

Intra-sexual competition modulates calling behavior and its association with secondary sexual traits

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Abstract

The expression of elaborate sexual displays is associated with individual quality ensuring reliable information about the bearers. However, the associated cost of expressing enhanced sexual traits is expected to change according to environmental circumstances. Specifically, the cost of maintaining or producing a signal is predicted to increase when environmental conditions are unfavorable, which may lead to a reduction in signal expression as shown in several species. Here, we compared the calling behavior of male red grouse *Lagopus lagopus scoticus* living in an area of experimentally increased intra-sexual competition to that of males living in a control area. Levels of intra-sexual competition were experimentally manipulated by testosterone implants in a subset of captured males. In addition, we compared the association between two sexual traits, calling behavior and comb size, of males living

in these two areas. Although call frequency was not affected by different levels of intra-sexual competition, males from the control area performed shorter calls than individuals from the treatment area. Additionally, a positive association between comb size and call duration was found only for males in the area of lower aggressiveness. We suggest that environmental conditions influence the expression of multiple plastic sexual traits, depending on the costs and the information conveyed about different individual qualities.

Significance statement

Although sexual signals are considered reliable indicators of individual quality, environmental heterogeneity may modulate their expression and reliability. We experimentally manipulated levels of intra-sexual competition in a wild population of red grouse by increasing testosterone levels using implants in a subset of males. We observed that the social context shapes the expression of sexual traits. In less competitive conditions, males performed shorter calls and call length was positively related to the size of the supra-orbital comb, a relevant secondary sexual trait. Under higher intra-sexual competition, this relationship was decoupled and investment was directed at increasing call length.

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Introduction

In animal communication, sexual signals are considered reliable indicators of individual quality. The cost of their expression must differ between high-quality and low-quality signalers in order to be evolutionarily stable (Grafen 1990; Zahavi 1993). However, the role of sexual selection as the

unique force driving the evolution and maintenance of sexual traits has been questioned because the association between sexual signals and measures of individual condition or reproductive success is widely variable (Chaine and Lyon 2008; Robinson et al. 2008). Variation in abiotic conditions, resource availability, or population structure may alter the costs of expressing sexual traits and, therefore, its reliability (Garant et al. 2004; Cothran and Jeyasingh 2010; Vergara and Martínez-Padilla 2012).

The idea that harsh environmental conditions reinforce the condition dependence of sexual signals has been evaluated in wild populations (e.g., Cothran and Jeyasingh 2010; Vergara et al. 2012a). Under severe environments, exaggerated sexual traits are only affordable for individuals of prime quality without compromising their condition (Cotton et al. 2004a). For instance, a positive association between the expression of a sexual trait and body condition only emerges when intra-sexual competition is strong, because only high-quality individuals can display enhanced sexual traits (Vergara et al. 2012a; Martínez-Padilla et al. 2014a). Accordingly, favorable environmental circumstances allow both low-quality and high-quality individuals to similarly invest in trait expression (Cotton et al. 2004a). Hence, understanding the role of environmental heterogeneity is key to gain a clear comprehension of the evolution of sexual traits (Cornwallis and Uller 2009). The most reliable way to explore the effects of environmental heterogeneity on the costs and expression of sexual traits relies on adequate manipulation of the environmental conditions in which individuals live. Unfortunately, such manipulations are seldom carried out in wild conditions.

In birds, male songs are sexual displays involved in territory defense and mate attraction (Gil and Gahr 2002) that may reliably signal individuals' quality, like the expression of many other secondary sexual traits (Grafen 1990). For instance, individuals of high quality have increased repertoire size and length (e.g., in the hoopoe *Upupa epops* Martín-Vivaldi et al. 1998, common nightingale *Luscinia megarhynchos* Kipper et al. 2006) or decreased call frequency (e.g., in barn swallows *Hirundo rustica* Galeotti et al. 1997, montezuma oropendolas *Psarocolius montezuma* Price et al. 2006, common scops owl *Otus scops* Hardouin et al. 2007). As in other sexual signals (Vergara and Martínez-Padilla 2012), it is expected that environmental context mediates the expression of these components of vocal performance and, consequently, the reliability of the message conveyed. Particularly, the social context has been shown to affect vocal signaling (in the hoopoe Galeotti et al. 1997, in the eurasian eagle owl *Bubo bubo* Penteriani 2003). However, the assessment of mate or competitor quality may be based on the integrated information obtained by multiple sexual signals (Candolin 2003). The *redundant signal hypothesis* states that several sexual traits inform about the same individual aspect but with a certain error, and thus, the simultaneous evaluation

of all signals ensures a more reliable information of individual quality (see review by Candolin 2003). The *multiple message hypothesis* proposes that different sexual signals convey information about different individual qualities, and the assessment of the overall quality requires the evaluation of all signals together (see Møller and Pomiankowski 1993; Candolin 2005; Chaine and Lyon 2008), or alternatively, each ornament can be the target of different receivers (Andersson et al. 2002; Karubian et al. 2009). However, studies are often done on isolated traits (but see, for instance, Chaine and Lyon 2008), which may yield unrealistic and biased conclusions on the evolution of sexual traits. This is particularly relevant in changing environmental contexts, where environmental variation may alter the reliability and the message conveyed by several sexual traits. Thus, exploring how environmental variability influences the joint expression of multiple sexual traits is required.

Here, we explored the role of environmental heterogeneity in the expression and association of two sexual traits in male red grouse (*Lagopus lagopus scoticus*). Our study species, the red grouse, is a tetraonid bird that inhabits the heather moorlands of the UK. Males are territorial during autumn, when defending territories is crucial for winter survival and during the breeding season in spring to mate and reproduce (Watson and Moss 2008). Territory size is such an essential component in the ecology of red grouse males that it has been suggested as a factor explaining the species' population cycles (*territorial behavior hypothesis*; Mougeot et al. 2003a; Redpath et al. 2006a; Martínez-Padilla et al. 2014b). It is well known that territory size is affected by levels of intra-sexual competition. As intra-sexual competition increases, competition for holding a territory does so (Mougeot et al. 2005c), and territory size decreases (Piertney et al. 2008). Unsurprisingly, intra-sexual competition in male red grouse has been shown to modify the kin structure and affect winter survival, reproductive success, and ultimately population cycles (Moss et al. 1994; Mougeot et al. 2003a; Vergara et al. 2012a; Martínez-Padilla et al. 2014a, c).

We chose calling behavior and comb size as target secondary sexual traits. The supra-orbital comb is a condition-dependent trait displayed in intra-sexual and inter-sexual encounters. Comb size can be considered as a proxy of individual quality in males, since individuals with bigger combs also have lower burdens of intestinal parasites, a better T cell-mediated immunity, and a better body condition (Mougeot et al. 2004, 2005b). Males with larger combs are more aggressive and preferred by females (Moss et al. 1979; Redpath et al. 2006b), and comb size of subordinate males decreases in the presence of aggressive males (Vergara and Martínez-Padilla 2012). Territorial calls and ground and flight displays are behaviors also involved in intra-sexual competition (Mougeot et al. 2005a) and can be considered as reliable proxies of individual quality. Particularly, territorial calling rate increases

in parasite-free males (Fox and Hudson 2001) and also increases in males implanted with testosterone, which are more aggressive toward territorial playbacks (Mougeot et al. 2005a). However, there is still lacking information on the role played by different components of the call in male red grouse within a sexual selection context and, thus, on how environmental variation may alter its structure.

As a source of environmental heterogeneity, we took advantage of an already published study where the level of intra-sexual competition of males was experimentally manipulated in a wild population of red grouse in order to assess how environmental conditions may modulate the reliability of comb size as a proxy of individual quality (Vergara and Martínez-Padilla 2012). The experiment was settled in the habitat of red grouse, homogeneous heather moorlands of *Calluna vulgaris* in Scotland (UK), and started in autumn. In the study area, two subareas about 1 km² were chosen and assigned as control and treatment. These two areas were equivalent in habitat structure and population density before manipulation. A subset of red grouse males captured in the treatment area were given testosterone implants, and aggressiveness levels were then assessed 1 month later. Levels of aggressiveness measured as rates of dominance-related behaviors increased in the area where individuals were implanted with testosterone (Vergara and Martínez-Padilla 2012). In addition, untreated males living in the area where aggressiveness was experimentally increased reduced their comb size but had similar testosterone levels to that of males implanted with exogenous testosterone, higher than the circulating levels found in untreated males living in the control area (Vergara and Martínez-Padilla 2012). Therefore, authors pointed out that other dominance-related traits like calling behavior, a testosterone-dependent signal in this species (Mougeot et al. 2005a), may vary accordingly to testosterone levels.

We evaluated the effect of increased levels of intra-sexual competition on calling behavior and its association with comb size in a wild population of red grouse. The expression of both comb size and calling rate is negatively affected by parasite burdens (Fox and Hudson et al. 2001; Mougeot et al. 2005b) and is enhanced by testosterone levels (Mougeot et al. 2005a). Therefore, we consider that these two signals might convey similar information about the bearer, in agreement with the redundant signal hypothesis. We aim to test two predictions framed within the redundant signal hypothesis and based on previous results of this experimental manipulation, showing that increased levels of aggressiveness reduced comb size of subordinate males holding a territory in areas of high male-male competition (Vergara and Martínez-Padilla 2012). First, if both signals reflect the dominant status, males of red grouse living in a high intra-sexual competitive area will perform shorter calls of higher frequency, similarly to the reduction in comb size of non-dominant individuals, reported by

Vergara and Martínez-Padilla (2012). This prediction is based on the following: (i) High levels of intra-sexual competition can reduce the expression of sexual signals, as suggested by the condition-dependent hypothesis (Cotton et al. 2004a, b). Accordingly, comb size of male red grouse is negatively associated with population density (Vergara et al. 2012b); (ii) low frequency and long calls are often performed by high-quality individuals in other species (e.g., hoopoe Martín-Vivaldi et al. 1998, common scops owl Hardouin et al. 2007); (iii) the reduction in parasite burdens and the increase in testosterone levels cause increased comb size and calling rate (Mougeot et al. 2005a, b; Martínez-Padilla et al. 2014a), suggesting that both signals change similarly. Second, since only under harsh environmental conditions individual quality and comb size are positively associated in red grouse (Vergara et al. 2012a; Martínez-Padilla et al. 2014a), we expect a positive association between call length and comb size and a negative relationship between frequency and comb size in areas of high male-male competition. Thus, only high-quality individuals would be able to confront the cost of expressing both signals.

Methods

Social context manipulation experiment

This study was part of an experiment aiming to modify the social context of a red grouse population (Vergara and Martínez-Padilla 2012). Briefly, the experimental design performed by Vergara and Martínez-Padilla (2012) was carried out in autumn 2011. In a heather *C. vulgaris* dominated moorland, two areas of 1 km² separated by 100-m buffer area were randomly assigned as treatment and control areas (hereafter T-area and C-area, respectively). In a first capture, half of the males in the T-area were treated with testosterone implants (two silastic tubes of 1.57 mm inner and 2.41 mm outer diameter, 20 mm long, filled with testosterone [Sigma-Aldrich Co. Ltd., Poole, Dorset, UK]) and the other half left as untreated males. In order to reject that implanting itself affects individuals, a subsample of non-treated birds from both T-area and C-area was given empty implants. Because no effects were detected, empty and non-implanted males were all considered as controls (more details in Vergara and Martínez-Padilla 2012). In the C-area, all individuals were untreated and therefore considered as control males.

Before testosterone manipulation of birds in the different areas, neither grouse density nor aggressiveness differed among them (Vergara and Martínez-Padilla 2012). After testosterone manipulation, levels of intra-sexual competition assessed by calling rate behavior were higher in the T-area than in the C-area, as described in Vergara and Martínez-Padilla (2012). In addition, testosterone levels of intact males

from the T-area increased as much as birds implanted with testosterone (see Vergara and Martínez-Padilla 2012). These two results confirm that the experiment was effective at increasing intra-sexual competition, thus creating a more competitive environment in the T-area. Despite that the experimental manipulation was performed in autumn, we expected high levels of aggressiveness also during the spring because of two main reasons. First, the peak of aggressiveness in red grouse occurs in spring (Mougeot et al. 2005a), a fact that may partially explain the delayed density-dependent population dynamics in red grouse (see Martínez-Padilla et al. 2014b for further details). Second, the effects of testosterone implants on male aggressiveness may last for a year, beyond the 3-month increase on circulating testosterone levels induced by implants (Mougeot et al. 2003a, b). This is particularly relevant for our study on male calls, because the effects of testosterone manipulation in autumn induce these dominant males to defend large territories until the next autumn (Mougeot et al. 2003b). This may even affect individual behavior (body size and the condition-dependent expression of sexual traits) at the second spring after manipulation (Vergara et al. 2012a). Thus, we are confident that our manipulation of aggressiveness at population level in autumn (Vergara and Martínez-Padilla 2012) was still effective during the following spring when we recorded male calls, although we did not measure territory size to corroborate this idea.

For the purpose of this study, 32 males in the C-area, 33 control males in the T-area, and 19 testosterone-treated males in the T-area were fitted with radio-collars with a unique frequency (TW3-necklace radio-tags; Biotrack, Wareham, Dorset, UK) in autumn 2011 to allow for finding and recapturing the following spring. Ten recaptured birds were located in the buffer area or in an area different than in the first capture (three control males from the C-area, two control males from the T-area, and five T-treated males). These ten individuals were excluded from the study. Males recaptured during the following spring were aged from plumage traits (Vergara and Martínez-Padilla 2012), and their comb size (comb length \times width, mm²) was measured. We considered three groups: (1) control males in the C-area, (2) control males in the T-area, and (3) treated males in the T-area.

Acoustical analysis of call records

Red grouse calls were recorded by RT the following spring between the 27th and 30th of March 2012 (portable sound recorder, Microtrack II, M-Audio, UK). The recording and analysis were performed blindly in relation to the experimental treatment each individual male belonged to, since RT only had the radio frequencies and autumn locations obtained by Vergara and Martínez-Padilla in the original experiment (Vergara and Martínez-Padilla 2012). Males were localized by radio-tracking during the day. The observer approached

until the individual flew away, at which point the flight call was recorded.

The call of the red grouse is a simple vocalization formed by two strophes, the last of which is not always displayed (Fig. 1; Online Resource 1). The first strophe is formed by one syllable clearly separated from a subsequent fast chain of several syllables, which spreads out over time. Each syllable presents two frequency areas of maximum power. For each call, we measured 17 variables related to the duration and structure (i.e., number of syllables and peak frequency) of the whole call or to each of the strophes (Table 1). To determine whether our flight calls recorded in response to human intruders differed from territorial calls displayed by interacting males, we compared a sample of calls from our dataset with male calls recorded in the same population in the spring 2011 in response to male call playbacks (JM-P, unpublished data). We only found significant differences for two variables from a total of 11 variables analyzed (Table 2). Therefore, we considered the flight calls we recorded to be reliable proxies of sexual displays.

Red grouse calls were analyzed using Raven Pro 1.4 with short-time Fourier transform (sample rate 44,100 Hz, window size 526, time resolution 0.012 s, frequency resolution 83.8 Hz, Hann window, overlap 50 %, smoothing turned off). Calls with high background noise were discarded. A total of 35 calls consisting of one bout of several strophes (see Online Resource 1) from 29 males (13 young and 15 adults) were analyzed. In order to ensure the accuracy of call structure variables, the same person (RT) analyzed all red grouse calls. In addition, RT repeated the measurements of 15 calls in three time-related variables (song duration, lapse time between syllable 1 and syllable train, duration syllable 1). Repeatability between measurement was high ($r = 1$, $F_{1,43} = 4288716$, $P < 0.001$).

Statistical analysis

We performed a principal component analysis (PCA) considering only the call variables related to strophe 1 since only eight calls displayed strophe 2. PCA axes were our call-related dependent variables and were fitted by general linear mixed models (GLMMs), considering treatments as a factor to determine the influence of social context in call expression. Because six individuals were recorded more than once, we used individual identity as a random effect. We also analyzed whether comb size differed between treatment areas with a GLMM, including again individual identity as a random factor. Finally, we aimed to explore the mediating effect of our treatment on the relationship between grouse call and comb size. We ran a GLMM considering comb size and its interaction with treatment as the explanatory variables, including individual identity as random effects. Age was also included as a fixed factor but was not significant ($P > 0.05$) and thus

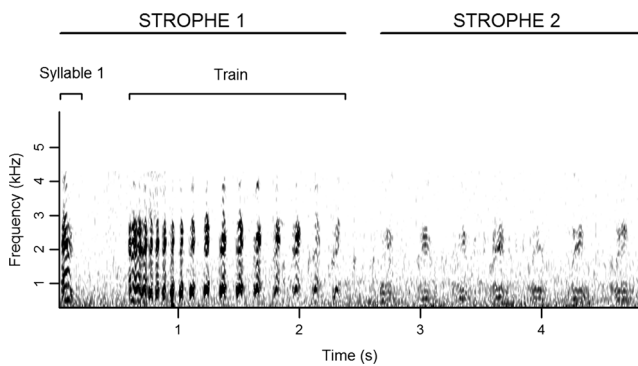


Fig. 1 Spectrogram of a red grouse call (corresponding with the audio file Redgrousecall.wav in Online Resource 1)

removed from the final models. Statistical analyses were performed in R v3.0.1 (R Core Team 2014).

Results

Only one male did not call when it flew away. We chose the first two axes of the PCA that explained 50.14 % of the variance and can be interpreted in the light of our aims (Table 3). By limiting the number of axes analyzed, we reduced the need to correct for multiple comparison in a small dataset. The first axis reflected the variation of the red grouse call duration (call

Table 2 Results for the *t* test of each call structure variable (only those related to strophe 1) between males recorded in spring 2012 in response to human intruders and males recorded in spring 2011 in response to male call playbacks

	<i>T</i> statistic	<i>P</i>
Strophe 1 duration	-1.69	0.116
Syllable train peak frequency	-0.92	0.378
Peak at low-frequency syllable train	6.16	<0.001
Peak at high-frequency syllable train	-2.16	0.05
Strophe 1 length	-0.87	0.397
Lapse time between syllable 1 and syllable train	-0.95	0.356
Syllable 1 peak frequency	-0.80	0.445
Peak at low frequency of syllable 1	4.17	<0.001
Peak at high frequency of syllable 1	-1.61	0.117
Duration syllable 1	-1.11	0.293
Duration syllable train	-1.50	0.161

duration axis), and the second one described the call frequency pattern (frequency axis).

The call duration axis was influenced by treatment group ($F_{2,22} = 3.84$; $P = 0.037$). Control males in the C-area displayed significantly shorter calls than control males in the T-area (Tukey's HSD; Fig. 2). The frequency axis ($F_{2,22} = 1.04$; $P = 0.371$) and comb size ($F_{2,19} = 2.11$; $P = 0.149$) did not differ between treatment groups.

Table 1 Mean \pm SE of the call structure variables measured in male red grouse of control areas (CC), control males in the testosterone-treated area (CT), and testosterone-treated birds (TT)

	Global	CC	CT	TT
Call length (number of syllables)	17.6 \pm 0.9	13.9 \pm 0.9 (<i>n</i> = 13)	19.9 \pm 1.4 (<i>n</i> = 15)	20.4 \pm 2.7 (<i>n</i> = 5)
Whole call duration (s)	2.612 \pm 0.263	1.832 \pm 0.208 (<i>n</i> = 13)	3.166 \pm 0.455 (<i>n</i> = 15)	3.281 \pm 0.882 (<i>n</i> = 5)
Strophe 1 duration (s)	1.974 \pm 0.093	1.673 \pm 0.118 (<i>n</i> = 13)	2.184 \pm 0.124 (<i>n</i> = 15)	2.176 \pm 0.342 (<i>n</i> = 5)
Strophe 1 length (number of syllables)	16.0 \pm 0.6	13.5 \pm 0.9 (<i>n</i> = 13)	17.5 \pm 0.8 (<i>n</i> = 15)	17.4 \pm 2 (<i>n</i> = 5)
Syllable 1 duration (s)	0.099 \pm 0.003	0.010 \pm 0.004 (<i>n</i> = 13)	0.099 \pm 0.005 (<i>n</i> = 15)	0.097 \pm 0.006 (<i>n</i> = 5)
Syllable 1 peak frequency (Hz)	1474.4 \pm 89.7	1504.0 \pm 163.5 (<i>n</i> = 13)	1458.1 \pm 142.9 (<i>n</i> = 14)	1429.8 \pm 209.4 (<i>n</i> = 5)
Peak at low frequency of syllable 1 (Hz)	1072.9 \pm 41.8	1023.7 \pm 77.0 (<i>n</i> = 13)	1045.9 \pm 42.2 (<i>n</i> = 14)	1102.5 \pm 50.2 (<i>n</i> = 5)
Peak at high frequency of syllable 1 (Hz)	2126.7 \pm 86.7	2040.7 \pm 97.1 (<i>n</i> = 13)	2214.8 \pm 186.6 (<i>n</i> = 14)	2084.4 \pm 97.8 (<i>n</i> = 5)
Lapse time duration between syllable 1 and syllable train (s)	0.450 \pm 0.013	0.419 \pm 0.022 (<i>n</i> = 13)	0.478 \pm 0.016 (<i>n</i> = 15)	0.464 \pm 0.030 (<i>n</i> = 4)
Syllable train duration (s)	1.411 \pm 0.089	1.153 \pm 0.106 (<i>n</i> = 13)	1.609 \pm 0.124 (<i>n</i> = 15)	1.549 \pm 0.412 (<i>n</i> = 4)
Syllable train peak frequency (Hz)	1463.4 \pm 101.7	1643.2 \pm 175.3 (<i>n</i> = 13)	1390.6 \pm 155.2 (<i>n</i> = 15)	1360.9 \pm 285.6 (<i>n</i> = 5)
Peak at low-frequency syllable train (Hz)	981.9 \pm 21.0	964.0 \pm 39.2 (<i>n</i> = 13)	987.7 \pm 23.9 (<i>n</i> = 15)	964.7 \pm 81.3 (<i>n</i> = 5)
Peak at high-frequency syllable train (Hz)	2142.7 \pm 24.8	2113.6 \pm 36.3 (<i>n</i> = 13)	2197.3 \pm 30.9 (<i>n</i> = 15)	1989.7 \pm 53.5 (<i>n</i> = 5)
Lapse time between strophe 1 and strophe 2 (s)	0.380 \pm 0.059	0.589 (<i>n</i> = 1)	0.386 \pm 0.079 (<i>n</i> = 5)	0.262 \pm 0.007 (<i>n</i> = 2)
Strophe 2 duration (s)	2.409 \pm 0.212	1.482 (<i>n</i> = 1)	2.556 \pm 0.275 (<i>n</i> = 5)	2.505 \pm 0.120 (<i>n</i> = 2)
Strophe 2 length (number of syllables)	7.0 \pm 0.6	5 (<i>n</i> = 1)	7.2 \pm 0.9 (<i>n</i> = 5)	7.5 \pm 0.5 (<i>n</i> = 2)
Strophe 2 peak frequency (Hz)	1690.4 \pm 220.2	516.8 (<i>n</i> = 1)	1757.1 \pm 212.5 (<i>n</i> = 5)	2110.2 \pm 172.2 (<i>n</i> = 2)

The overall values are also shown as well as sample sizes

^a Peak frequency is the frequency at which maximum power is reached

Table 3 Results of the two principal component analysis axes indicating the original call variables influencing red grouse call feature variation

	PC1	PC2
Call length	-0.440	0.076
Song duration	-0.395	-0.230
Strophe 1 duration	-0.446	0.116
Strophe 1 length	-0.353	0.413
Duration syllable train	-0.440	0.148
Syllable train peak frequency	0.008	-0.153
Peak at low-frequency syllable train	0.203	0.552
Peak at high-frequency syllable train	-0.202	0.027
Lapse time between syllable 1 and syllable train	-0.184	-0.208
Syllable 1 peak frequency	-0.037	-0.101
Peak at low frequency of syllable 1	0.085	0.584
Peak at high frequency of syllable 1	0.055	0.010
Duration syllable 1	-0.073	-0.109
Explained variance (%)	35.52	14.62

Treatment mediated the relationship between comb size and call duration (treatment \times comb size $\chi^2 = 12.29$, $P = 0.002$). Call duration was positively associated with comb size only in control males in the C-area ($F_{1,7} = 19.88$, $P = 0.003$; control males in the T-area $F_{1,7} = 0.010$, $P = 0.924$; testosterone males in the T-area $F_{1,2} = 9.14$, $P = 0.094$; Fig. 3).

Discussion

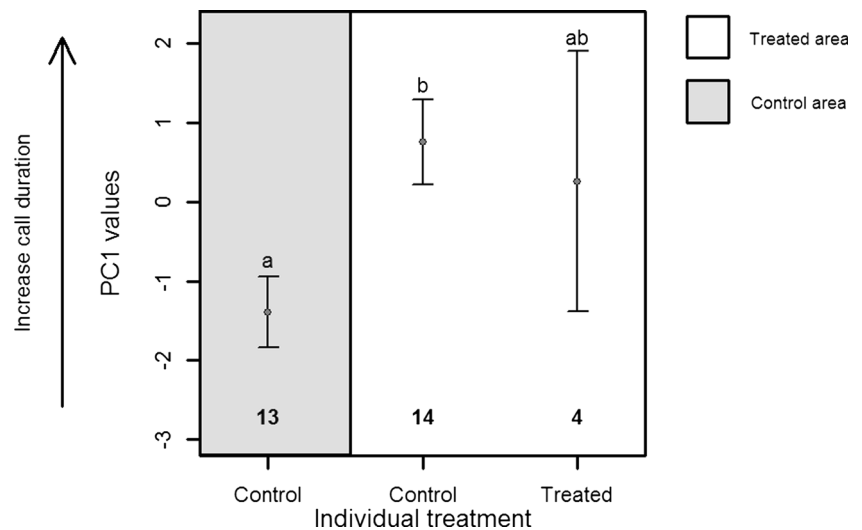
Our results show that increased intra-sexual competition influenced red grouse vocal signaling by increasing call duration. Moreover, we found a positive association between call duration and comb size only in areas where intra-sexual competition was lower. However, we did not find an effect on the frequency of red grouse calls. One plausible explanation is that morpho-anatomical constraints on the vocal apparatus may limit the feasible variation on the frequency bandwidth of male red grouse. Other possibility is that flight and ground calls differ in the expression of frequency bandwidth, although we only found differences between the two call types for 2 variables out of 11 (“peak at low frequency of syllable train” and “peak at low frequency of syllable 1”—Tables 2 and 3). Therefore, further studies should clarify the role of frequency bandwidth in sexual selection.

Previous studies suggest the role of aggressive behaviors like calling behavior in male-male competition in red grouse and its importance on territory maintenance (e.g., Moss et al. 1994; Mougeot et al. 2005a). In accordance with the redundant signal hypothesis, which predicts that the simultaneous evaluation of sexual traits informing about the same individual aspect ensures a more reliable interpretation of individual

quality (Candolin 2003), we expected that control males exposed to higher competitive conditions (T-area) reduced call length in accordance with the decreased in comb size found in autumn (Vergara and Martínez-Padilla 2012). However, our results do not support the predictions derived from the redundant signal hypothesis. Harsher environmental circumstances caused by increased intra-sexual competition may force low-quality individuals to decrease signal expression, comb size in this case, in order to avoid risky encounters with dominant males (Candolin 2000; Vergara and Martínez-Padilla 2012). Instead, we found that males exposed to higher competitive conditions performed longer calls. Previous findings in song sparrows suggested no effects of dominant (testosterone-treated) males on calling behavior of intact male neighbors (Wingfield 1984). However, our experiment explored the effect of increased aggressiveness on intact males defending territories in areas of increased levels of intra-sexual competition, while Wingfield (1984) compared male calling behavior of intact individuals neighboring testosterone-treated males without increasing intra-sexual competition at population level as we did, preventing comparisons among studies. Our experiment recreates a scenario with a higher proportion of dominant males in a given area (Mougeot et al. 2005b), which may push non-dominant males to increase their call length to avoid intra-sexual encounters or male intrusions into their territories. Although we predicted a decrease in call length under high male-male competition, following the redundant signal hypothesis (Candolin 2003), the escalation of aggressiveness when individuals confront high intra-sexual competition is very often found for behavioral traits (Leitão and Riebel 2003; de Kort et al. 2009; Morales et al. 2014). Contrary to call length, previous results of this field experiment showed that the comb size of intact red grouse males living in areas of experimentally increased intra-sexual competition decreased while testosterone levels increased (Vergara and Martínez-Padilla 2012). The authors hypothesized that other sexual traits different from comb size may increase in accordance with testosterone levels. Our results support this hypothesis since call length of control males living in the testosterone-treated area increased but comb size did not, suggesting that investment in both sexual traits might be either too costly, or alternatively, each signal informs about different individual qualities.

It is important to elucidate the different role of calling structure and comb size in red grouse, in order to tease apart the relative influence of these two traits on male-male competition contests, because their outcome can determine the successful establishment of territories, a critical resource for this species (Mougeot et al. 2003b). Our results shed some light at this respect. We found a positive association between call length and comb size in intact males when intra-sexual competition is relaxed but no association when intra-sexual competition is high. This means that levels of intra-sexual competition may differently affect the expression of these signals, whose costs and benefits may change depending on environmental

Fig. 2 Mean \pm SE of the call duration axis (PC1) for the different treatment types. Different letters indicate significant differences between treatment types ($P < 0.05$, Tukey's HSD). Call duration axis was multiplied by -1 to facilitate interpretation of the results



conditions, as suggested for comb size (Martínez-Padilla et al. 2014a). Therefore, our results suggest that comb size and call length can inform about different characteristics of the signaler (see review in Candolin 2003), being in agreement with the multiple message hypothesis. It might be possible that calling behavior can be favored over comb size to reduce intra-sexual encounters when intra-sexual competition is intense. Assuming that comb size and call length are candidates to trigger agonistic encounters between males and that the assessment of comb size requires a closer approach, increasing call length may be a better way to reduce the number of agonistic encounters in more competitive circumstances.

According to our results, comb size and calling behavior may suffer different selective pressures depending on the particular environmental conditions. Several studies demonstrate

the key role that parasites and testosterone play on the population dynamics of this species (e.g., Fox and Hudson 2001; Martínez-Padilla et al. 2014b) and how they also modulate the expression of these sexual traits. Individuals free of nematode parasites are able to gain more behavioral contests (Fox and Hudson 2001), to increase calling rate (Mougeot et al. 2005b), and to increase comb size (Mougeot et al. 2005b), whereas high testosterone levels also cause an increase on the expression of comb size and calling rate (Mougeot et al. 2005a; Martínez-Padilla et al. 2014a). Given the relevant role that parasites and testosterone have on territory defense and ultimately on population dynamics, and the effects of intra-sexual competition on comb size and call length showed by our study, it might be possible that the role of comb size over calling structure changes throughout the population cycle. From a population dynamics perspective, viability selection (survival due to territory acquisition) acting on comb size might vary with different levels of intra-sexual competition. Specifically, when intra-sexual competition is high, males reduce their comb size (Vergara and Martínez-Padilla 2012; Vergara et al. 2012b) but instead perform longer calls, as our results suggest. The strength of selection on call length could peak when population density, and therefore intra-sexual competition, reaches its maximum during the population cycle. Meanwhile, the selective strength on comb size should diminish at this moment of the population cycle. Our findings encourage future studies that investigate how differential selective pressures over the population cycle of the red grouse maintain the honesty of these two sexual signals.

We acknowledge the shortcomings of our experimental approach. The study was based on a limited sample size, and each area (treated vs. control) constitutes a single plot. This lack of replication likely constitutes the main weakness of our study, and future replicated experiments are needed to corroborate the patterns described here. One might think that habitat structure, predation rate, or parasite burdens between the treatment

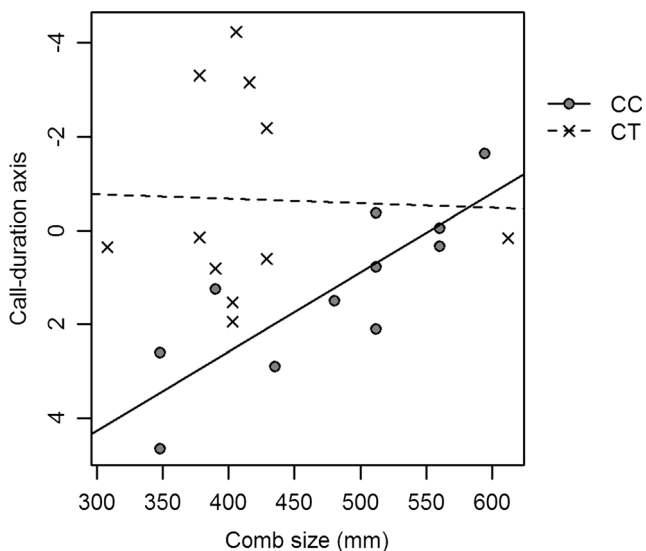


Fig. 3 Relationship between call duration axis (PC1) and comb size for control males (CC control males in the control area, CT control males in the testosterone area). Call duration axis was multiplied by -1 to facilitate interpretation of the results

and control area may differ, affecting our results. However, these environmental factors are equivalent among treatment and control areas. The study site is managed by game keepers to facilitate heather grow and is under severe legal avian and mammal predator control, so habitat structure and predation pressure are similar within the study site. It has not been described spatial variation of abundance or prevalence of nematode parasites in red grouse within grouse moors, so we consider unlikely that abundance of parasite may differ among treatment and control areas. Remarkably, as described in Vergara and Martínez-Padilla (2012), population density did not differ among areas. In addition, we did not evaluate the association between call length and testosterone levels, although this is likely to occur because calling rate of males increased when their testosterone levels were experimentally increased (Mougeot et al. 2005a). Future studies should explore the role of testosterone on the calling structure of red grouse, as it occurs in other species (Fusani et al. 1994; Apfelbeck et al. 2012).

Our study supports the idea that intra-sexual competition mediates the coordinated expression of multiple sexual signals. Dynamic changes in sexual traits due to fluctuating levels of male-male competition may compromise the reliability of the signals and modify the distribution of sexual phenotypes across space and time, which may affect the magnitude and direction of sexual selection. Research on the evolution of sexual traits should move toward a more ecological framework, with attempts to experimentally manipulate environmental conditions, linking the variation in the expression of multiple secondary sexual traits induced by social or ecological environmental heterogeneity.

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Compliance with ethical standards

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Informed consent This article does not contain any studies with human participants performed by any of the authors.

References

- Andersson S, Pryke S, Örnborg J, Lawes MJ, Andersson M (2002) Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. *Am Nat* 160:683–691
- Apfelbeck B, Kiefer S, Mortega KG, Goymann W, Kipper S (2012) Testosterone affects song modulation during simulated territorial intrusions in male black redstarts (*Phoenicurus ochruros*). *PLoS ONE* 7, e52009
- Candolin U (2000) Increased signalling effort when survival prospects decrease: male-male competition ensures honesty. *Anim Behav* 60: 417–422
- Candolin U (2003) The use of multiple cues in mate choice. *Biol Rev* 78: 575–595
- Candolin U (2005) Why do multiple traits determine mating success? Differential use in female choice and male competition in a water boatman. *Proc R Soc Lond B* 272:47–52
- Chaine AS, Lyon BE (2008) Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science* 319:459–462
- R Core Team (2014) R Foundation for Statistical Computing, Vienna, www.r-project.org
- Cornwallis CK, Uller T (2009) Towards an evolutionary ecology of sexual traits. *Trends Ecol Evol* 25:145–152
- Cothran RD, Jeyasingh PD (2010) Condition dependence of a sexually selected trait in a crustacean species complex: importance of the ecological context. *Evolution* 64:2535–2546
- Cotton S, Fowler K, Pomiankowski A (2004a) Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc R Soc Lond B* 271:771–783
- Cotton S, Fowler K, Pomiankowski A (2004b) Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *Evolution* 58:1038–1046
- de Kort SR, Eldermire ERB, Cramer ERA, Vehrencamp SL (2009) The deterrent effect of bird song in territory defense. *Behav Ecol* 20:200–206
- Fox A, Hudson PJ (2001) Parasites reduce territorial behaviour in red grouse (*Lagopus lagopus scoticus*). *Ecol Lett* 4:139–143
- Fusani L, Beani L, Dessì-Fulgheri F (1994) Testosterone affects the acoustic structure of the male call in the grey partridge (*Perdix perdix*). *Behaviour* 128:301–310
- Galeotti P, Saino N, Sacchi R, Møller AP (1997) Song correlates with social context, testosterone and body condition in male barn swallows. *Anim Behav* 53:687–700
- Garant D, Sheldon BC, Gustafsson L (2004) Climatic and temporal effects on the expression of secondary sexual characters: genetic and environmental components. *Evolution* 58:634–644
- Gil D, Gahr M (2002) The honesty of bird song: multiple constraints for multiple traits. *Trends Ecol Evol* 17:133–141
- Grafen A (1990) Biological signals as handicaps. *J Theor Biol* 144:517–546
- Hardouin LA, Reby D, Bavoux C, Burneleau G, Bretagnolle V (2007) Communication of male quality in owl hoots. *Am Nat* 169:552–562
- Karubian J, Swaddle JP, Varian-Ramos CW, Webster MS (2009) The relative importance of male tail length and nuptial plumage on social dominance and mate choice in the red-backed fairy-wren *Malurus*

- melanocephalus*: evidence for the multiple receiver hypothesis. *J Avian Biol* 40:559–568
- Kipper S, Mundry R, Sommer C, Hultsch H, Todt D (2006) Song repertoire size is correlated with body measures and arrival date in common nightingales, *Luscinia megarhynchos*. *Anim Behav* 71:211–217
- Leitão A, Riebel K (2003) Are good ornaments bad armaments? Male chaffinch perception of songs with varying flourish length. *Anim Behav* 66:161–167
- Martínez-Padilla J, Pérez-Rodríguez L, Mougeot F, Redpath SM (2014a) Intra-sexual competition alters the relationship between testosterone and ornament expression in a wild territorial bird. *Horm Behav* 65:435–444
- Martínez-Padilla J, Redpath SM, Zeineddine M, Mougeot F (2014b) Insights into population ecology from long-term studies of red grouse *Lagopus lagopus scoticus*. *J Anim Ecol* 83:85–98
- Martínez-Padilla J, Pérez-Rodríguez L, Mougeot F, Ludwig SC, Redpath SM (2014c) Experimentally elevated levels of testosterone at independence reduce fitness in a territorial bird. *Ecology* 95:1033–1044
- Martín-Vivaldi M, Palomino JJ, Soler M (1998) Song structure in the Hoopoe (*Upupa epops*) - Strophe length reflects male condition. *J Ornithol* 139:287–296
- Møller AP, Pomiankowski A (1993) Why have birds got multiple sexual ornaments? *Behav Ecol Sociobiol* 32:167–176
- Morales MB, Casas F, García de la Morena E, Ponjoan A, Calabuig G, Martínez-Padilla J, García JT, Mañosa S, Viñuela J, Bota G (2014) Density dependence and habitat quality modulate the intensity of display territory defence in an exploded lekking species. *Behav Ecol Sociobiol* 68:1493–1504
- Moss R, Kolb HH, Marquiss M, Watson A, Treca B, Watt D, Glennie W (1979) Aggressiveness and dominance in captive cock red grouse. *Aggress Behav* 5:59–84
- 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* 258:175–180
- Mougeot F, Redpath SM, Leckie F, Hudson PJ (2003a) The effect of aggressiveness on the population dynamics of a territorial bird. *Nature* 421:737–739
- Mougeot F, Redpath SM, Moss R, Matthiopoulos J, Hudson PJ (2003b) Territorial behaviour and population dynamics in red grouse *Lagopus lagopus scoticus*. I. Population experiments. *J Anim Ecol* 72:1073–1082
- Mougeot F, Irvine JR, Seivwright L, Redpath SM, Piertney S (2004) Testosterone, immunocompetence, and honest sexual signaling in male red grouse. *Behav Ecol* 15:930–937
- Mougeot F, Dawson A, Redpath SM, Leckie F (2005a) Testosterone and autumn territorial behavior in male red grouse *Lagopus lagopus scoticus*. *Horm Behav* 47:576–584
- Mougeot F, Evans SA, Redpath SM (2005b) Interactions between population processes in a cyclic species: parasites reduce autumn territorial behaviour of male red grouse. *Oecologia* 144:289–298
- Mougeot F, Piertney SB, Leckie F, Evans S, Moss R, Redpath SM, Hudson PJ (2005c) Experimentally increased aggressiveness reduces population kin structure and subsequent recruitment in red grouse *Lagopus lagopus scoticus*. *J Anim Ecol* 74:488–497
- Penteriani V (2003) Breeding density affects the honesty of bird vocal displays as possible indicators of male/territory quality. *Ibis* 145:E127–E135
- Piertney SB, Lambin X, Maccoll ADC et al (2008) Temporal changes in kin structure through a population cycle in a territorial bird, the red grouse *Lagopus lagopus scoticus*. *Mol Ecol* 17:2544–2551
- Price JJ, Earnshaw SM, Webster MS (2006) Montezuma oropendolas modify a component of song constrained by body size during vocal contests. *Anim Behav* 71:799–807
- Redpath SM, Mougeot F, Leckie FM, Elston DA, Hudson PJ (2006a) Testing the role of parasites in driving the cyclic population dynamics of a gamebird. *Ecol Lett* 9:410–418
- Redpath SM, Mougeot F, Leckie FM, Evans SA (2006b) The effects of autumn testosterone on survival and productivity in red grouse, *Lagopus lagopus scoticus*. *Anim Behav* 71:1297–1305
- Robinson MR, Pilkington JG, Clutton-Brock TH, Pemberton JM, Kruuk LEB (2008) Environmental heterogeneity generates fluctuating selection on a secondary sexual trait. *Curr Biol* 18:751–757
- Vergara P, Martínez-Padilla J (2012) Social context decouples the relationship between a sexual ornament and testosterone levels in a male wild bird. *Horm Behav* 62:407–412
- Vergara P, Martínez-Padilla J, Mougeot F, Leckie F, Redpath SM (2012a) Environmental heterogeneity influences the reliability of secondary sexual traits as condition indicators. *J Evol Biol* 25:20–28
- Vergara P, Redpath SM, Martínez-Padilla J, Mougeot F (2012b) Environmental conditions influence red grouse ornamentation at a population level. *Biol J Linn Soc* 107:788–798
- Watson A, Moss R (2008) Grouse. Collins, London
- Wingfield J (1984) Environmental and endocrine control of reproduction in the song sparrow, *Melospiza melodia*: 2. Agonistic interactions as environmental information stimulating secretion of testosterone. *Gen Comp Endocrinol* 56:417–424
- Zahavi A (1993) The fallacy of conventional signalling. *Philos T Roy Soc B* 340:227–230