

CORRELATES OF AGGRESSION: THE INTERPLAY BETWEEN BOLDNESS,
TESTOSTERONE, AND TERRITORIALITY IN MALE SONG SPARROWS,
MELOSPIZA MELODIA, IN URBAN AND RURAL HABITATS

By

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A thesis presented to the faculty of the Graduate School of
Western Carolina University in partial fulfillment of the
requirements for the degree of Master of Science in Biology

By

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ABSTRACT

CORRELATES OF AGGRESSION: THE INTERPLAY BETWEEN BOLDNESS, TESTOSTERONE, AND TERRITORIALITY IN MALE SONG SPARROWS, MELOSPIZA MELODIA, IN URBAN AND RURAL HABITATS

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Animals colonizing urban habitats are often noticeably bold in the presence of humans, and such boldness is typically thought to arise as urban individuals habituate to the repeated presence of humans. However, recent studies in animal behavior suggest that: 1) boldness may be an inherent trait as opposed to a learned behavior and 2) some individuals exhibit behavioral syndromes that restrict behavioral plasticity, and may limit an individual's ability to adapt to environmental change. In a recent study, we examined differences in urban and rural populations of song sparrows and confirmed that urban birds were bolder toward humans, but also, this boldness was correlated with higher levels of territorial aggression. This study also examined the correlation between testosterone, a hormone associated with aggression in birds, and variation in aggression and boldness in urban and rural song sparrows (*Melospiza melodia*). Despite significant differences in aggression and boldness between urban and rural populations, this study found no evidence of differences in testosterone between the two populations. It is possible that other mechanisms (e.g. sensitivity to circulating testosterone) may be

involved, and further examination into these possibilities will provide better understanding of these behavioral differences.

BACKGROUND

Animals colonizing urban habitats are often noticeably bold in the presence of humans, and such boldness is typically thought to arise as urban individuals habituate to the repeated presence of humans. (Anderson et al. 1999; Metcalf et al. 2002). If boldness in the presence of humans is a result of habituation, then the process is a learned behavior and humans are not considered to be predators by the animals (Evans et al. 2010). However, recent studies in animal behavior suggest that: 1) boldness is an inherent trait, not a learned behavior, which may determine which individuals invade an urban environment (Sol & Lefebvre 2000), and 2) some individuals exhibit behavioral syndromes that restrict behavioral plasticity, a phenomenon that is correlated with the ability of an individual to adapt to changes in an environment (Sih et al. 2004). In this case, one or more of the behavioral traits associated with the behavioral syndrome may be exaptation(s), arising with other adaptive behavioral traits because of coexpression by some mechanism such as hormones (Ketterson & Nolan 1999).

In recent studies of behavioral differences between urban and rural populations of song sparrows (*Melospiza melodia*), we found that urban birds were bolder toward humans, but surprisingly, we also found that urban birds had higher levels of territorial aggression (Evans et al. 2010). Though we did not expect urban song sparrows to be more aggressive, a correlation between boldness and aggression has been shown in several species including sticklebacks (*Gasterosteus aculeatus*) (Bell & Stamps 2004), fiddler crabs (Reaney & Backwell 2007), and funnel web spiders (*Agelenopsis aperta*) (Riechert & Hedrick 1993). This raises the possibility that urban habitats may select for

bold birds and that high levels of aggression arise merely as a correlated consequence of urban habitats selecting for bold birds. Alternatively, urban habitats may actually select for high aggression for reasons that are currently unclear.

One possible reason why birds in urban habitats may need to be more aggressive is that this habitat type may actually be ideal for nesting and survival, leading to higher competition and territorial aggression for urban habitat. Such possible benefits of urban habitats might be warmer temperatures, or greater light availability that can result in earlier dawn chorusing (Miller 2006) which may be preferred by females (Otter et al. 1997). In great tits (*Parus major*) dawn chorus is used as a territorial mechanism and earlier singing males may be able to devote greater activity to territorial defense prior to their mates becoming active for the day (Slagsvold et al. 1994). Urban male song sparrows could benefit from more light availability, and thus earlier dawn chorusing, by having more time available for territorial singing and defense without taking away from other important activities (e.g. foraging and mate guarding).

Alternatively, urban habitats may actually be a poor habitat type, and increased risk of predation and greater territory turnover may lead to higher competition for territories. Several studies suggest that urban habitats possess greater abundance of predators (Jokimaki & Huhta 2000; Sorace 2002). In urban habitats where birds are generally bolder, animals may spend more time in the open, which could lead to higher rates of predation, and such a factor could lead to higher territory turnover and more territory boundary instability (Evans et al. 2010). In this case, greater territory turnover could lead to higher levels of aggression within urban bird populations as males compete to takeover and maintain territories. Additionally, greater food availability in areas with

high population density has led to higher competition, and in some cases, may lead to reduced adult survivorship (Shochat 2004; Arcese & Smith 1988). Lastly, urban habitats may contain limited ideal nesting sites for breeding pairs and male song sparrows must increase their territorial aggression against would be intruders to retain their boundaries.

Previous studies in a number of species have demonstrated a correlation between aggression and high levels of testosterone (T), showing that higher levels of T are associated with higher levels of territorial aggression during the breeding season, and that T implants can induce high levels of aggression in males outside the breeding season (reviewed in Wingfield 1994; Goyman et al. 2007). This association between increased levels of circulating T and territorial aggression is most likely the outcome of male-male interactions during the breeding season and may decline as these interactions decrease in frequency, a phenomenon known as the “challenge” hypothesis (Wingfield et al. 1990).

If having higher circulating T results in stronger territorial aggression, then more aggressive males may gain the benefit of better reproductive success through acquisition of better territories or more mates. Monogamous male birds implanted with T showed an increase in territory size and aggression, resulting in polygynous behavior and increased mating success (Wingfield et al. 1990, see also Raouf et al. 1997). Another potential benefit of having higher T and aggression is better mate-guarding success. In instances where extra-pair fertilizations (EPFs) are a factor, male dark-eyed juncos (*Junco hyemalis*) with higher T had fewer extra pair young in their home nest than control subjects, presumably a result of more effective mate-guarding of their fertile partners (Raouf et al. 1997).

While there are benefits to expressing higher T levels there are also potential costs to having higher T, one possibility being decreased survivorship. Male mountain spiny lizards (*Sceloporus jarrovi*) that were implanted with T showed an increase in territorial aggression, and higher aggression increased daily movements and territorial displays, resulting in fewer foraging attempts, decreased lean body mass, and presumably greater predation risks (Marler & Moore 1989). Another potential cost may be decreased parental care, resulting in fewer surviving offspring. In some circumstances, T-implanted males experienced a decrease in parental behavior such as a lower rate of nestling feeding (Saino & Møller 1994; Stoehr & Hill 2000) and lower annual output of fledglings (Raouf et al. 1997). Lastly, current research has shown that both experimentally elevated levels of T (Duffy et al. 2000; Castro et al. 2001) and endogenous levels of T (Greives et al. 2006) may compromise immune system function.

As natural habitats are removed or fragmented as a result of urban sprawl, it is important to understand how human development impacts selection and diversity. If urban habitats are selecting specifically for bold and aggressive animals, then the diversity of behavioral phenotypes observed could be impacted. Selection for specific behaviors (e.g. reduced vigilance and defense in the presence of a predator) can result in small population extinctions (Sutherland 1998). Human-induced behavioral selection can also have a detrimental impact on conservation and reintroduction of a species as certain temperament traits hinder the species from adapting to the wild (McDougall et al. 2005). The purpose of this research presented here was to test for a correlation between testosterone level (a hormonal mechanism) and boldness/aggression (a behavioral syndrome) in song sparrows living in urban and rural habitats.

METHODS AND MATERIALS

STUDY SITES

Two sites were selected for this project: the campus of Western Carolina University in Cullowhee, NC (35°18'N, 83°11'W, elevation 640m) was used as the urban population, and Yonaguska Kituwah Cultural, Bryson City, NC (35°26'N, 83°24'W, elevation 546m) was used for the rural population. The rural population was found in habitats that consisted of farm plots, hedge and shrub rows in open fields or along a creek bank, dense weeds alongside a railroad track, a stream, and a riparian depression wetland. The urban population was found in habitats consisting of open lawns and parking lots with ornamental shrubs and hedges alongside university buildings or near campus roads. Urban habitats have been described as human developed areas containing more than 2500 people at the center (Dumouchel, 1975; cited in Chace & Walsh 2006), and there were over 3000 students residing on the campus between the Fall of 2009 and Spring 2010 (Barnett, pers. comm.).

TERRITORIAL MAPPING AND PLAYBACK

Territories of male song sparrows were mapped at each site by extensive observation of the locations of singing subjects and their interactions with neighbors during the breeding season when males are most active in territorial defense. Males were captured using mist nets and either playback of conspecifics, recorded distress calls, or by random catch. Territorial male subjects were banded using a unique color band combination and a USFW aluminum band for individual identification.

Levels of aggression were determined by measuring response to playback of conspecific song (Hyman & Hughes 2006). To perform playback experiments, a speaker (Amp Can, Fender, Inc.) was set up under a known song perch on an individual male's territory and a conspecific song was broadcasted for 6 minutes. Individual male's responses to playback song (number of times countersinging and distance to speaker) were recorded for the 6 minutes of song broadcast and for an additional 3 minutes after the playback concluded, for a total of 9 minutes. The proximity to the speaker and the number of songs males sang during and after the playback broadcast was used as a measure of the level of aggression in the individual male as described in Searcy et al. (2006).

DETERMINING BOLDNESS

Bold behavior in each territorial male was determined using a measure of flight initiation distance (FID) or the distance at which the territorial male being approached takes evasive action (hiding or flying away) in response to human presence (Blumstein et al. 2003). FID was measured by walking at a steady and quiet pace toward a singing male and measuring: 1) the distance at which we began our approach, 2) the distance to the subject at the point where the male took evasive action (e.g. flew away), and 3) the approximate height where the male was singing prior to the evasive action. FID was then calculated following protocol set in Blumstein (2006).

BLOOD SAMPLING AND TESTOSTERONE ANALYSIS

Blood samples from captured males were obtained from the brachial vein using a 27 gauge heparinized needle during a time when males were also being banded for identification. Blood was collected in capillary tubes and stored in a cooler that contained ice packs to preserve the blood in the field until the samples could be transported back to the lab. Each plasma sample was spun down in a centrifuge at 13000 rpm for 10 minutes to separate plasma from blood cells. Plasma was drawn using a pipette and transferred to a clean microcentrifuge tube and vortexed for 1 minute. Plasma samples were then placed in a freezer (-20°C) for later hormonal analysis.

Hormone analysis (159 samples total) was conducted in the lab of Dr. Jodie Jawor at the University of Southern Mississippi, Hattiesburg, MS. Plasma T levels were determined using a ELISA immunoassay (EIA) kit (Assay Designs, Ann Arbor, Michigan #901-065) as described in Clotfelter et al. (2004). The EIA procedure is a three day process (described more fully below): Day 1) samples are aliquotted and radiolabeled T is added in small amounts to determine extraction efficiency, Day 2) ether extraction (2 times) is performed followed by a reconstitution of the samples, and Day 3) EIA analysis is performed.

On the first day, frozen plasma was thawed and vortexed for approximately one minute. Plasma samples were transferred to a correspondent 15mL conical centrifuge tube using a Hamilton syringe. Ideally, a standard amount of 30 μ L of plasma was used for analysis, and if the desired amount was less, then distilled water was added to bring the total volume to this level. The amount of actual plasma aliquotted was recorded along with the plasma identification for future correction and analysis. Several test tubes with a

solution of known T concentrations were spaced randomly throughout the assay for the determination of inter- and intra-assay variation. For this analysis, three known concentration 'samples' were used per plate. In the next step, 20 μ L of labeled T (approximately 2000 cpm of H³-T) was added to the plasma and standards with another 20 μ L distributed into scintillation vials (along with 2.5 mL of UltimaGold scintillation fluid), vortexed, and stored in. The addition of labeled T was for the purpose of calculating extraction recoveries.

Next, extractions were performed using diethyl ether (2 extractions) 1mL of diethyl ether was added to each sample, vortexed, and set aside for 20 minutes to allow phase separation. Next, the test tubes were placed in a snap freeze bath with dry ice and methanol for approximately 30 seconds and the supernatant was poured off into clean 13x100 test tubes. Between each extraction, diethyl ether was evaporated by placing the test tubes in a water bath under nitrogen gas. Plasma extractions were then resuspended in 50 μ L of ethanol and diluted to 350 μ L with assay buffer from the EIA kit.

The final procedure of the T hormonal analysis was to establish the standard curve for assay calculation. From each of the reconstituted sample, 100 μ L were used to determine recoveries, and duplicate quantities of 100 μ L were used in the EIA. T levels were determined with a 10 parameter logistic curve-fitting program (Microplate Manager; Bio-Rad Laboratories, Hercules, California) and corrected for any incomplete recoveries and initial sample volume. Intraplate variation ranged from 1 to 36% (mean 14%), and interplate variation was 2.8%. Several standard points had rather high values (ranging from 7.23-11.5), and a plate correction factor was used to adjust the T levels for each plate accordingly.

STATISTICAL ANALYSES

Statistical analyses were performed using Systat software (Systat Software, Inc., Chicago, IL.) and SSPS 19 (SSPS, Inc., Chicago, IL.). Pearson's r was used to determine the correlation between boldness and aggression for both populations. Differences in boldness and aggression between the urban and rural populations were determined using a two-tailed, unequal sampling, t -test. T data was transformed using natural log (\ln) prior to analysis to a normalized the data distribution. The data was tested using a linear mixed model (LMM) with restricted maximum likelihood estimation to account for repeated sampling from each individual male.

RESULTS

Boldness (measured using FID) differed strongly between urban and rural populations, ($t = -3.910$, $df = 124$, $P < 0.001$; Figure 1), with urban male song sparrows allowing a significantly closer approach by a human observer. Additionally, aggression, which was determined using the response of focal birds to playbacks of conspecific song, differed significantly between the two sites ($t = -3.037$, $df = 166$, $P = 0.003$; Figure 2), urban males responded more strongly to playbacks than males at our rural site making a closer approach to the speaker. These results support observations from our previous study, which male urban males were more bold and aggressive than their rural counterparts (see Evans et al. 2010).

There was a significant correlation between boldness and aggression in the two populations when considered together (Pearson Correlation: $r_p = 0.28$, $N = 90$, $P = 0.008$, Figure 3). However, individuals at the rural study site (Kituwah) showed a significant correlation between boldness and aggression (Kituwah Pearson Correlation: $r_p = 0.351$, $N = 41$, $P = 0.024$), while the urban site did not show a significant correlation (Campus Pearson Correlation: $r_p = 0.10$, $N = 57$, $P = 0.452$).

Testosterone analysis revealed no significant difference in plasma T levels between the two populations (LLM: $df = 60$, $P = 0.175$, Table 1, Figure 4). There was a significant effect between Date and T levels when sampling for the two populations were considered together (LLM: $df = 60$, $P = 0.001$, Table 1).

Table 1: Table 2: Linear mixed model. Study site (grouping Kituwah and Campus with their respected InT plasma levels) was entered into the model as a fixed effect. Because plasma T was collect throughout the breeding season, Date was also entered into the model as a fixed effect. Bird ID (not shown) was used the error term.

Type I Tests of Fixed Effects

Fixed Effects	F-ratio	P-value
Site	1.888	.175
Date	3.444	.001

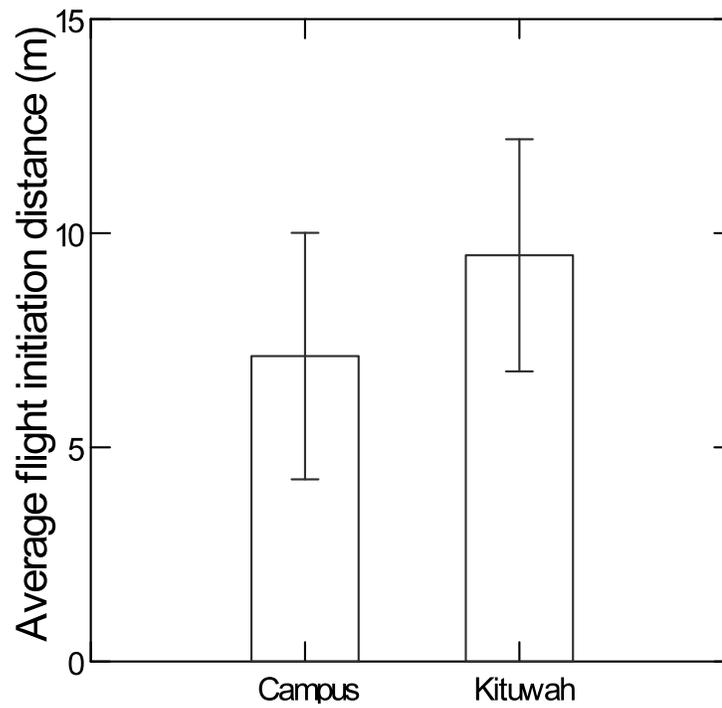


Figure 1: Average flight initiation distance in response to a human observer ($\bar{x} \pm SD$) for birds at two study sites: Campus, an urban habitat on the campus of Western Carolina University, in Cullowhee, NC, and Kituwah, a rural habitat located near Bryson City, NC, and owned by the Band of Eastern Cherokee Indians (EBCI). Urban birds have significantly smaller FID than rural birds (two sampled t-test: $t = -3.910$, $df = 124$, $P < 0.001$)

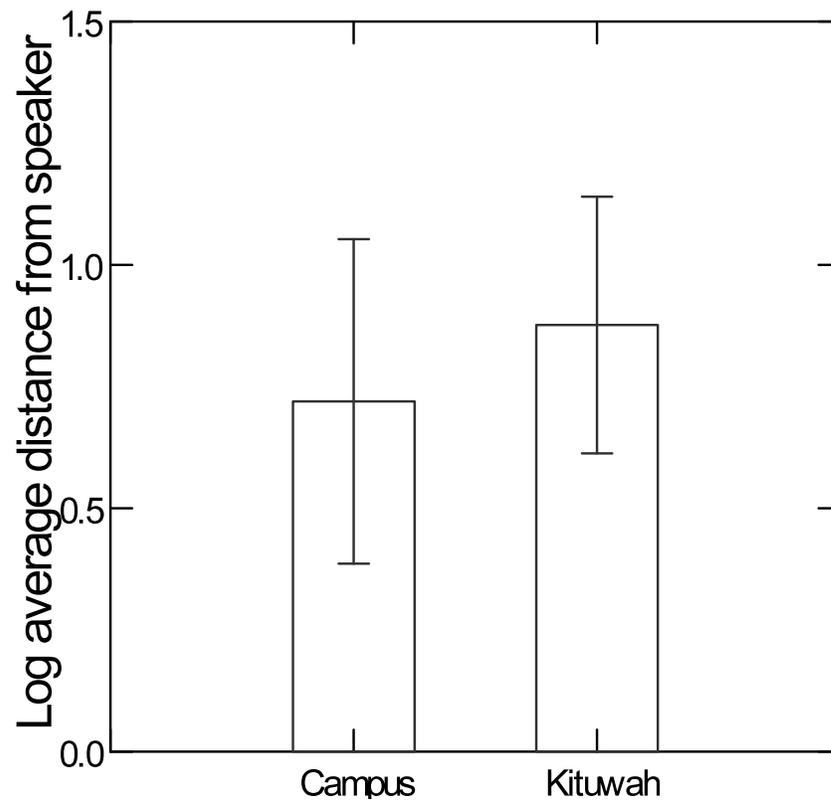


Figure 2: Average distance to speaker (log transformed, $x \pm SD$) for birds at two study sites. Birds in the urban population (Campus) show a significantly closer approach to playback than rural birds (Kituwah) (two sample t-test: -3.037 , $df = 166$, $P = 0.003$)

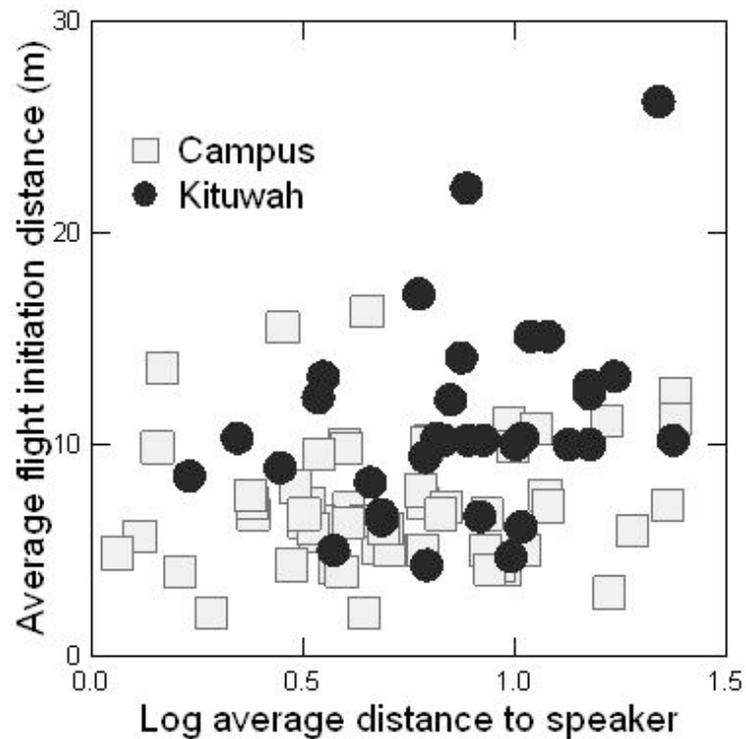


Figure 3: Correlation between boldness (flight initiation distance) and territorial aggression (log distance to playback) for birds at two study sites. Overall, there was a significant correlation between boldness and aggression in urban and rural populations (Pearson Correlation: $r_p = 0.28$, $N = 90$, $P = 0.008$). The rural site (Kituwah) also showed a significant correlation (Kituwah Pearson Correlation: $r_p = 0.351$, $N = 41$, $P = 0.024$). The urban site (Campus) did not show any correlation between boldness and aggression (Campus Pearson Correlation: $r_p = 0.10$, $N = 57$, $P = 0.452$).

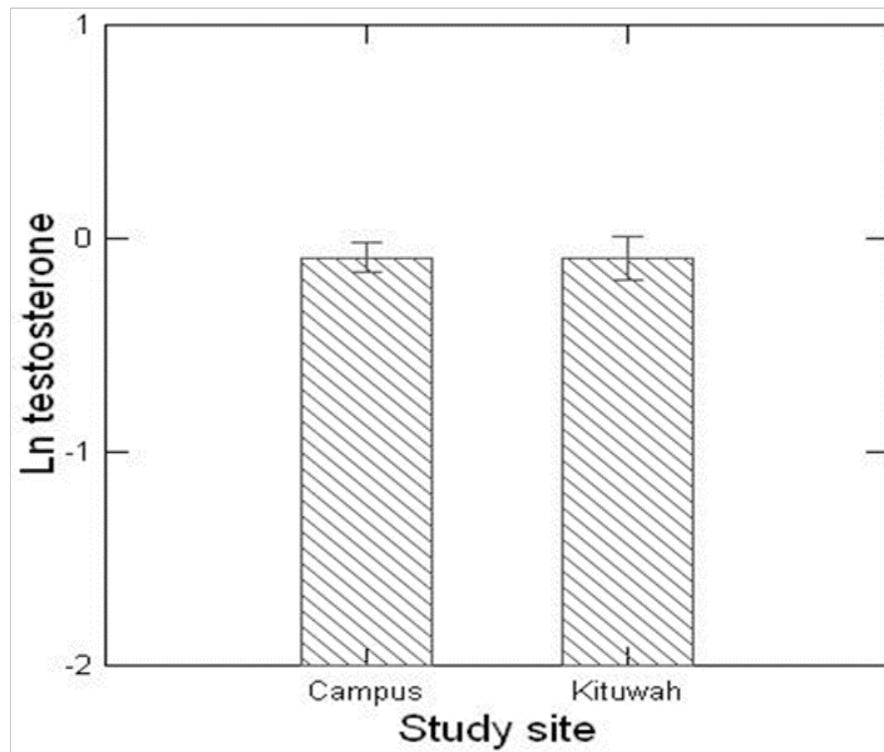


Figure 4: Potential differences between natural-log transformed testosterone levels (lnT) in urban and rural song sparrows. Differences in lnT levels were analyzed using a linear mixed model (LLM: $P = 0.175$).

DISCUSSION

In a previous study of song sparrow behavior, we determined that boldness and aggression were higher in urban song sparrow population than their rural counterparts (Evans et al. 2010). Boldness and aggression were correlated in rural, but not urban, populations. This study supports the previous findings. Urban male song sparrows displayed greater aggression and boldness, but these behaviors remained uncoupled as a behavioral syndrome.

It is unclear why urban male song sparrows show no correlation between boldness and aggression, despite repeated studies showing higher expression of these behaviors when compared to rural birds. Perhaps, the best possibility to explain this phenomenon is the influence of the environment on animal behavior. A study in damselfish (*Stegastes leucostictus*) showed that aggression and courtship were linked in natural, but not artificial, habitats (Snesker et al. 2008). For whatever undetermined factors within urban habitats, male song sparrows lack a behavioral syndrome, possibly, the result of adjusting to a changing environment. Testing for potential environmental factors that may account for uncorrelated behaviors in urban sites should be the focus of future studies.

Presently, the only environmental factor that has been shown to generate correlated behaviors is the risk of predation. High risk of predation may create a correlation between bold and aggressive behaviors, a phenomenon that has been shown in threespined sticklebacks (*Gasterosteus aculeatus*) (Bell & Sih, 2007). Though research that has examined abundance of avian predators in urban habitats is conflicting (Jokimaki & Huhta 2000; Sorace 2002; Chace & Walsh 2006), few avian predators have been

observed at the campus site (Boudreau, pers. obs.). With this in mind, the lack of any correlation between boldness and aggression in our studies may be the result of low predation rates that would otherwise generate a correlation, such as the correlation that is evident in the rural population in this study.

We are still left with the question as to why both boldness and aggression remain higher in urban males. In the previous study, urban males possessed greater boldness and aggression compared to the two rural study sites (Evans et al., 2010). Additionally, present data from this research also showed greater expression of these behaviors in urban birds (see Figures 1 & 2). Again, characteristics within the urban environment may influence the expression of these behaviors. There are two possible scenarios that may might explain why aggression is higher: 1) urban habitats may be ideal sites for breeding males, leading to higher competition and territorial aggression, or 2) urban habitats are poor sites, and certain urban characteristics may lead to higher competition for territories.

Within the urban environment, there are several characteristics that may be beneficial to territorial males. Urban areas contain extensive edge habitats that may create ideal territories for male song sparrows, and these preferred edge sites could generate higher aggression as males increase territorial defense against intruders. A study in painted buntings (*Passerina ciris*) showed that males that acquired edge habitats were behaviorally more aggressive toward possible intruders than those that settled in interior habitats (Lanyon & Thompson 1986). Another potentially beneficial characteristic of urban environments is greater light availability. Several studies have shown that greater light availability in urban habitats may create favorable conditions for territorial males, such as earlier dawn chorusing (see Slagsvold et al. 1994; Otter et al. 1997). Male song

sparrows in urban habitats have been heard singing as early as 2:30 AM in areas with abundant artificial light (Boudreau, pers. obs.). The use of earlier dawn chorusing for a territorial male song sparrow may provide the benefit of broadcasting their territory boundaries to potential intruders for longer periods of time. Future studies could examine the differences in time intervals at which male song sparrows sing in urban and rural habitats to see if earlier dawn chorusing is correlated with greater aggression.

Conflictingly, characteristics within urban habitats may also have poor qualities that encumber breeding males. Generally, urban habitats possess higher levels of noise pollution than rural sites. Greater noise pollution can have a negative effect on breeding males using song either to attract mates (Fernández-Juricic et al. 2005) or proclaim territorial boundaries (Brumm 2004). In perspective, noise pollution may dampen the ability for male song sparrows to broadcast song that advertises territory boundaries thus requiring greater behavioral aggression to enforce ownership of a territory. Recent documentation in song sparrow behavior revealed males singing at higher frequencies in noisier locations (Wood & Yezerinac 2006), though the potential effects on territoriality are unknown. Examining the influence of urban noise pollution on territorial aggression could be a promising study. Another potentially detrimental property of urban habitats is supplemental food resources. Supplemental food resources can have a negative affect on adult survival in song sparrow populations, presumably the result of increased population sizes and competition and the stress associated with that competition (Arcese & Smith 1988). It is quite possible that higher aggression may be the result of supplemental food resources within these territories, and urban males may be allocating more time for defense and less time for important activities like feeding and parental care.

There are several possibilities that may explain greater boldness in urban males. Presumably, animals exhibit greater boldness in urban habitats as the result of habituation to increased human activities (Anderson et al. 1999; Metcalf et al. 2002). Alternatively, bolder males in urban habitats may be the result of differential settlement between the two sites (Evans et al. 2010). In the first case, animals learn that humans are not a threat and become bolder through habituation. In the latter, animals possess an inherent trait that generates the bolder personality, which allows them to successfully inhabit urban environments. A recent study on FIDs in urban and rural populations revealed shorter FID scores in urban birds with consistent variation across several generations since urbanization, suggesting that tameness in urban birds is indicative of an adaption to urban environments (Møller 2008).

Alternatively, Snesker et al. (2008) proposed the possibility that certain behavioral types may be linked with inherent qualities, but individuals may be able to alter their personalities in response to environmental assessment. This suggests that, though certain behaviors can be inherent, environmental factors can control the expression of these behaviors. With urban male song sparrows, this may suggest that individuals have a natural tendency to be bolder, and the habituation to the presence of humans further influences the level of expression for this behavior. Presently, we cannot conclude that our urban male song sparrows express greater boldness as the result of a learned or inherent behavior. Future investigations on behavioral tests in captive birds from urban and rural habitats may shed light on this debate.

One facet of this study examined the relationship between hormonal mechanisms, in this case testosterone (T), and differences in behavioral expression between the two

populations. T levels did not vary significantly between the urban and rural populations, suggesting the possibility that higher circulating plasma T may not be the factor influencing higher aggression in urban males. However, the method for obtaining T data from both populations may have influenced the results. We collected blood from territorial males throughout the breeding season (May-August), and there was a significant correlation between date and T. As a result, T levels for both sample populations most likely reflect seasonal and not the ideal male-male responsiveness levels (Wingfield, pers. comm.). Ideally, for this study, measurements of T levels between the urban and rural populations would reflect the interaction between males as they vie for territories. This study provides evidence that seasonal T levels may not differ between two populations with strikingly different levels of territorial aggression.

T levels increase significantly during certain times within the breeding season when interactions with competitors and females are high (Wingfield et al. 1990; Goymann et al. 2007). Potentially, during these times, circulating levels of T are higher in urban than rural males (urban males are more aggressive). However, supposing that T levels do not vary between the two populations, there are other possible mechanisms underlying higher aggression in urban male song sparrows. First, urban male song sparrows may be hypersensitive to circulating plasma T. A similar event has been described as the “testosterone hypersensitivity hypothesis” where testosterone-dependent behaviors become sensitive to low levels of circulating T (Wingfield et al. 2001). There is also the possibility that another hormone may play an important role in territorial expression. One study in male song sparrows showed a decrease in territorial aggression when corticosterone (CORT) implants were administered, even though levels of T were

within the normal range necessary for territorial defense (Wingfield & Silverin 1986). Research with dark eyed juncos (*Junco hyemalis*) and white throated sparrows (*Zonotrichia albicollis*) suggests that birds lack specific sex steroid-binding proteins, and over 90% of T may normally be bound to CORT-binding proteins (CBGs) (Deviche et al. 2001; Swett 2007). Furthermore, birds with more proactive personalities (such as bold and aggressive behaviors) have been shown to have lower levels of CORT (Cockrem 2007). Urban male song sparrows, with more proactive personalities, may have lower levels of CORT, and this would allow more transport of T to its target sites leading to greater levels of aggression.

A recent study of two desert birds, the Curve-billed thrasher (*Toxostoma curvirostre*) and Abert's towhee (*Melospiza aberti*), revealed higher territorial behaviors in urban over rural populations, though there were no differences in T and CORT levels (Fokidis et al. 2011). Our results seem to agree however, boldness was not measured in this study, and boldness and aggression have been shown to be linked to lower levels of CORT (Cockrem 2007). This data does not necessarily refute the possibility that lower CORT in urban song sparrows leads to greater T transport to target tissues, which results in greater territorial aggression. Future studies should examine the potential correlation of CORT levels with greater boldness and aggression in urban males.

There is evidence that higher levels of circulating T can have detrimental effects on survivorship, parental care, and immune system function (Marler & Moore 1989; Saino & Møller 1994; Grieves et al. 2006). Additionally, male dark eyed juncos implanted with T showed an increase in CORT, which suggests a potential selection against prolonged elevation of T as a result of this association (Ketterson et al. 2004).

Assuming T levels do not vary, it is possible that urban male song sparrows have developed an adaptive characteristic that carries the benefit of greater expression of territoriality towards conspecifics without the potential costs associated with elevated T levels. Studies that examine T levels and aggression in individuals in urban and rural habitats, along with assessment of habitat quality, body condition, and fitness will provide valuable insight.

Understanding how urbanization impacts behavior in residing animals will provide pertinent information that may be applied towards issues in conservation, such as small population extinctions, reducing the risk of extinction by predation from introduced species, and failures in captive breeding programs (Sutherland 1998). Growing evidence suggests that animals inhabiting urban environments might exhibit life history traits that reflect an adaptation to human presence which differs from their rural counterparts, differences that may create difficulties for biologists or wildlife managers (Ditchkoff et al. 2006). Indeed, behavior plays an important role in an individual's life history. The significant differences in the expression of boldness and aggression in urban song sparrows may provide insight in urban wildlife management and provide new tools for applying conservation in populations that live in both urban and rural settings.

In summary, this research, combined with our past study, has shown that both boldness and aggression are higher - and remain uncoupled - in urban male song sparrows. Presumably, expression of greater boldness, whether inherent or learned, may be influenced by urban habitat characteristics (e.g. more human activity). Greater aggression (territoriality) may be linked to how a male perceives habitat quality in urban environments, as demonstrated in a recent study on male song sparrows where there was

an association between increased territorial aggression and habitat quality (Scales 2009). T levels between the two populations did not vary significantly. Though T levels reflected seasonal and not male-male androgen responsive as described in Goymann et al. (2007), our study may be the first to show that T levels do not differ between urban and rural male song sparrows despite differences in territorial expression throughout the breeding season.

LITERATURE CITED

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