Social context decouples the relationship between a sexual ornament and testosterone levels in a male wild bird

Pablo Vergara [a],[*],[1], Jesús Martínez-Padilla [a],[b],[1],[2]

[a] *University of Aberdeen, School of Biological Sciences, Tillydrone Avenue, Aberdeen AB24 2TZ, UK*
[b] *Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, 28006. Madrid, Spain*

ARTICLE INFO

ABSTRACT

In order to maximise fitness individuals should adjust their level of signalling according to their surrounding social environment. However, field experiments showing such adjustment of current signalling associated to changes in social context are lacking. Here, we manipulated levels of male aggressive- and dominance-related displays in a wild bird in our treated area by increasing testosterone levels using implants in a subset of males. We then compared the expression of sexual signals (i.e. comb size) between non-treated red grouse *Lagopus lagopus scoticus* males from control and treatment areas. We further explored the potential endocrinological mechanism linking social environment and signal expression by analysing testosterone levels in all males. Our treatment successfully increased overall aggressive- and dominance-related behaviours in the treatment area. Furthermore, testosterone-implanted birds increased their comb size as repeatedly shown in previous studies in male red grouse. Interestingly, untreated males living in the treatment area decreased their comb size, whilst increasing testosterone levels. Since comb size is a signal of dominance, untreated males from the treatment area may have perceived themselves as subordinate individuals and decreased their signalling levels to avoid confrontations with testosterone-treated, dominant individuals. In conclusion, our findings show that social context has the potential to regulate sexual signalling and testosterone levels. Our results highlight the role of social context when exploring the link between testosterone and behaviour, as it may reverse the relationship between both traits. Our results suggest that social context affects signalling and testosterone independently.

© 2012 Elsevier Inc. All rights reserved.

Introduction

In animal communication, signals can be defined as phenotypic traits produced by the signaller that have evolved because they change the behaviour of receivers in ways that benefit the signaller (Maynard Smith and Harper, 2003). The presence and quality of receivers changes over time and space so, to maximise the benefits associated with signalling, the signallers have to alter the expression of their signal according to their surrounding environment. It is therefore expected that individuals adjust the expression of a signal according to the context in which they live (e.g. Setchell and Dixon, 2001). Social context is among the most important factors that affect animal communication, specifically the expression and reliability of signals (Burmeister and Wilczynski, 2000; Vergara et al., 2012a). Changes in population sex-ratio (Gautier et al., 2008) or dominance hierarchy can also affect the expression of social or sexual signals, as socially subordinate individuals often reduce investment in signalling (Burmeister et al., 2005; Karubian et al., 2011; Setchell and Dixon, 2001; Zuk and Johnsen, 2000). However, experimental manipulations of

the social context that explore how it mediates signal expression remain limited, and the scarcity of field experiments is particularly noteworthy (Vergara et al., 2012a).

Among the physiological mechanisms linking social context and signals in general, and sexual signals in particular, the role of the endocrine system is of particular interest (Fusani, 2008). The social environment may have profound effects on androgen, particularly testosterone levels. For instance, it has been shown that testosterone levels increase when intra-sexual competition is more intense (Cristóbal-Azkarate et al., 2006; Wingfield and Hahnn, 1994). Androgens are supposed to be the link between the environment and ornament expression, and as such they influence the expression of male sexual signals in a wide variety of taxa (Folstad and Karter, 1992). It is widely assumed that implants of exogenous testosterone typically enhance sexual traits, although the increase in androgen levels is not always associated with a concomitant increase in ornament expression (Adkins-Regan, 2005). The link between androgens and aggressive behaviour has largely been considered the most plausible explanation for the dominance often exhibited by most ornamented males (Senar, 2006). Under such a scenario, we could expect an indirect effect of the social context on sexual traits via changes in testosterone levels, i.e. enhanced sexual signalling after intense levels of intra-sexual competition because of an increase in endogenous testosterone levels (McGraw et al., 2003; Setchell

---

* Corresponding author. Fax: +44 1224 272396.
 E-mail address: vergara@abdn.ac.uk (P. Vergara).
[1] Both authors contributed equally.
[2] Fax: +34 915645078.

et al., 2008). However, results are often inconsistent among and within species, and in fact, the way that social context influences sexual signals through changes in testosterone levels is likely much more complex (Adkins-Regan, 2005; Burmeister and Wilczynski, 2000). Changes in intra-sexual competition might not be associated with concomitant changes in sexual signalling, despite increases in testosterone levels, suggesting that social context affects both androgens and signalling independently (e.g. Burmeister and Wilczynski, 2000). In addition, androgens and sexual signalling levels are not always correlated, or if so, such relationship might be context-dependent (Adkins-Regan, 2005; Burmeister and Wilczynski, 2001; Setchell et al., 2008). Therefore, further research is needed to understand the effects of social context on animal communication. Our approach here is to experimentally manipulate the social context in a population by means of testosterone implants in a subset of males, and analyse both sexual ornament expression and androgen levels in control (un-implanted) males.

The red grouse *Lagopus lagopus scoticus* is a tetraonid bird living in the moorlands of northern UK. Male red grouse are territorial during spring, corresponding with the breeding season, but also during autumn and sporadically throughout winter (Watson and Moss, 2008). Autumn territorial behaviour is vital to males, as those that fail to establish or maintain a territory have low survival prospects (Mougeot et al., 2003b; Watson, 1985). In agreement, aggressiveness, intra-sexual displays and testosterone levels increase during autumn (early–middle October, see Mougeot et al., 2005a). Male–male aggressive encounters are mediated by a variety of dominance-related signals, such as territorial calls, ground and flight displays and supra-orbital combs (Mougeot et al., 2005a; Watson, 1985; Watson and Jenkins, 1964). Males erect their combs during intra- and inter-sexual encounters, and males displaying bigger combs are typically dominant, more aggressive and preferred by females (Moss et al., 1979; Redpath et al., 2006). Experimental studies have shown that comb size is a condition- and testosterone-dependent trait (Martínez-Padilla et al., 2010; Mougeot et al., 2004, 2006), although both properties can be context-dependent (Moss et al., 1996; Vergara et al., 2012a,b). Furthermore, males can rapidly (i.e. in a few days) increase or reduce the size of their combs depending on their current position in a dominance hierarchy (Moss et al., 1979).

Here we manipulated the social environment within a population by implanting some of the territorial males with exogenous testosterone in autumn. Previously, it has been shown that males implanted with testosterone increase their aggressive behaviour and sexual displays (Moss et al., 1994; Mougeot et al., 2004, 2005a, 2006). In particular, wild grouse implanted with testosterone show higher call rate, higher rate of aggressive encounters with other males and more intrusions to neighbouring territories (Moss et al., 1994; Mougeot et al., 2005a). Unsurprisingly, manipulations of testosterone at a population level also modified the social environment: increased overall aggressiveness, reduced recruitment of young individuals back into the population and subsequent population density, modified kin structure (fewer kin groups and related individuals with the male populations) and decreased body condition (Moss et al., 1994, 1996; Mougeot et al., 2003a, 2005b; Vergara et al., 2012a). These effects suggest that manipulations of testosterone at a population level increase intra-sexual competition (Vergara et al., 2012a). Following previous experimental designs, during autumn, ca. 40% of males living in a given area were treated with testosterone to modify social context. We then compared the expression of sexual signal (i.e. comb size) and testosterone levels of intact, unmanipulated, males living within the Treatment-area (T-area) with individuals living in a neighbouring and non-manipulated Control-area (i.e. C-area, see below). As comb size is a signal of dominance adjusted to the current position in the hierarchies (Moss et al., 1979), we first expected that non-treated individuals living in the T-area would decrease their comb size to avoid confrontations with dominant and more aggressive, implanted individuals. Second, as a result of the expected overall increase in aggressiveness in the T-area, we expected higher levels of testosterone in non-implanted individuals living in the T-area than in those males living in the C-area, because of the positive effect of aggressive environments in testosterone levels (Oliveira, 2004).

Material and methods

Manipulation of social context

Our experiment was carried out on Invermark moor (56° 54′N, 02° 50′W) in autumn 2011. Two 1-km^2 areas of heather *Calluna vulgaris* dominated moorland, separated by a 100 m wide buffer area were randomly assigned as Treatment or Control areas. During early autumn (12–27 September, see ESM), we caught 88 and 82 males in the T- and C-areas respectively, which corresponds to 85% and 79% of the estimated number of territorial males living in these areas (estimated density = 104 males/km^2; see below). We caught male red grouse by dazzling and netting them at night. We ringed each male and determined its age (i.e. young, <1-year-old or adult, >1-year-old) from plumage. We measured comb area (maximum comb length × width, mm^2) and body mass (g) as indices of ornament size and body condition respectively (Mougeot et al., 2006; Vergara et al., 2012a,b). Comb size was measured by both P.V. and J.M.-P. and repeatability between researchers was high (r = 0.96, $F_{7, 8} = 64.18$, $P < 0.001$; Lessells and Boag, 1987). To avoid bias in the day of capture, we randomly selected which area to work on each night.

Testosterone and empty implants

In the T-area, 39 males (44 % of captured males within this area), were implanted with testosterone (Treated-males), while the rest of the individuals were left un-manipulated. Treated birds were implanted with two silastic tubes (1.57 mm inner and 2.41 mm outer diameter, 20 mm long) filled with testosterone (Sigma Aldrich Co. Ltd., Poole, Dorset, UK) and sealed with glue at both ends. Implants were inserted between skin and breast muscles on the flank, under local anaesthesia. We previously determined the length of the tubing during trials on captive grouse with the implants increasing circulating testosterone levels by 2 SD of the autumn values for 2–3 months (S. Redpath and F. Mougeot, unpublished data). Males in the control area were not implanted with testosterone. Empty implants do not have detectable effects on body condition, breeding success or survival (Moss et al., 1994). However, to reject any potential confounding effect of the implanting itself on our results, a subsample of non-treated individuals captured within both T-area (n = 15) and C-area (n = 22) were given equivalent sized, empty implants, in order to compare their body mass, comb size and testosterone levels with those of non-implanted birds. Empty implants did not influence any of the study variables (all $P > 0.22$). Thus, we will not distinguish between empty- and non-implanted individuals hereafter and all were considered as control males. We had therefore considered three experimental groups:

1) Control individuals living in the control-area.
2) Control individuals living in the treatment-area.
3) Treated individuals living in the treatment-area.

Radio-tagging

A subsample of birds (32 males in C-area, 33 control males in T-area and 19 treated males in the T-area) were fitted with a radio-collar with a unique frequency (TW3-necklace radio-tags; Biotrack, Wareham, Dorset, UK) to facilitate relocation and recapture. Radio-collared birds did not differ from non radio-collared individuals in any of the study variables (all $P > 0.18$). We chose to include more males within the control groups because we were interested in studying the effect of social context rather than the effect of exogenous testosterone. We recaptured 56 radio-collar males 18 ± 0.4 days

(mean ± S.E.M.) after the first capture (control males in C-area n = 20; control males in T-area, n = 21; treated males in T-area, n = 15). Two birds died (natural mortality) during the study (1 control in C-area and 1 control in T-area). Ten out of the 56 recaptured birds were located in the buffer area or in a different area than in the first capture (3 control males from the C-area, 2 control males from the T-area and 5 treated males). These individuals were excluded from the analyses. Recaptures started at least 11 days after the last male was implanted with testosterone (see ESM). At the second capture, comb size and body mass were measured again to assess within-individual changes in ornamentation and body condition in relation to the experiment. We also took a blood sample to quantify testosterone levels in the second capture only (see below).

Testosterone assays

Blood samples (1 ml) from males were collected by intravenous extraction from the brachial vein. Plasma was separated by centrifuging blood for 10 min at 8000 rpm and storing it at −80 °C until analysis for measuring testosterone. We measured plasma testosterone concentration using a commercially available testosterone enzyme immunoassay (Elisa Kit EIA-1559 from DRG Diagnostics, Marburg, Germany), an assay that has been developed and validated for determining testosterone levels in small volume (20 μl) avian plasma samples (Washburn et al., 2007). Duplicate sample analyses showed that the testosterone assays were highly repeatable ($r = 0.86$, $F_{15, 16} = 13.90$, $P < 0.01$; Lessells and Boag, 1987). All measurements are given in ng ml^{-1}.

Grouse density and calling behaviour

We estimated grouse density by a method previously validated for red grouse (Evans et al., 2007). Briefly, we conducted playbacks at 6–8 points (3–4 in each area), 250 m apart. At each point, we played male territorial calls (flight and ground calls), four times, once in each of the four cardinal directions, using a portable tape player. Before and after the playback, we scanned the surrounding area with binoculars for five minutes and recorded males that responded by calling or were seen within a 100 m radius of the survey point. Playbacks were conducted in the morning (8:00–12:00) avoiding days with strong wind or rain (Evans et al., 2007). Densities (grouse or male per km^2) in each area were calculated using the equations provided in

Evans et al. (Evans et al., 2007). We calculated the proportion of males calling as the total number of males calling/total number of males seen per point. We conducted these surveys 20th of April (i.e. before the manipulation) and 10th of October, more than two weeks after the last implant of testosterone was given (see ESM).

Statistical analyses

First, we tested for changes over time (before treatment vs. after treatment) in density and the proportion of males calling using generalised linear models (GENMOD procedure in SAS 9.2). Models were fitted to the data using a Poisson error distribution corrected for over dispersion and a log-link function. Second, we tested for treatment effects (three-level fixed factor) on changes over time (first capture, before treatment, vs. second capture, after treatment) in comb size and body mass using general linear mixed models (GLMM, PROC Mixed procedure). In these models, individual identity was considered as a random effect, to account for repeated measures. The individual identity × time interaction was also included as a random term to correct for potential bias in random intercept models (Schielzeth and Forstmeier, 2009). Only males with measurements at both captures were included in these models. Finally, we used GLMs to test for treatment effects on the data collected upon recapture (testosterone levels and its association with comb size and body mass). Age was included as a two-level fixed factor (young vs. adult) in the previous models to account for age-class differences in the study variables. We used the Satterthwaite method to calculate degrees of freedom. Testosterone concentration was log-transformed to achieve a normal distribution (log-transformed values S–W test; $P = 0.67$).

Results

Effect of the experiment on male calling behaviour and density

Changes over time in the proportion of males calling differed between areas, but not in density (Table 1). Before implanting, density ($F_{1,6} = 0.11$, $P = 0.75$) and the proportion of males calling ($F_{1,5} = 0.09$, $P = 0.77$) did not differ significantly between areas (median ± SD; calling behaviour: T-area = 0.58 ± 0.23; C-area = 0.58 ± 0.41; density: T-area = 102 ± 42; C-area = 102 ± 48). After the implanting the number of individuals remained similar in both T- and C-area ($F_{1,4} = 0.58$, $P = 0.48$;

Table 1
Effect of the experiment on the change in (a) population density (grouse/km^2), and (b) calling behaviour (proportion of males calling per playback point) before and after the implanting, effect of the experiment on the change in (c) comb area (mm^2) and (d) body mass (g) between captures and (e) the effect of the experiment in log-transformed testosterone levels (ng ml^{-1}). The models analysing (a) and (b) were run using generalised linear models. The models analysing (c) and (d) were run using general linear mixed models (GLMMs) and also included individual identity and its interaction with time as random factors. In all models, Time was included as a two-level factor (before implanting vs. after implanting). In models (a) and (b), Area was included as a two-level factor (C-area vs. T-area). In models (c), (d) and (e), Treatment was included as a three-level factor: 1) Control individuals living in the C-area; 2) Control individuals living in the T-area; 3) Testosterone-treated individuals living in the T-area. Parameter estimates for "Area" correspond to C-area. Parameter estimates for "Treatment" correspond to control males living in the C-area. Parameter estimates for "Time" correspond to surveys before implanting in models a) and b) and to the first capture in models c) and d).

Dependent variables	Explanatory variables	Statistics
a-Density	Area	$F_{1,11} = 0.43$, $P = 0.52$, *estimate* ± SE = 0.28 ± 0.42
	Time	$F_{1,12} = 3.12$, $P = 0.07$, *estimate* ± SE = 0.74 ± 0.42
	Area × Time	$F_{1,10} = 0.66$, $P = 0.43$, *estimate* ± SE = 0.73 ± 0.90
b-Calling behaviour	Area	$F_{1,9} = 3.84$, $P = 0.08$, *estimate* ± SE = 0.08 ± 0.24
	Time	$F_{1,9} = 0.29$, $P = 0.60$, *estimate* ± SE = 0.34 ± 0.23
	Area × Time	$F_{1,9} = 5.66$, **P = 0.04**, *estimate* ± SE = −0.89 ± 0.38
c-Comb area	Treatment	$F_{2,41} = 17.72$, $P < 0.01$, *estimate* ± SE = −166.3 ± 18.2
	Time	$F_{1,42} = 93.97$, $P < 0.01$, *estimate* ± SE = −165.7 ± 11.0
	Treatment × Time	$F_{2,42} = 108.79$, **P < 0.01**, *estimate* ± SE = 149.3 ± 13.3
d-Body mass	Treatment	$F_{2,41} = 1.18$, $P = 0.31$, *estimate* ± SE = −21.9 ± 18.1
	Time	$F_{1,42} = 32.12$, $P < 0.01$, *estimate* ± SE = 24.4 ± 7.0
	Treatment × Time	$F_{2,42} = 0.60$, $P = 0.55$, *estimate* ± SE = −9.4 ± 8.7
e-Testosterone	Treatment	$F_{2,37} = 3.71$, **P = 0.03**, *estimate* ± SE = 0.4 ± 0.1

Control area   **Treatment area**
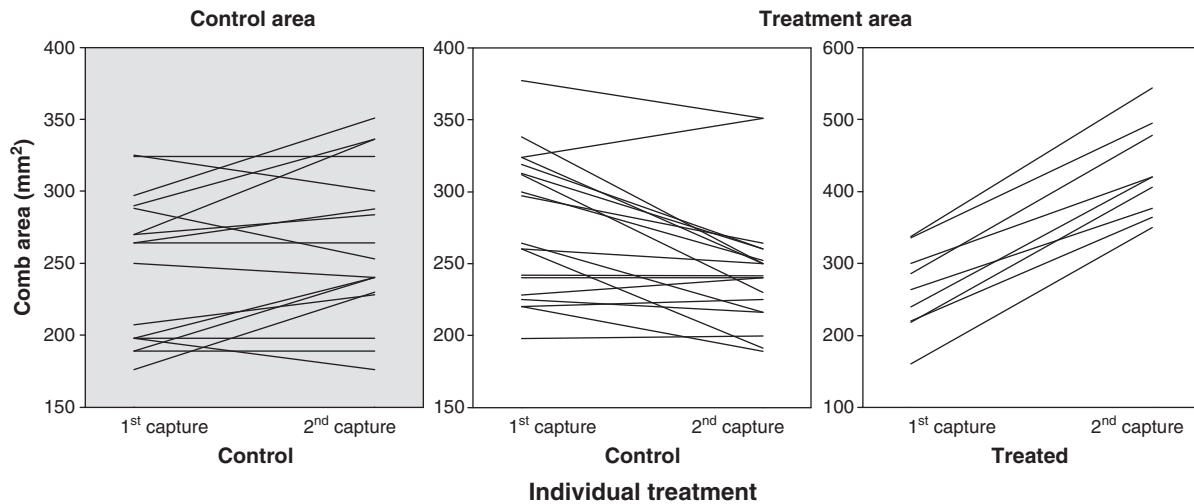
Fig. 1. Changes in comb size (mm^2) between captures (before vs. after implanting) according to individual treatment and area.

median $\pm$ SD, T-area $= 86 \pm 130$; C-area $= 117 \pm 255$), but males living in the T-area called more frequently than those in the C-area ($F_{1,4} = 10.13$, $P = 0.03$, median $\pm$ SD; T-area $= 1.00 \pm 0.09$; C-area $= 0.33 \pm 0.22$).

Effect of the experiment on comb size

Before testosterone implanting, comb area and body mass did not differ significantly between both areas and treatment groups (all $P > 0.11$). The experimental manipulation of testosterone influenced levels of sexual signalling. First, we found that changes in comb size within individuals significantly differed between treatments (Table 1, Fig. 1). Treated birds increased comb size after testosterone implants more than control males from T- and C-areas (GLMM, interaction treatment $\times$ time, both $P < 0.01$, Fig. 1). In addition, the change in comb size between experimental groups differed between control birds from the C- and T-area (GLMM, interaction treatment $\times$ time, $F_{1,34} = 18.31$, $P < 0.01$). Specifically, control males from the T-area decreased their comb size in relation to control males from the C-area (Fig. 1). In addition, control males from T-areas decreased their comb size (within experimental group GLMM, time, $P < 0.01$, Fig. 1) whereas control males from C-areas increased them (within experimental group GLMM, time, $P = 0.04$, Fig. 1). Average comb size from the second capture also differed between treatments (GLM, treatment $F_{2,41} = 62.25$, $P < 0.01$). Comb size in treated males (428 ± 17 mm^2), was bigger than the comb size of males from the two other groups (post-hoc test both $P < 0.01$). Comb size of control birds from the C-area (263 ± 12 mm^2) did not differ from that of control birds from the T-area (246 ± 12 mm^2) (post-hoc test $P = 0.11$). Body mass decreased between capture and recapture, but this change did not differ among treatments (Table 1). None of the previous results changed when including day of capture in the models described above.

*Effect of the experiment on circulating testosterone levels*

Upon second capture, testosterone levels differed significantly between experimental groups (Table 1, Fig. 2). Testosterone levels did not differ between treated and control individuals from the T-area (post-hoc test $P = 0.88$, Fig. 2), and both groups showed testosterone levels significantly higher than those of individuals from the C-area (post-hoc tests: control males from C-area vs. control males from T-area, $P = 0.01$; control males from C-area vs. treated males, $P = 0.04$, Fig. 2). Testosterone levels did not differ between age-classes ($F_{1,36} = 2.57$, $P = 0.11$) and the age $\times$ treatment interaction was not significant ($F_{2,34} = 0.26$, $P = 0.77$). Comb size (dependent

variable) and testosterone levels were not significantly related ($F_{1,37} = 0.52$, *estimate* $\pm$ SE $= 24.9 \pm 34.6$, $P = 0.47$, Fig. 3), irrespective of the treatment (testosterone $\times$ treatment $F_{1,33} = 2.80$, $P = 0.07$, Fig. 3; within treatment relationships: control birds C-area $F_{1,11} = 0.40$, $P = 0.53$; control birds T-area $F_{1,16} = 0.61$, $P = 0.44$; treated birds T-area $F_{1,6} = 3.56$, $P = 0.10$) and age-classes (testosterone $\times$ age $F_{1,35} = 0.60$, $P = 0.44$). Body mass was not correlated with testosterone levels, irrespective of treatment and age (all $P > 0.29$). None of these results changed when including sampling date in the models.

Discussion

Sexual signalling and social context

Animal communication theories predict that social environment can affect signal expression, yet experimental tests of this prediction in the field are lacking. Here, we were able to increase aggressive- and dominance-related displays, elements of social context, and provided experimental evidence that it modified sexual signalling levels in a wild vertebrate. Specifically, we showed that male red grouse decreased their sexual signalling levels (i.e. comb size) when levels of
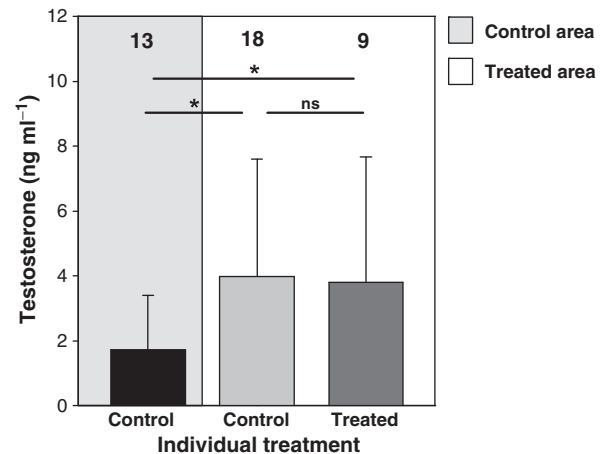
Fig. 2. Testosterone levels (ng ml^{-1}) $\pm$ SD according to treatment and area. n.s. $P > 0.05$, *$P < 0.05$. Sample sizes are given above bars. Note that analyses were run using log-transformed testosterone levels.
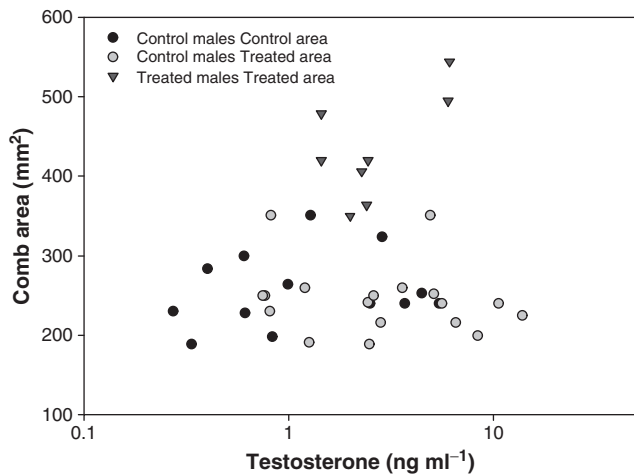
Fig. 3. Relationship between comb size and testosterone levels (ng ml^{-1}). Different treatments are presented with different symbols. Note that analyses were run using log-transformed testosterone levels.

calling behaviour and the size of the combs in a subset of individuals were experimentally increased. Exploring the endocrinological mechanism behind such changes in sexual trait expression, we found that such a decrease in ornament size was not explained by testosterone levels. Instead, the decrease in comb size was associated with an increase in testosterone levels.

In agreement with previous studies, treated birds increased comb size after testosterone implanting, reaching similar sizes to those naturally displayed by males in spring (see Mougeot et al., 2006). In addition, and as expected because of the autumnal peak in intra-sexual competition in red grouse (Mougeot et al., 2005a), individuals living in the C-areas increased their comb size. Most interestingly, we found that control, non-treated, males living in a more competitive area (T-area) decreased their comb size after the experimental manipulation of social context. The reason behind such a decrease in male comb size might be that such males became subordinate to treated males. Treated males increase their comb size and that increase can be interpreted as a proxy of aggressiveness potential and dominance (Moss et al., 1979; Zuk and Johnsen, 2000). As a consequence, control, subordinate males may react to the increased comb size of their treated conspecifics by decreasing their ornament size, in order to avoid confrontations with dominant individuals (e.g. Moss et al., 1979; Zuk and Johnsen, 2000). This is perhaps the most plausible reason, but we cannot rule out similar reasoning with other dominance-related behavioural (e.g. aggressiveness, calling, flight displays) or phenotypic (e.g. coloration) traits that are also associated with both testosterone levels and dominance in red grouse (Martínez-Padilla et al., 2010; Moss et al., 1994; Mougeot et al., 2005a). Among these, calling behaviour is a testosterone-dependent trait that deserves further speculation. Calling behaviour is a testosterone-dependent signal in red grouse and it has been considered an important signal mediating male–male territorial confrontations (Mougeot et al., 2005a). Interestingly, we found that the proportion of males calling increased in the T-area after the implanting. Therefore, similar to comb size, calling behaviour could be interpreted as a signal of dominance, an idea that would require further confirmation.

Higher testosterone levels after increased levels of male aggressive behaviours

As predicted by the feedback between androgens and social context (reviewed in Oliveira, 2004), we found that experimentally increased male–male competition promotes an increase in testosterone levels. Furthermore, the increase in testosterone levels shown in control individuals living in the T-area, was similar to that found in T-implanted males (Fig. 2). Treated males showed the expected increase in

testosterone levels with respect to the average autumn values in this species (Mougeot et al., 2005a). However, the high levels of testosterone of control, non-implanted males, living in the T-area is somewhat surprising. The reason behind such increase can be explained because of the high level of intra-sexual competition in the study area, regardless of the experimental treatment. Intra-sexual competition in red grouse increases along population density (Mougeot et al., 2003a). Population density in the study area was extremely high: 104 males/km^2 as compared with densities of around 40 males/km^2 shown in the same area a few years before (authors' unpublished data). Furthermore, testosterone levels of individuals living in the C-area were above the average autumn values in red grouse (e.g. Bortolotti et al., 2009). This suggests high and natural intra-sexual competition level in the study population that we boosted when implanting with exogenous testosterone some males in the T-area. This created an extremely competitive environment that helps us to understand the high testosterone levels found in non-implanted birds from the T-area.

The role of androgens in regulating comb size

Our results suggest an inconsistency regarding the link between testosterone and ornament expression (Fusani, 2008). On the one hand, we found that comb size increased in males implanted with exogenous testosterone, as shown in previous experiments (Martínez-Padilla et al., 2010; Mougeot et al., 2004). On the other hand, we found that control, untreated, males living in the treatment area also had increased levels of endogenous testosterone but did not increase comb size. Furthermore, we also found that testosterone and comb size were not correlated (Fig. 3). These inconsistencies show that the relationship between circulating testosterone levels and sexual signalling is not simple, as repeatedly reported (Adkins-Regan, 2005). Indeed, the relationship can be complex (e.g. non-linear) and context- or condition-dependent (Burmeister and Wilczynski, 2001; Perez-Rodriguez et al., 2006; Safran et al., 2008).

It has been proposed that the relation between testosterone and ornamentation may be mediated by other hormones released by the endocrine system, such as corticosterone. This idea has received support in the study species and in a recent experiment it was shown that the effect of testosterone implants on comb size was mediated by corticosterone levels of individuals (Bortolotti et al., 2009). Specifically, they found that the more stressed the birds were (i.e., higher corticosterone levels), the less testosterone-dependent the expression of the ornament was (Bortolotti et al., 2009). In addition, equivalent manipulations to the one described in our experiment in red grouse showed that stressful conditions of the T-area promote a loss of male body condition of individuals living in this area (Vergara et al., 2012a). Although, we did not find such expected negative effect of the experiment on the condition of individuals, likely because of the limited time between captures, we cannot rule out that corticosterone levels were affected. Perhaps corticosterone is the hormone that may regulate the ornament expression under given stressful circumstances. We cannot disregard however, that control individuals living in the T-area use their endogenous testosterone to increase other androgen-dependent displays (e.g. coloration, calling or flight displays) and behaviours (e.g. aggressiveness) not measured here, thereby explaining the decrease in their comb size. Further studies will help to clarify the mechanism behind the changing testosterone-dependence of comb size.

At this point we should however acknowledge the limitations of our experimental approach. The study was based on a limited sample size in the sense that each area (Treated vs. Control) constitutes a single plot. However, our "Before vs. After" comparison design, and the fact that the experiment was carried out in natural conditions provide robustness to our main conclusions, but further, if possible replicated, experimental studies are needed to corroborate the present findings.

In conclusion, we provided an experimental demonstration in wild conditions that social context can regulate both sexual signalling

and testosterone levels. Males decreased their dominance-related signalling in response to a more competitive environment, but enhanced their testosterone levels as expected when faced with increasing male–male competitiveness. Our findings suggest that social context may regulate sexual signalling and testosterone independently, and that testosterone levels and ornament expression do not have to be necessarily linked in a simple way.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh.2012.07.007.

References

Adkins-Regan, E., 2005. Hormones and Animal Social Behaviour. Princeton University Press, New Jersey.
Bortolotti, G.R., Mougeot, F., Martínez-Padilla, J., Webster, L.M.I., Piertney, S.B., 2009. Physiological stress mediates the honesty of social signals. PLoS One 4, e4983.
Burmeister, S.S., Wilczynski, W., 2000. Social signals influence hormones independently of calling behavior in the treefrog (*Hyla cinerea*). Horm. Behav. 38, 201–209.
Burmeister, S.S., Wilczynski, W., 2001. Social context influences androgenic effects on calling in the green treefrog (*Hyla cinerea*). Horm. Behav. 40, 550–558.
Burmeister, S.S., Jarvis, E.D., Fernald, R.D., 2005. Rapid behavioral and genomic responses to social opportunity. PLoS Biol. 3, e363.
Cristóbal-Azkarate, J., Chavira, R., Boeck, L., Rodríguez-Luna, E., Veàl, J.J., 2006. Testosterone levels of free-ranging resident mantled howler monkey males in relation to the number and density of solitary males: a test of the challenge hypothesis. Horm. Behav. 49, 261–267.
Evans, A.E., Mougeot, F., Redpath, S.M., Leckie, F., 2007. Alternative methods for estimating breeding density in an upland game bird, the red grouse *Lagopus lagopus scoticus*. Wildl. Biol. 13, 130–139.
Folstad, I., Karter, A.J., 1992. Parasites, bright males and the immunocompetence handicap. Am. Nat. 139, 603–622.
Fusani, L., 2008. Testosterone control of male courtship in birds. Horm. Behav. 54, 227–233.
Gautier, P., Barroca, M., Bertrand, S., Eraud, C., Gaillard, M., Hamman, M., Motreuil, S., Sorci, G., Faivre, B., 2008. The presence of females modulates the expression of a carotenoid-based sexual signal. Behav. Ecol. Sociobiol. 62, 1159–1166.
Karubian, J., Lindsay, W.R., Schwabl, H., Webster, M.S., 2011. Bill coloration, a flexible signal in a tropical passerine bird, is regulated by social environment and androgens. Anim. Behav. 81, 795–800.
Lessells, C.M., Boag, P.T., 1987. Unrepeatable repeatabilities: a common mistake. Auk 104, 116–121.

Martínez-Padilla, J., Mougeot, F., Webster, L.M.I., Pérez-Rodríguez, L., Piertney, S.B., 2010. Testing the interactive effects of testosterone and parasites on carotenoid-based ornamentation in a wild bird. J. Evol. Biol. 23, 902–913.
Maynard Smith, J., Harper, D., 2003. Animal Signals. Oxford University Press, Oxford, UK.
McGraw, K.J., Dale, J., Mackillop, E.A., 2003. Social environment during molt and the expression of melanin-based plumage pigmentation in male house sparrows *Passer domesticus*. Behav. Ecol. Sociobiol. 53, 116–122.
Moss, R., Kolb, H.H., Marquiss, M., Watson, A., Treca, B., Watt, D., Glennie, W., 1979. Aggressiveness and dominance in captive cock red grouse. Aggress. Behav. 5.
Moss, R., Parr, R., Lambin, X., 1994. Effects of testosterone on breeding density, breeding success and survival of red grouse. Proc. R. Soc. Lond. B Biol. Sci. 258, 175–180.
Moss, R., Watson, A., Parr, R., 1996. Experimental prevention of a population cycle in red grouse. Ecology 77, 1512–1530.
Mougeot, F., Redpath, S.M., Leckie, F., Hudson, P.J., 2003a. The effect of aggressiveness on the population dynamics of a territorial bird. Nature 421, 737–739.
Mougeot, F., Redpath, S.M., Moss, R., Matthiopoulos, J., Hudson, P.J., 2003b. Territorial behaviour and population dynamics in red grouse *Lagopus lagopus scoticus*. I. Population experiments. J. Anim. Ecol. 72, 1073–1082.
Mougeot, F., Irvine, J.R., Seivwright, L., Redpath, S.M., Piertney, S., 2004. Testosterone, immunocompetence, and honest sexual signaling in male red grouse. Behav. Ecol. 15, 930–937.
Mougeot, F., Dawson, A., Redpath, S.M., Leckie, F., 2005a. Testosterone and autumn territorial behavior in male red grouse *Lagopus lagopus scoticus*. Horm. Behav. 47, 576–584.
Mougeot, F., Piertney, S.B., Leckie, F., Evans, S., Moss, R., Redpath, S.M., Hudson, P.J., 2005b. Experimentally increased aggressiveness reduces population kin structure and subsequent recruitment in red grouse *Lagopus lagopus scoticus*. J. Anim. Ecol. 74, 488–497.
Mougeot, F., Redpath, S.M., Piertney, S., 2006. Elevated spring testosterone increases parasite intensity in male red grouse. Behav. Ecol. 17, 117–125.
Oliveira, R.F., 2004. Social modulation of androgens in vertebrates: mechanisms and function. Adv. Study Behav. 34, 165–239.
Perez-Rodriguez, L., Blas, J., Vinuela, J., Marchant, T.A., Bortolotti, G.R., 2006. Condition and androgen levels: are condition-dependent and testosterone-mediated traits two sides of the same coin? Anim. Behav. 72, 97–103.
Redpath, S.M., Mougeot, F., Leckie, F., Evans, A.D., 2006. The effects of autumn testosterone on survival and productivity in red grouse *Lagopus lagopus scoticus*. Anim. Behav. 71, 1297–1305.
Safran, R.J., Adelman, J.S., McGraw, K.J., Hau, M., 2008. Sexual signal exaggeration affects physiological state in a social vertebrate. Curr. Biol. 18, R461–R462.
Schielzeth, H., Forstmeier, W., 2009. Conclusions beyond support: overconfident estimates in mixed models. Behav. Ecol. 20, 416–420.
Senar, J.C., 2006. In: Hill, G.E., McGraw, K.J. (Eds.), Color Displays as Intrasexual Signals of Aggression and Dominance. Harvard University Press, pp. 87–137.
Setchell, J.M., Dixon, A.F., 2001. Changes in the secondary sexual adornments of male mandrills *Mandrillus sphinx* are associated with gain and loss of alpha status. Horm. Behav. 39, 177–184.
Setchell, J.M., Smith, T., Wickings, E.J., Knapp, L.A., 2008. Social correlates of testosterone and ornamentation in male mandrills. Horm. Behav. 54, 365–372.
Vergara, P., Martínez-Padilla, J., Mougeot, F., Leckie, F., Redpath, S.M., 2012a. Environmental heterogeneity influences the reliability of secondary sexual traits as condition indicators. J. Evol. Biol. 25, 20–28.
Vergara, P., Mougeot, F., Martínez-Padilla, J., Leckie, F., Redpath, S.M., 2012b. The condition-dependence of a secondary sexual trait is stronger under high parasite infection level. Behav. Ecol. 23, 502–511.
Washburn, B.E., Millspaugh, J.J., Morris, D.L., Schulz, J.H., Faaborg, J., 2007. Using a commercially available enzyme immunoassay to quantify testosterone in avian plasma. Condor 109, 181–186.
Watson, A., 1985. Social class, socially-induced loss, recruitment and breeding of red grouse. Oecologia 67, 493–498.
Watson, A., Jenkins, D., 1964. Notes on the behaviour of the red grouse. Br. Birds 57, 137–170.
Watson, A., Moss, R., 2008. Grouse. Collins, London.
Wingfield, J.C., Hanhn, T.P., 1994. Testosterone and territorial behaviour in sedentary and migratory sparrows. Anim. Behav. 47, 77–89.
Zuk, M., Johnsen, T.S., 2000. Social environment and immunity in male red jungle fowl. Behav. Ecol. 11, 146–153.