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Non-migratory stonechats show seasonal changes in the hormonal regulation of non-seasonal territorial aggression

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ABSTRACT

In many birds and mammals, male territorial aggression is modulated by elevated circulating concentrations of the steroid hormone testosterone (T) during the breeding season. However, many species are territorial also during the non-breeding season, when plasma T levels are basal. The endocrine control of non-breeding territorial aggression differs considerably between species, and previous studies on wintering birds suggest differences between migratory and resident species. We investigated the endocrine modulation of territorial aggression during the breeding and non-breeding season in a resident population of European stonechats (*Saxicola torquata rubicola*). We recorded the aggressive response to a simulated territorial intrusion in spring and winter. Then, we compared the territorial aggression between seasons and in an experiment in which we blocked the androgenic and estrogenic action of T. We found no difference in the aggressive response between the breeding and the non-breeding season. However, similarly to what is found in migratory stonechats, the hormonal treatment decreased aggressive behaviors in resident males in the breeding season, whereas no effects were recorded in the non-breeding season. When we compared the aggressive responses of untreated birds with those obtained from migratory populations in a previous study, we found that territorial aggression of resident males was lower than that of migratory males during the breeding season. Our results show that in a resident population of stonechats T and/or its metabolites control territorial aggression in the breeding but not in the non-breeding season. In addition, our study supports the hypothesis that migratory status does modulate the intensity of aggressive behavior.

Introduction

The role of testosterone (T) in the activation of male sexual and territorial behavior in reproductive contexts has been well documented (reviewed by Balthazart, 1983; Fusani, 2008b; Nelson, 2005; Wingfield et al., 1987, 1990). In general, male–male aggressive interactions can be reduced by castration and this effect is restored by T replacement (Becker et al., 1992; Nelson, 2005). In addition, most male vertebrates in the temperate zones display territorial aggression only during the breeding season, when circulating T levels are elevated and gonads enlarged, and exhibit non-territorial behavior during the non-breeding season, when plasma T levels are low and gonads regressed. However, in some species the positive relationship between the hormone and the behavior is not maintained throughout the year (e.g. Fusani, 2008a) and males establish and defend territories also outside the reproductive context (e.g. Caldwell et al., 1984; Canoine and Gwinner, 2002a; Canoine et al., 2007; Gwinner et al., 1994; Hau et al., 2004a; Moore and Marler, 1987; Moore et al., 2003; Schwabl and Kriner, 1991; Scotti et al., 2008; Soma et al., 1999; Wiley and Goldizen, 2003; Wingfield and Hahn, 1994).

Species that display territorial aggression year-round despite a distinct reproductive window and seasonal fluctuations in T concentration are good models to study seasonal or life history stage differences in territorial behavior (Canoine and Gwinner, 2002a; Hau et al., 2004a; Landys et al., 2010; Logan and Wingfield, 1990; Schwabl, 1992; Silverin et al., 1989; Soma et al., 2003; Wikelski et al., 1999). In these species non-breeding territorial aggression is often qualitatively and quantitatively similar to that shown in the breeding season (Wingfield and Hahn, 1994; Wingfield and Soma, 2002) and can persist following castration (Wingfield, 1994, in song sparrows, *Melospiza melodia morphna*; see also Pinxten et al., 2000, in European

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starlings, *Sturnus vulgaris*) or treatment with androgen blocker (Schwabl and Kriner, 1991, in European robins, *Erithacus rubecula*). In some tropical birds, basic year-round territorial aggression may be expressed independently of T levels, which are generally low throughout the year (e.g. Canoine, 2006; Canoine et al., 2007; Dittami and Gwinner, 1990; Fedy and Stutchbury, 2006; Gill et al., 2008; Goymann et al., 2006; Hau et al., 2008; Wiley and Goldizen, 2003; Wikelski et al., 1999, 2000). Further, tropical birds that establish a seasonal territory have generally higher T levels than year-round territorial species (Goymann et al., 2004). These findings suggest that the regulation of aggression is more complex and more plastic than previously thought (e.g. Soma et al., 2008), and factors other than seasonality might influence the effects of T.

Testosterone can exert its effects either directly by binding to androgen receptors or indirectly by binding to estrogen receptors after conversion into 17β-estradiol (E2) by the enzyme aromatase within the brain (Balthazart et al., 1997; Lephart et al., 1996; Remage-Healey et al., 2009; Schlinger and Callard, 1990; Steimer and Hutchison, 1981). It was suggested that estrogenic metabolites of T might be involved in the control of non-breeding territorial aggression (Beletsky et al., 1990; Schlinger and Callard, 1990). In fact, in male song sparrows territorial behavior (and song) in the non-breeding season is reduced by aromatase inhibition and restored by E2 administration, despite very low plasma levels of T and E2 in this season (Soma et al., 2000a, 2000b). In male European stonechats (*Saxicola torquata rubicola*), however, Canoine and Gwinner (2002a) found that the simultaneous inhibition of T and its conversion into E2 reduced territorial aggression in spring, but had no effect on winter aggression. Interestingly, the song sparrows investigated by Soma et al. (2000a, 2000b) were resident and wintered at, or close to, their breeding grounds, whereas European stonechats studied by Canoine and Gwinner (2002a) were migratory, with distinct breeding and non-breeding territories hundreds or thousands of kilometers apart.

Altogether, these studies suggest that differences in the regulation of aggressive behavior, particularly during the non-breeding season, are related to differences in social and life history strategies between migratory and non-migratory birds (e.g. Wingfield and Hahn, 1994; Rodl et al, 2004). Birds that do not migrate may have a stronger motivation to defend their territory in winter since they will need it to reproduce. This is obviously not the case for migrants, which have distinct territories in the reproductive season.

In this study, we asked whether the territorial response to an intruder and its control by testosterone and/or its estrogenic metabolites are seasonally different in a population of European stonechat that does not migrate. Stonechats have the peculiarity that pairs establish territories during both the reproductive and the non-reproductive period, and seasonal comparison of territorial behavior can be conducted within the same social context, i.e. male–female pairs. Resident stonechats, in addition, do not change territories between seasons. We first recorded in spring and winter the aggressive responses of free-living resident stonechats towards a stuffed decoy and compared our results with those obtained from a previous study in male stonechats from migratory populations (Canoine and Gwinner, 2002a). Then, we performed an experiment in which we carried out the simulated territorial intrusion after blocking both the androgenic and estrogenic action of T in a group of males. We predicted that territorial aggression does not changes seasonally in resident stonechats reflecting a more stable social environment compared to migratory populations which have to establish territories twice a year (see Wingfield, 1994; Wingfield and Hahn, 1994), and that the response to the blockage of T and/or its estrogenic metabolites does not change between seasons in contrast with a previous study in stonechats from migratory populations (Canoine and Gwinner, 2002a).

Material and methods

Study sites and subjects

Experiments were conducted on a resident stonechat population in Tuscany (Italy) during both the breeding season (March 2008–April 2008) and the non-breeding season (November 2008–December 2008). Several birds of this stonechat population have been observed and ring-banded, confirming that they establish and hold territory pairwise throughout the year (Canoine, Del Testa and Furlani, unpublished results). However, no male used for the present study was either caught or handled in previous investigations.

Experimental design

All field experiments were authorized by the Regional Government of Tuscany after positive evaluation by the National Institute for Wildlife (ISPRA). We applied the same experimental protocols used by Canoine and Gwinner (2002a) for migratory stonechat populations. Before the experiments started, we observed each stonechat pairs from up to 20 days to determine the extent of their territories and locate the preferred perches. Then, we conducted a first series of Simulated Territorial Intrusion (STI) tests to quantified territorial aggressive response of all males. A stuffed male stonechat was placed in the middle of the subject's territory as a decoy. Behavioral responses were recorded for 30 min (see next section). Following these first STI tests, males (spring, N = 14; autumn, N = 11) were caught with spring-traps and banded with a unique combination of two color bands. Males were then divided in two groups: one group received two implants of silastic tubing filled with an androgen receptor blocker and an aromatase inhibitor (ATD + F; see Hormonal manipulation) whereas the second group (control A) were given empty implants. An additional control group (B) was composed of males that were tested with an STI but were neither caught nor implanted. Seven to 11 days (median: 9) after implantation we performed a second series of STI test as described earlier for birds of all groups. Testing was conducted in parallel, randomized and balanced among experimental groups (ATD + F, control A, control B).

Behavioral observations

During the STI tests, we recorded to the nearest minute the aggressive responses of the focal territorial male towards the decoy for 30 min or until it attacked the decoy with physical contact. The following variables were recorded: a) Latency to the first attack — time interval between the beginning of the test and the first attack to the decoy (min); b) Distance of approach to the decoy, measured in relation to fixed landmarks (trees, bushes, fences, stones or roads) with a precision of 0.5–5 m depending on the distance of the bird from the focus area where the dummy was placed — i.e. short distances to the dummy were measured with greater precision than larger distances. In case of a physical attack the decoy was removed immediately to prevent its destruction. Males that did not attack the decoy were assigned a latency of 30 min and a distance of approach of 30 m. Song was not recorded because stonechats do not sing during the non-breeding season.

Hormonal manipulation

Males of the experimental group (ATD + F) (N = 8 breeding season; N = 6 non-breeding season) received one implant (silastic tubing, Dow Corning, inner diameter 1.47 mm, outer diameter 1.96 mm, effective length = 8 mm) containing the androgen receptor blocker Flutamide (F, Eg Spa, Milano, Italy) and one implant of the same size containing the steroidal aromatase inhibitor 1-4-6 androstatrien3,17 dione (ATD, Steraloids Inc. Ltd, UK). The ends of