Desert
BE	–	–	5.3	0.4
TEnt	3.9	1.1	10.7	24.9
TEt	37.4	6.4	5.6	0.0
Con	–	–	0.2	0.9
M	0.8	0.0	5.9	2.6
D	1.9	0.0	4.3	6.0
Sh	16.5	0.9	51.2	17.4
Hg	0.5	0.0	0.6	0.2
Sag	0.3	11.6	–	–
TbC	6.6	19.4	0.0	0.2
ChC	0.5	0.6	–	–
BuC	0.6	7.9	0.5	0.0
PP	14.0	13.3	1.4	0.0
BC	0.3	4.5	0.2	0.3
Cr	3.4	13.9	3.8	25.6
Yuc	0.0	0.6	–	–
Ag	2.1	1.8	3.6	0.9
Cb	3.8	0.0	3.0	0.0
Oco	0.3	8.7	0.1	0.2
W	0.2	0.2	0.0	8.3
TP	1.1	0.0	0.0	1.2
TW	0.8	0.0	0.6	1.7
T	–	–	0.0	0.2
Fen	2.4	0.0	1.2	1.0
Sig	1.8	0.0	0.6	1.3
Lig	0.8	0.0	0.4	1.9
Cow	0.0	0.2	0.9	3.0

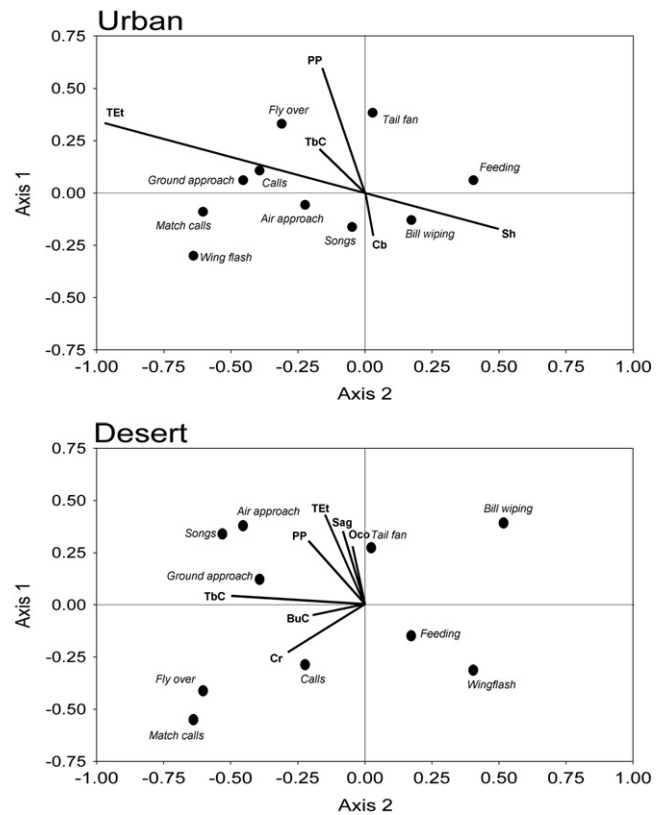


Fig. 4. Canonical correspondence analysis plot relating habitat variables to territorial behaviors demonstrated by urban and desert populations of Curve-billed Thrashers in response to simulated territorial intrusion. Length and direction of vectors correspond to the degree of association between habitat and behavior variables. See Table 1 for abbreviations to habitat variables.

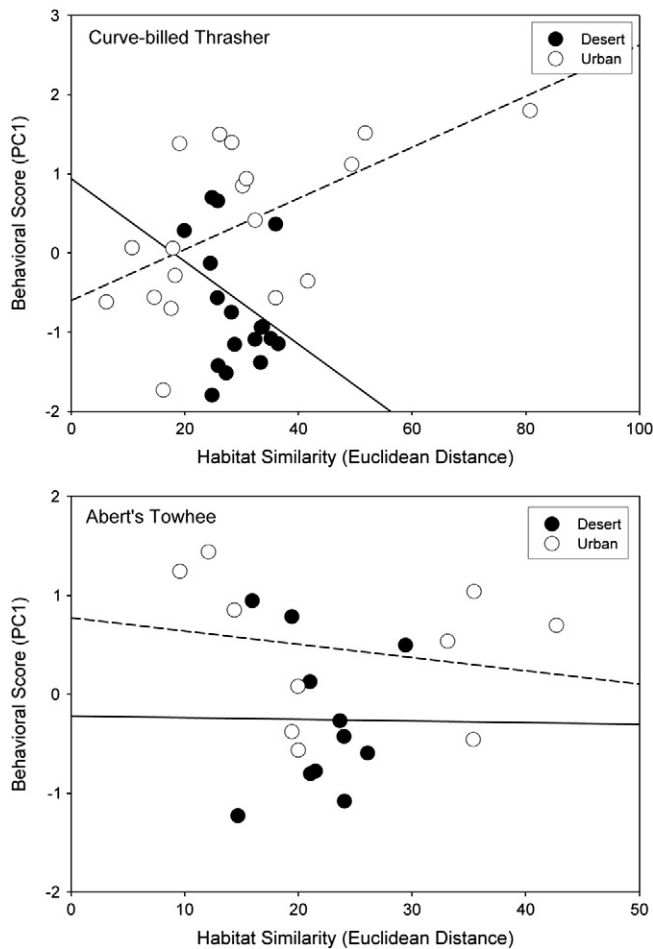


Fig. 5. Relationship between territorial behaviors (principal component “PC” score) to simulated territorial intrusion and the degree of similarity in habitat composition between sites of capture and randomly sampled sites as assessed by Euclidean distances between points. Solid and dashed lines indicate relationships in desert and urban populations, respectively.

Discussion

We compared territorial behavior and its association with plasma steroids, population density, and potential nest site availability, in urban and desert populations of two Sonoran Desert breeding bird species. Urban birds of both species responded with more territorial behaviors during a simulated territorial intrusion than their desert counterparts, but this difference was apparently not related to plasma T or CORT. Contrary to the predictions of the “challenge hypothesis,” prolonged exposure to simulated territorial intrusion did not alter plasma hormone levels in either species (Goymann et al., 2007; Moore, 2007; Wingfield et al., 1990). Territoriality during the breeding season is related to plasma T in some (Ferree et al., 2004; Goymann et al., 2007; Lynn et al., 2000; Moore, 2007; Wingfield et al., 2001) but not other species (Lynn and Wingfield, 2005; Lynn et al., 2005, 2007). In our study, neither thrashers nor towhees showed an association between territorial behavior and circulating total or free T levels. Experimentally elevating plasma T often decreases paternal care (Lynn et al., 2000) and Lynn (2008) proposed that the relationship between male territorial behavior and T may be uncoupled in species showing a high degree of male parental investment. The two species that we studied are monogamous and exhibit biparental care, as seems to be the case of many desert-dwelling birds (Tweitt, 1996; Tweitt and Finch, 1994). As deserts have highly variable food resource availability, biparental care may be needed to successfully fledge young of desert-dwelling birds (Vleck, 1993). Plasma T in many monogamous birds is elevated only at the beginning of the

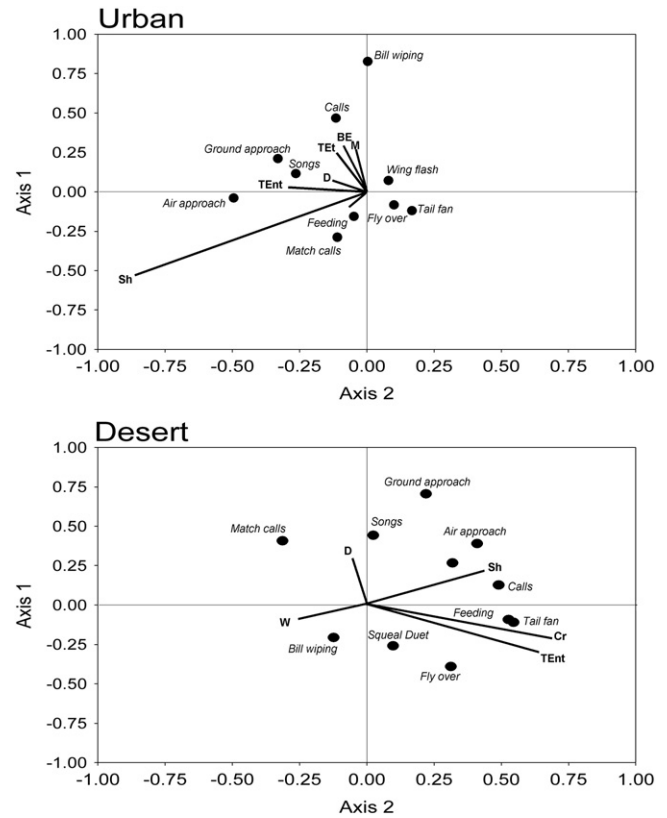


Fig. 6. Canonical correspondence analysis plot relating habitat variables to territorial behaviors demonstrated by urban and desert populations of Abert's Towhees in response to simulated territorial intrusion. Length and direction of vectors correspond to the degree of association between habitat and behavior variables. See Table 1 for abbreviations to habitat variables.

breeding season. This seasonal pattern has been reported in thrashers (Vleck, 1993), but not towhees. In thrashers, Vleck (1993) reported high plasma T levels from January to March and lower hormone levels during the rest of the year. In this study, we did not detect any change in plasma T in either species during the sampling period (March to July), which is consistent with the above observations. Despite seemingly low T levels through most of the year, thrashers and towhees exhibit territorial responses to playback year round.

The relationship between plasma T and CORT

An objective of the present work was to research relationships between steroids and the expression of territorial behavior. We found no relationship between plasma total T and behavior. One explanation may be that our study design, which did not include a live decoy, may have been sufficient to elicit a behavioral response, but was not sufficient to elicit a hormonal response to territorial intrusion. There is very little information concerning how the presence/absence of a live decoy can alter territorial behavior, however decoy type altered both behavioral and hormonal responses of European Robins, *Erithacus rubecula*, (Scriba and Goymann, 2008). Although this may be an important consideration, the observation in our two study species that territorial behavior in response to solely song playback induced without elevating plasma T levels suggests increasing total concentrations of this hormone is not directly required for territorial behavior during a short-term intrusion. Changes in the plasma free T (i.e., T not bound to CBG) may be more critical for the regulation of behavior than total plasma T (Breuner and Orchinik, 2002; Deviche et al., 2001; Lynn et al., 2007). We investigated this hypothesis by also measuring plasma CBG and CORT and estimating how an elevation in plasma CORT, such as resulting from 30 min of capture and restraint,

alters free T in plasma. In thrashers and towhees, CORT and T bind competitively to “CBG” with high affinity (Fokidis et al., 2009). Therefore, an increase in plasma CORT presumably increases the number of binding sites on CBG occupied by CORT and thus decreases the number of binding sites for T, in turn increasing plasma free T. Thus, if territorial behavior is modulated by short-term changes in plasma T, an increase in plasma CORT induced by territorial intrusion, as observed in other studies (Landys et al., 2007; Van Duyse et al., 2004), may increase territorial behavior. However, we found no association between territorial behavior and total or free plasma CORT. Furthermore, song playback did not elevate plasma total or free CORT or T levels and plasma hormone levels did not differ between birds that we caught passively or using song playbacks. Collectively these data suggest that territory holders do not perceive simulated territorial intrusion as stressful, at least in terms of stimulating glucocorticoid secretion.

The acute 30 min of capture and handling protocol that we used in the present study increased total plasma CORT and decreased total plasma T in thrashers and towhees. These results are consistent with those of other studies (Baird and Hews, 2007; Husak et al., 2007; Quillfeldt et al., 2006). The mechanism by which acute stress decreases plasma T in birds is not entirely understood and likely involves multiple pathways acting at various levels of the reproductive endocrine axis (see review in Wingfield and Sapolsky, 2003). In a recent study on male Rufous-winged Sparrows, *Poecetes gramineus*, suggests that this decrease partly results from a direct impairment of endocrine testicular function (DeViche et al., 2010). In our study 30 min of capture and restraint led to elevated free CORT in towhees but not thrashers and did not affect free T levels in either species. The lack of effect of this acute stress on plasma free T suggests that CBG is not directly involved in the control of territorial behavior.

Thrashers and towhees maintain territorial behavior year-round, even during the non-breeding season when gonadal steroid secretion is presumably low. Territorial behavior outside the breeding season in birds may be mediated either by steroids other than T and such as the androgen precursor dehydroepiandrosterone (DHEA: Hau et al., 2004; Soma, 2006; Soma et al., 2004) or through changes in brain levels of aromatase, which may alter territorial behavior independent of plasma T (Schlinger and Callard, 1989; Balthazart and Foidart, 1993; Soma et al., 2003). Another possibility is that differences in the brain levels of arginine vasotocin (AVT), a neuropeptide known to influence aggression in birds (Goodson, 1998a, 1998b; Goodson et al., 2009), explain the differences in territorial behavior between urban and desert birds. Administration of AVT to thrashers elevated plasma CORT more in urban than desert birds, suggesting higher sensitivity of pituitary gland corticotrophic cells in urban than desert thrashers (Fokidis and DeViche, in review). Testosterone administration to rats increases AVT immunoreactivity in several brain regions associated with aggression (e.g., amygdala). Similarly, in Dark-eyed Juncos, *Junco hyemalis*, castration reduced AVT immunoreactivity in several areas of the brain associated with social behavior and this reduction was reversed by T administration that resulted in high physiological circulating levels of this hormone (Plumari et al., 2004). How changes in AVT brain expression regulate territorial behavior during competitive bouts remains, however, unclear (reviewed in De Vries and Panzica, 2006). Together, these suggest that the central AVT system is involved in territorial responses to changes in androgens levels, but how habitat type may impact this physiological system is unknown. Future studies should compare levels of these endocrine mediators of territorial behavior between or within species that occupy different habitat types and should incorporate hormone inhibition and replacement studies to elucidate mechanisms.

Why would urban birds be more territorial?

Few studies have investigated the mechanisms that control context-specific territorial behavior in free-ranging animals. Under-

standing the specific factors that potentially contributed to the evolution of territoriality in birds may assist in determining its regulation. We measured two ecological parameters that differ between urban and desert populations of our study species: Population density and vegetation composition. Among urban towhees, higher urban population densities were associated with increased territorial behavior. This association was not observed in thrashers or in towhees inhabiting desert areas. Towhees in Phoenix are found at similar or higher densities than in appropriate desert habitat, whereas the opposite is seen in thrashers. Increased territorial behavior by urban towhees may facilitate their establishment of breeding territories in areas where intrusions by conspecific birds is more common due to higher densities. Previous studies also found a positive association between territorial behavior level and population density (Marvin, 1998; Vervust et al., 2009), but this observation cannot explain all our behavioral data. Habitat-related differences in territorial behavior in towhees were not related to variation in plasma T or CORT and their physiological basis is presently unclear.

Urban thrashers showed a positive association between territorial behavior and the degree of difference in vegetation composition between the capture site and surrounding randomly sampled areas (i.e., habitat similarity). This observation suggests that higher levels of territorial behavior are exhibited by urban thrashers that defend “unique” territories, relative to surrounding areas, compared to birds that defend more homogeneous territories. In urban and desert thrashers, the more territorial behaviors of thrashers were associated with “thorny” desert-type vegetation such as cholla, prickly pear cacti, and mesquites. These plant types, particularly the cholla, serve as important nest sites for this species (Tweit, 1996), suggesting that increased territorial behavior is associated with defense of high quality nesting grounds (i.e., sites with extensive thorny vegetation). In towhees, this type of association was less prominent in both urban and desert habitats. Towhees are largely riparian birds and nest primarily in mesquite (*Prosopis* spp.), coyote bush (*Baccharis* spp.), and wolfberry (*Lycium* spp.; Tweit and Finch, 1994). In urban areas, Abert's Towhees can nest in any dense shrub available (Fokidis, personal observation). Collectively these results can be interpreted as evidence for habitat-dependent territorial behavior that is regulated independently of plasma T or CORT. We propose that birds alter their territorial responses based on perceptions of habitat quality (as in thrashers) or cues associated with population density (as in towhees), and not as a function of differences in plasma T or CORT.

Another factor that may explain differences in territorial behavior between urban and desert birds is variation in signal transmission between noisy urban and quieter rural habitats (Brumm, 2006; Slabbekoorn and den Boer-Visser, 2006; Slabbekoorn and Ripmeester, 2008; Warren et al., 2006). In noisier (urban) habitats, birds may need to rely increasingly on territorial displays in close proximity, as vocal communication from a distance may not be sufficient to ward off a potential intruder.

Intraspecific studies comparing levels of territorial behavior between populations of free-living animals are few. A study comparing Dark-eyed Juncos from a natural population with birds living in a recently colonized urban area (San Diego, California) showed that territorial responses to song playback were decreased in the urban population (Newman et al., 2006). The decreased territorial behavior may be associated with a decrease in sexual selection due, at least in part, to a lengthened breeding season in the milder urban climate (Price et al., 2008). However, another study found no correlation between territorial responses of Noisy Miners *Manorina melanocephala* and the proximity to road edges (Maron, 2009). These studies highlight how territorial behavior can be modulated by local environmental factors and differences between species can vary depending on what territorial behavior is meant to defend.

Urban birds may cope with the fragmentation of nesting habitat in cities by promoting territorial behaviors that ultimately may increase

fitness. Historically in birds, territorial behavior has been thought to be largely T-mediated. Our research suggests that in urban birds with year-round territorial behavior, this interaction is independent of T and CORT regulation and may be mediated by neuropeptides or hormones not examined in the present study. Further studies are warranted to explore which neural and hormonal mechanisms regulate such territorial behavior in urban animals, and how these mechanisms are shaped by local ecological factors.

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