



Environmental conditions influence red grouse ornamentation at a population level

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Theory suggests that condition-dependent sexual displays should be more weakly expressed under adverse conditions than under more favourable ones. Here, we tested this hypothesis in wild red grouse *Lagopus lagopus scoticus* using a data set of nearly 1500 individuals from nine populations over 8 years, covering varying environmental conditions. We analysed whether male and female ornament expression (i.e. comb size) in a given site and year varied with various indices of environmental conditions: population density, *Trichostrongylus tenuis* nematode infection at the population level, and climate conditions [measured as winter North Atlantic oscillation (NAO) index]. We found that average comb size in males, but not in females, negatively correlated with population density, parasite infection levels, and winter NAO index. Furthermore, the coefficient of variation (CV) of comb size was higher in females than in males. CVs in both males and females were not clearly associated with the studied environmental variables. Our results support the idea that the expression of condition-dependent sexual traits should be lower under more stressful environmental conditions, but only in males. We discuss the potential reasons behind the effect of environmental conditions on secondary sexual traits, and why these effects differ between sexes. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, 107, 788–798.

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INTRODUCTION

Secondary sexual traits displayed by males or females may have evolved to signal individual quality, with stronger signals providing fitness benefits by influencing either mating decisions or the outcome of intra-sexual interactions (Andersson, 1994; Amundsen, 2000). Ornaments are often condition-dependent (Andersson, 1994), with only individuals in prime

condition displaying the brightest or largest sexual traits (Zahavi, 1975). This condition-dependence of sexual traits implies that average ornament expression levels within a population should be lower under adverse environments than under more favourable conditions, because of physiological trade-offs associated with resource limitation (reviewed by Cotton, Fowler & Pomiankowski, 2004a). However, teasing apart the impact of environmental conditions on ornaments in the field is challenging. Most studies have addressed this question by modifying environmental conditions through methods such as brood- or

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food-manipulations (e.g. Gustafsson, Qvarnström & Sheldon, 1995; Fitze, Tschirren & Richner, 2003). But fewer studies have used specific environmental factors as proxies of environmental quality (Endler, 1995; Garant, Sheldon & Gustafsson, 2004; Mysterud *et al.*, 2005; Hegyi *et al.*, 2007; Vanpé *et al.*, 2007). The latter approach can be a powerful one but it requires sufficient variation in conditions across populations or years with the obvious difficulty of data collection.

Social context is among the environmental factors known to affect sexual ornamentation (Mysterud *et al.*, 2005; Vanpé *et al.*, 2007). For instance, in a context of intense intra-sexual competition, dominance hierarchies can strongly affect the expression of sexual traits because subordinate individuals reduce their investment in signalling (Moss *et al.*, 1979; McGraw, Dale & Mackillop, 2003; Karubian *et al.*, 2011). Another component of social context that may affect sexual ornamentation is population density, but whether a higher density promotes or decreases levels of sexual displays remains controversial. On the one hand, population density has been shown to have a negative impact on condition-dependent ornamentation because a greater competitiveness associated with a higher density may lead to a loss in body condition (Mysterud *et al.*, 2005; Vanpé *et al.*, 2007). Hence, in less favourable high-density areas, average ornament expression levels should be lower (Mysterud *et al.*, 2005; Vanpé *et al.*, 2007). On the other hand, higher density areas could be better quality areas (e.g. with more resources, or fewer predators) where individuals are in better condition. If so, positive relationships between density and the level of condition-dependent ornamentation could also be found. Positive relationships between population density and ornaments, particularly those whose expression is androgen-dependent, would be also predicted because of an indirect effect of the social context on sexual traits via changes in hormone levels (Moss, Watson & Parr, 1996). Androgen levels, and in particular testosterone levels, are often increased after intense episodes of intra-sexual competition (according to the so-called 'Challenge Hypothesis'; Wingfield *et al.*, 1990). If so, increased levels of androgen-dependent ornamentation should be found in highly densely, more competitive areas (Moss *et al.*, 1996).

Other sources of environmental quality variation that have received particular interest within a sexual selection framework are climate conditions and parasite infection levels. Both climate and parasites have strong effects on the body condition, reproduction, and survival of individuals (Hudson, Dobson & Newborn, 1998; Przybylo, Sheldon & Merilä, 2000). Hence, according to the condition-dependence of sexual traits, harsh climatic conditions and high parasite

abundance should promote lower ornament expression levels (Evans, 1991; Møller, Christe & Lux, 1999; Mysterud *et al.*, 2005).

The hypotheses regarding the role that environmental factors may have in mediating ornament expression, although initially developed for males, could similarly be applied to the condition-dependent traits displayed by females. Indeed, recent studies have shown an enhanced investment in female sexual signalling as competition for males increases (Svensson *et al.*, 2009) or under more favourable climatic conditions (Hegyi *et al.*, 2008). However, the generality of these relationships still needs to be confirmed.

The red grouse *Lagopus lagopus scoticus* is a monogamous, territorial game species that lives in the moorlands of the UK. Males establish their territories during autumn, and defend them over winter until the next spring, when mating and breeding take place (Watson & Moss, 2008). This species shows unstable cyclical dynamics in abundance, so density varies greatly between years, from only a few tens to several hundreds of pairs per km² (Moss & Watson, 2001; Redpath *et al.*, 2006a). Red grouse are affected by the nematode *Trichostrongylus tenuis*, with well-documented negative effects on its body condition, breeding outcome, survival, and ornamentation in both sexes (Delahay *et al.*, 1995; Hudson *et al.*, 1998; Redpath *et al.*, 2006a; Martínez-Padilla *et al.*, 2010, 2011; Vergara *et al.*, 2011, 2012b). Red grouse display a main ornament, supra-orbital combs, whose size is condition-dependent in both sexes, and testosterone-dependent in males (Mougeot *et al.*, 2004; Mougeot, Redpath & Piertney, 2006; Martínez-Padilla *et al.*, 2011). In addition, comb size positively correlates with fitness components in both sexes (MacColl *et al.*, 2000; Mougeot *et al.*, 2004, 2006; Redpath *et al.*, 2006b). In males, comb size acts as an indicator of quality in both intra- and inter-sexual contexts (Moss *et al.*, 1979; Redpath *et al.*, 2006b). In females, the functions of comb size are not as well known as in males, but empirical evidence suggests possible functions in female–female aggressive encounters and in male mate choice (Watson & Jenkins, 1964; Redpath *et al.*, 2006b; Martínez-Padilla *et al.*, 2011).

In this study, we analysed whether average male and female comb size in a given site and year varies with indices of environmental conditions, specifically population density (grouse km⁻²), average *T. tenuis* parasite infection levels within grouse populations, and climatic conditions. We use a data set of nearly 1500 individuals from nine populations over 8 years, capturing a large degree of environmental variation to adequately assess the response of sexual traits to environmental stressors (Cotton *et al.*, 2004a). Overall, we expected lower trait size under more adverse environmental conditions, as predicted by the

condition-dependence of sexual traits (Cotton *et al.*, 2004a; Cotton, Fowler & Pomiankowski, 2004b). Environmental conditions affect ornament expression at the time that the trait is growing or displayed. Because ornaments such as feathers or horns are often displayed during long time periods, studies on such sexual traits have focused on the delayed effects of environmental conditions (e.g. Garant *et al.*, 2004; Hegyi *et al.*, 2008). However, comb size is a highly dynamic trait whose size can change rapidly (within a few days) in response to changing conditions (Mougeot *et al.*, 2004; Martínez-Padilla *et al.*, 2011). Therefore, we analysed the effect of current (during the same season), rather than previous, environmental conditions on comb size. Specifically, we expect negative relationships between mean comb size and population density and parasite infection levels, given that both environmental factors should negatively affect resources available for ornamentation and the physiological condition of individuals (Mougeot *et al.*, 2006; Myrsterud *et al.*, 2005; Martínez-Padilla *et al.*, 2010, 2011; Vergara *et al.*, 2012a, b). For parasites, we recently showed a negative relationship between average parasite load and average male comb size across populations (Vergara *et al.*, 2012b) and its condition-dependence in both male and female grouse (Vergara *et al.*, 2011, 2012b). However, the relationship between average comb size and parasite load at the population level across populations remains unexplored in females, and may be different from that of male populations. As a proxy of climate conditions we used the winter North Atlantic oscillation NAO index (see Methods for more details), which in our study area (moorlands of northern UK) is positively correlated with rainfall and temperature and negatively with snow cover (Murphy & Washington, 2001; Fowler & Kilsby, 2002; Trivedi *et al.*, 2007). We assume that dry and cold winters are more favourable for grouse, with longer periods of snow cover associated with lower parasite infection (Hudson, Dobson & Newborn, 2002) and better foraging conditions during the spring (Myrsterud *et al.*, 2001). Therefore, we predicted a negative relationship between mean comb size and NAO index. We also explore whether the studied environmental factors affect female comb size. We expected that environmental quality positively affects female comb size, as for males. However, given the greater degree of exaggeration of comb size and a probably greater importance in sexual selection processes in males than in females, we expected stronger relationships for males than for females (Cotton *et al.*, 2004b). In addition to studying variations in the mean level of sexual trait expression, we also analysed variation in its variance, as it can inform the opportunity for selection on a given trait. For this, we analysed variance of comb size for males and females.

Adverse environments may increase the differences between low- and high-quality individuals, thus potentially increasing variance in trait expression under more restrictive conditions (Cotton *et al.*, 2004b; Cothran & Jeyasingh, 2010).

MATERIALS AND METHODS

During spring (February–April) of 2000–2004 and 2009–2011, we captured and individually tagged a total 1449 red grouse (952 males and 497 females) in nine different populations within the UK (see Table 1 and Martínez-Padilla *et al.*, 2011 for more details on the location of these study areas). Captures were not carried out every year in all the study populations, but overall, we sampled a total of 31 site-years (Table 1). Individuals were aged as young (< 1 year old) or adult (> 1 year old) from their plumage (Cramp & Simmons, 1980), and comb area (maximum length \times width of flattened combs, in mm²) was measured as a index of ornament size (Mougeot, Redpath & Leckie, 2005a; Martínez-Padilla *et al.*, 2010, 2011).

POPULATION DENSITY

Population density (number of grouse km⁻²) was estimated in each studied site-year by counting grouse during spring (March–April) with the aid of trained dogs or by recording the number of grouse responding to the playback of a male territorial calls, methods that report comparable numbers and that have been previously validated for red grouse (Evans *et al.*, 2007). Briefly, we conducted playbacks at points randomly distributed, 250 m apart. At each point, we played male territorial calls in each of the four cardinal directions, using a portable tape player. Following the playback, we scanned the surrounding area for 5 min and recorded the number of males that responded by calling or showing within a 100-m radius of the survey point. The average number of male responses per site were transformed into total number of grouse following the equations provided in Evans *et al.* (2007).

PARASITE INFECTION

Parasite infection at the population level, hereafter referred to as 'parasite population mean', was calculated as the geometric mean of the *T. tenuis* abundances per individual in a given site and year (Redpath *et al.*, 2006a; Vergara *et al.*, 2011, 2012b). To estimate *T. tenuis* abundance for each individual (number of worms per grouse), we used faecal egg counts and standard techniques developed and previously validated for red grouse (see Seivwright *et al.*, 2004). Faecal samples for parasite counts were

Table 1. Location and environmental factor variation in the studied populations

Population	Location	Altitude (m a.s.l.)	Density (no. km ⁻²)	<i>T. tenuis</i> (geometric mean)	Years	<i>N</i> males	<i>N</i> females
Edinglassie, Scotland	57°12'N, 03°07'W	470	70 (50–77)	652 (371–1376)	2001, 2003–04, 2006	76 (2–44)	13 (13)
Glen Dye, Scotland	56°55'N, 02°34'W	400	67 (67)	1202 (1202)	2003	18 (18)	–
Glen Muick, Scotland	56°56'N, 03°06'W	560	45 (32–70)	199 (106–387)	2000–04	151 (6–120)	26 (6–12)
Invercauld, Scotland	57°07'N, 03°15'W	450	42 (42)	1514 (1514)	2000	143 (143)	–
Invermark, Scotland	56°54'N, 02°50'W	400	61 (41–105)	549 (166–1174)	2001, 2009–11	136 (3–104)	91 (12–48)
Millden, Scotland	56°55'N, 02°45'W	300	32 (18–50)	572 (178–838)	2000–04	121 (2–91)	15 (3–7)
Catterick, England	54°20'N, 01°51'W	360	59 (38–90)	1917 (530–4810)	2002–04, 2009–10	105 (9–40)	148 (7–47)
Geltsdale, England	54°52'N, 02°38'W	430	74 (61–86)	486 (285–687)	2009–10	54 (22–32)	34 (15–19)
Moorhouse, England	54°44'N, 02°23'W	560	79 (29–193)	2260 (1147–4765)	2002–04, 2009	148 (6–105)	170 (3–127)

Ranges are given in parentheses.

collected from a subsample (mean = 28, range 9–107) of both males and females captured in each site and year, and that were kept overnight in individual boxes for this purpose (Redpath *et al.*, 2006a; Vergara *et al.*, 2011, 2012b).

CLIMATIC CONDITIONS

As a proxy of the climatic conditions generally affecting the studied populations, we used the winter (December–March) NAO index, where higher values are associated with increased precipitation and higher temperatures over northern Europe (see Stenseth *et al.*, 2003 for further descriptions), including the UK (Murphy & Washington, 2001; Fowler & Kilsby, 2002). In addition, snow cover may be maintained for longer when winter temperatures are low and prevent thawing. Therefore, negative winter NAO index values are typically associated with longer periods of snow cover in Northern UK at all altitudes, particularly at mid to low elevations (below 450 m) (Trivedi *et al.*, 2007). The average altitude of the studied sites is 437 m (Table 1). We obtained the winter NAO index from the web (<http://www.cgd.ucar.edu/cas/jhurrell/indices.html>).

STATISTICAL ANALYSES

We analysed the data using General Linear Mixed Models (GLMM) implemented in SAS 9.2 (PROC MIXED procedure). First, we analysed whether the environmental variables (i.e. spring population density, parasite population mean and NAO index) were intercorrelated (site being included in models as a random factor). Population density and parasite population mean were log-transformed hereafter, to meet assumptions of normality (although results remained similar when analysing un-transformed variables). Secondly, we tested if comb size varied with the studied environmental factors, and whether these relationships differed between sexes. Comb area was the response variable, and population density, parasite population mean, and NAO index were the explanatory terms. Sex was also included as an explanatory term, and we tested for its potential interactions with the studied environmental factors. We also included in our models the variable age (young vs. adult) as a fixed factor, capture date (i.e. julian date) as a covariate, and site and year as random factors to account for multiple measures in a given site and year. We also included year as a fixed effect (covariate) in models to account for potential directional temporal changes in comb size. We used the Satterthwaite method to calculate degrees of freedom. Non-significant terms and interactions ($P > 0.05$) were sequentially removed from models

following a backward procedure. To avoid potential pseudoreplication problems, we only used one data point from the same individual in our models (when the same individuals had been sampled more than once, we considered only the data from the first capture event). Finally, we analysed whether the coefficients of variation ($CV = \sigma/\mu$) of comb area per site and year differed between sexes and were related to the studied environmental factors (site and year being also included as random factors in these models).

RESULTS

Population density, NAO index, and parasite population mean were not intercorrelated (all $P > 0.16$). After standardizing comb data by sex, age, and capture date (males and older birds had bigger combs and comb size increased with sampling date during spring; see Table 2), comb size was related to population density, although only through its interaction with sex (Table 2, Fig. 1). Comb area negatively correlated with population density in males ($F_{1,1515} = 11.19$, $estimate = -60.4 \pm 18.0$, $P = 0.001$), but not in females ($F_{1,52.5} = 0.17$, $estimate = 3.7 \pm 9.1$, $P = 0.680$). Similarly, comb size was negatively related to parasite population mean through its interaction with sex (Table 2). The expected negative relationship between parasite population mean and comb size was significant in males ($F_{1,13.9} = 11.16$, $estimate = -42.8 \pm 12.8$, $P = 0.004$), but not in females ($F_{1,13.6} = 0.00$, $estimate = 0.2 \pm 6.9$, $P = 0.971$). Finally, mean comb size decreased with increasing NAO index values in males

($F_{1,5.64} = 7.30$, $estimate = -10.41 \pm 3.58$, $P = 0.037$), but not in females ($F_{1,4.24} = 2.55$, $estimate = -4.37 \pm 2.73$, $P = 0.181$), with the sex \times NAO index interaction being significant (Table 2, Fig. 1). To verify that unequal sample sizes between site-year were not an issue (Table 1), we repeated these previous analyses using a balanced subsample of ten randomly selected males and females per site and year. If a given sex has fewer than ten individuals per site-year, those individuals were not considered in these analyses. The significance of these previous analyses was not modified when using this reduced but balanced data set.

The coefficients of variation of comb size differed between sexes ($F_{1,51} = 21.26$, $P < 0.001$), comb size being less variable in males than in females (LS means \pm SE: males, 0.133 ± 0.007 ; females, 0.184 ± 0.008). These coefficients of variations were not significantly related to any of the studied environmental factors, neither in males nor in females (all $P > 0.27$).

DISCUSSION

As predicted, we found that the expression of a male secondary sexual trait, the size of the supra-orbital combs, was positively correlated with indices of environmental quality in the red grouse (i.e. higher expression under favourable conditions). Our findings are consistent with the idea that male comb size could act as an indicator of phenotypic quality, as predicted by the models of condition-dependence of sexual traits (reviewed by Cotton *et al.*, 2004a).

Table 2. Relationships between comb area in males and females and population density, parasite population mean, and winter North Atlantic oscillation (NAO) index

Dependent variable: Comb area				
Explanatory terms	d.f.	<i>F</i>	<i>P</i>	<i>estimate</i> \pm SE
Density	1, 95.7	1.45	0.231	-42.5 \pm 12.5
Density \times Sex	1, 1281	20.92	< 0.001	58.2 \pm 12.7
Parasite population mean (PPM)	1, 17.8	6.01	0.024	-42.0 \pm 9.7
PPM \times Sex	1, 950	18.14	< 0.001	39.2 \pm 9.2
NAO	1, 6.67	2.98	0.130	-9.52 \pm 3.72
NAO \times Sex	1, 1316	7.24	0.007	6.19 \pm 2.30
Age	1, 1430	29.61	< 0.001	-14.3 \pm 2.6
Sex	1, 1381	187.46	< 0.001	-467.9 \pm 34.1
Day of capture	1, 1335	132.44	< 0.001	1.12 \pm 0.09
Year (continuous)*	1, 4.98	4.13	0.097	4.04 \pm 1.98

The model also included site ($estimate = 136.9 \pm 100.5$, $Z = 1.36$, $P = 0.086$) and year ($estimate = 409.1 \pm 254.7$, $Z = 1.61$, $P = 0.054$) as random factors. Estimates in terms with sex and age correspond to females and young respectively.

*Rejected terms.

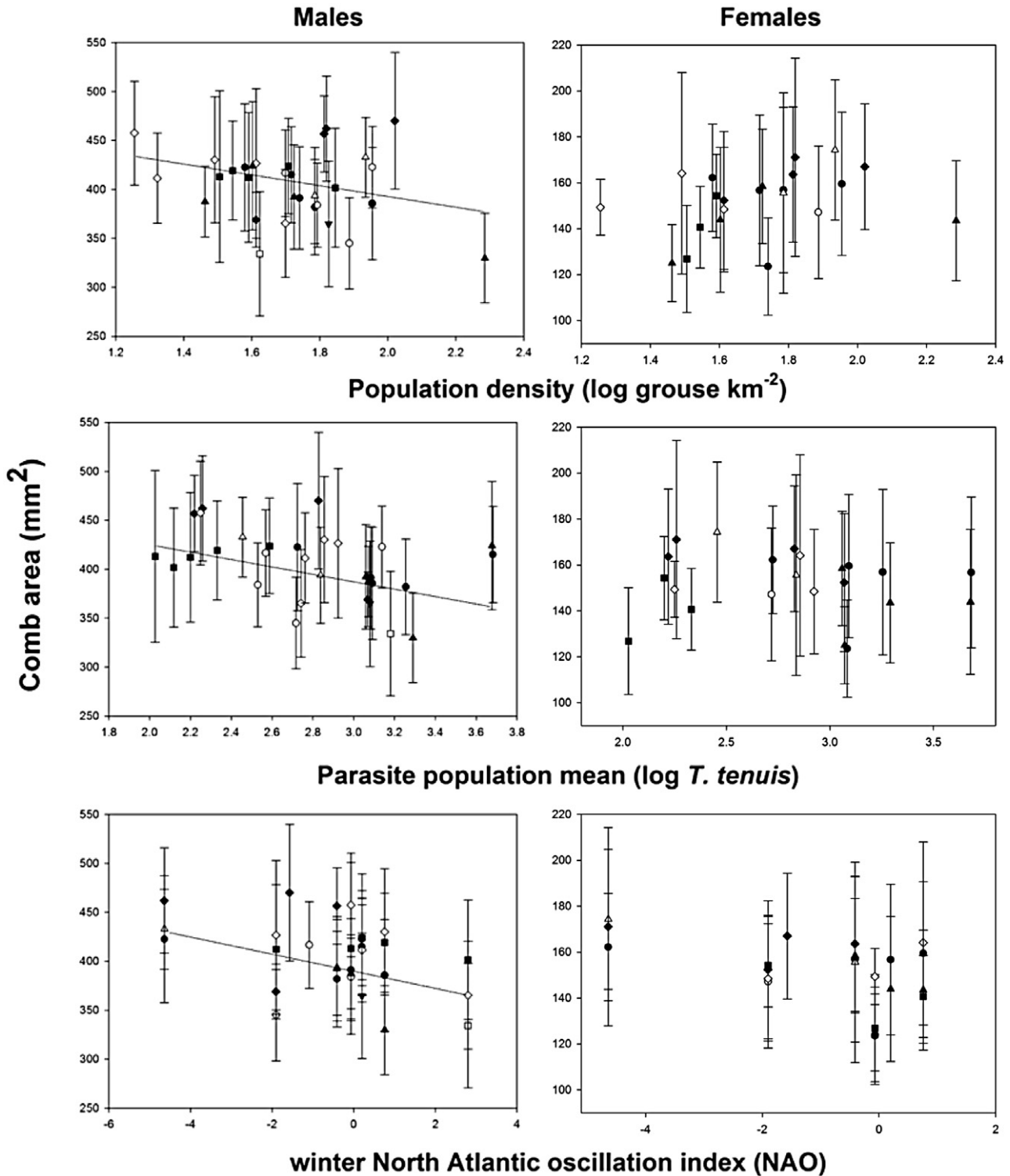


Figure 1. Variation in mean comb area (mm²) (\pm SD) of males and females in each population and year according to population density, *T. tenuis* parasite population mean, and winter NAO index. Significant relationships are highlighted with solid lines. Note that in the figure raw data are shown, but in models differences in age (young vs. adults), day of capture, year, and population (see Methods for more details) have been accounted for. Each population is represented by a different symbol.

POPULATION DENSITY AND COMB SIZE:
THE ROLE OF MALE–MALE AGGRESSIVENESS

The condition-dependent nature of sexual traits predicts lower ornament expression levels under more stressful environments. High density areas can be considered stressful environments because of the strong competition for limited resources (food, territories, or mates) that individuals living in such areas face. Here, we showed a similar trend: male grouse living in high-density areas displayed smaller combs. In agreement, negative relationships between population density and sexual displays have been found in other vertebrates (Jirotkul, 1999), particularly in mammals (e.g. Mysterud *et al.*, 2005). Under high densities, individuals can be constrained in terms of food resources, which would negatively affect their body condition and thereby average levels of condition-dependent trait expression (Mysterud *et al.*, 2005). However, the lack of effect of population density on female comb size does not fully agree with a direct role of food conditions in mediating the density-dependent effect on comb size. We might expect that female comb size would be negatively associated with density as both sexes feed in the same sites and might be similarly nutritionally constrained. Perhaps, food becomes a critical resource only above a given level of population density, and then studies analysing such a threshold would clarify the reason why both positive and negative relationships between ornament expression and density can be found.

An alternative explanation is that high densities are associated with increased male–male aggressiveness that imposes a cost for those individuals living under such stressful conditions. In red grouse, there is a strong competition between males for territories because holding a territory is necessary to survive during winter and breed (Watson & Miller, 1971; Watson, 1985). This male–male competition for territories should be more intense under higher abundances, as space becomes limited (Moss *et al.*, 1996; Mougeot *et al.*, 2003; Piertney *et al.*, 2008). Under such circumstances, individuals should increase their aggressiveness to obtain or maintain a territory (Moss *et al.*, 1996; Piertney *et al.*, 2008). But aggressiveness is energetically costly, and may have negative consequences on the resources available to invest sexual displays, probably explaining the negative comb–density relationship that we found here. From a mechanistic point of view, it has been suggested that corticosterone levels may mediate the honesty of sexual signals in red grouse (Bortolotti *et al.*, 2009). Specifically, ornament expression is ultimately a product of the physiological stress experienced by individuals. In a more competitive environment, such

as at high population density, individuals may face higher levels of environmental stress and thus the analysis of integrative indexes of stress and density may help to clarify this possibility (Bortolotti *et al.*, 2009).

However, the likely mechanism behind such increases in aggressiveness, i.e. enhanced testosterone levels, would promote the opposite trend between population density and comb size because the expression of this trait is testosterone-dependent (Mougeot *et al.*, 2004, 2006). Indeed, Moss *et al.* (1996) showed that comb size in spring positively correlated with current density in one area, although they also found no clear relationship in a neighbouring area. These authors explained the positive relationship as being a result of the enhanced effect of density on testosterone levels and subsequently on testosterone-dependent comb size. However, and similarly to our findings, Piertney *et al.* (2008) showed that comb size in autumn negatively correlates with population density in the subsequent spring in a Scottish population studied during an 8-year period. These changing comb–density relationships are difficult to explain. They may reflect the fact that the relationship between male aggressiveness and density is complex (Moss *et al.*, 1996; Matthiopoulos, Halley & Moss, 2005), as aggressiveness during one year can be affected by density or aggressiveness levels during previous years (Mougeot *et al.*, 2005b; Piertney *et al.*, 2008). If so, direct indices of male–male aggressiveness such as fights or territorial calls (e.g. Mougeot *et al.*, 2003) should explain more variance in the mean comb size across sites and years than population density itself. In other words, density may be a fairly crude index of aggressive conditions. We cannot exclude that other mechanisms such as differential mortality or genotype \times environment interactions also explain the observed associations between male comb size and population density and the contrasted results among studies. Longitudinal studies of the same individuals over different years as well as experimental works are needed to clarify these non-exclusive possibilities (Garant *et al.*, 2004).

NEGATIVE BUT SEX-DEPENDENT EFFECT OF
PARASITE INFECTION ON COMB SIZE

Parasites have been considered a key environmental factor in the evolution of sexual ornaments (Møller *et al.*, 1999). Because of the negative effects that parasites have on host fitness, they may mediate the trade-offs between investment in ornamentation and other costly activities such as self-maintenance (Møller *et al.*, 1999). Here, we add support to these ideas by reporting that average comb size in males was negatively correlated with *T. tenuis* infection

levels across populations. This negative relationship also agrees with previous findings at individual and population levels in this species (Martínez-Padilla *et al.*, 2010, 2011; Vergara *et al.*, 2012b). The new and important addition here is that this negative relationship remained after statistically controlling for other potential confounding variables, including host population density and climatic conditions, two other environmental factors commonly associated with parasite abundance levels (Hudson *et al.*, 2002; Altizer *et al.*, 2006; Loiseau *et al.*, 2010), particularly in the *T. tenuis* – red grouse system (Moss *et al.*, 1993).

In addition, we found that the negative association between *T. tenuis* and comb size was evident in males but not in females. Previous studies at individual level found a negative effect of *T. tenuis* on female comb size, although its effect was subtle, even after experimental manipulation (Martínez-Padilla *et al.*, 2011), and overall much less clear than that found in males (e.g. Mougeot *et al.*, 2010). The evolutionary pressures, investment trade-offs, and the mechanisms underlying ornament expression may differ between males and females, promoting sex-dependent effects of stressors on sexual traits (LeBas, 2006). The greater exaggeration of comb size in males suggests that sexual selection in that sex is more intense than in the other (Cotton *et al.*, 2004b). If so, male traits should have heightened condition-dependence as compared with female traits, and in consequence will be more affected by stressors (Cotton *et al.*, 2004b). In addition, females have costly reproduction-associated activities, such as egg formation that may also constrain investment in sexual traits, and probably influence the relationships between stressors and sexual traits (e.g. Morales, Velando & Torres, 2009). Previous experimental studies have demonstrated that *T. tenuis* nematodes have negative impacts on female physiology and reproductive investment in red grouse (Delahay *et al.*, 1995), but at the same time, may constrain comb size (Martínez-Padilla *et al.*, 2011). If so, the link between parasites and sexual traits is more complex in females than in males, which are not as constrained by reproductive duties. Other non-exclusive explanations are that the sexes differ in the mechanisms behind the expression of the trait (e.g. different hormonal regulation) or that the genetic architecture of the trait is partially discrete between the sexes (Wright *et al.*, 2007).

NAO INDEX: INDIRECT EFFECT OF CLIMATE ON COMB SIZE THROUGH PARASITE OR FOOD?

Climate conditions may affect ornament expression in both males and females, but the mechanism underlying such effects is likely to be specific to each study system (Garant *et al.*, 2004; Mysterud *et al.*, 2005;

Hegyi *et al.*, 2007, 2008). Here, we found that comb size in males was negatively correlated with the winter NAO index. In the moorlands of the UK, negative winter NAO values are associated with dry and cold winters (Trivedi *et al.*, 2007). We therefore expected fewer parasites and larger ornaments after winters with negative NAO values. However, site-year winter NAO index and *T. tenuis* abundance were not correlated (see Results). We still cannot discard the idea that parasites other than *T. tenuis* and affected by climate could negatively affect grouse comb size. Alternatively, the effect of climate on comb size could be through an indirect effect on plant production, in this case heather *Calluna vulgaris*, the main food of grouse (Savory, 1983). Winters with long snow cover would enhance the quality of the plant diet in the subsequent spring, for example by increasing the period with new forage, with some obvious benefits on the condition and reproduction of individuals living in such areas (e.g. Mysterud *et al.*, 2001). The diet of red grouse mainly consists of fresh parts of heather (Savory, 1983). Enhanced heather conditions have been shown to positively affect red grouse body condition and productivity (Savory, 1983; Watson, Moss & Parr, 1984) and to increase grouse density (Watson *et al.*, 1984). In addition, heather quality can be negatively affected by low snow cover (Hancock, 2008), possibly mediating the indirect effect of climate on red grouse comb size, although further research is needed to confirm this idea. The same reasoning proposed above, as well as the rarity of years with extreme weather conditions when sampling females, as that years are those apparently showing heightened effect on comb size (see Fig. 1), would explain the weak relationship between the studied female trait and the winter NAO index.

COEFFICIENTS OF VARIATION IN MALES AND FEMALES AND ENVIRONMENTAL FACTORS

We also showed that the coefficient of variation (CV) of comb size, an index of the likelihood of a trait to be under selection, is higher in females than in males. This is an a priori unexpected result. Traits under stronger sexual selection commonly show greater variance than those characters under weak or null sexual selection pressures (Cotton *et al.*, 2004b). It has been shown that female traits may have high CVs, and even similar to those shown by males for the same traits (e.g. Hegyi *et al.*, 2008). We have no obvious explanation for the higher degree of CVs in the expression of a female trait apparently less affected by sexual selection. It is possible that the timing that males and females display their highest comb size may differ. Comb size in females is a highly dynamic trait that can change within a few days (e.g.

Martínez-Padilla *et al.*, 2011). Because of the intense intra-sexual competition during late winter, males should display their largest combs from the very beginning of the season. If females delay such an increase in comb size it could be possible that some of the captured females showed 'non-breeding combs' that are significantly smaller than those displayed during the breeding season (e.g. Martínez-Padilla *et al.*, 2011). Furthermore, CVs in males and females were not clearly associated with the studied environmental factors. Therefore, our data do not support the idea that adverse environments increase the variance in the expression of sexual traits, in contrast to results under captive conditions (Cotton *et al.*, 2004b; Cothran & Jeyasingh, 2010).

In conclusion, our study supports a role for some specific indices of environmental conditions (population density, nematode parasite infection, and climate) in explaining some of the natural variations in the expression levels of secondary sexual traits in red grouse. The prediction that ornament expression should be lower under more adverse conditions as stated by the condition-dependent nature of sexual traits was only supported in males. Further studies are needed to clarify the mechanism behind the negative effects of population density and climate on comb size, as well as the differences between sexes in the environmental effects on the studied traits.

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REFERENCES

- Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, Rohani P. 2006. Seasonality and the dynamics of infectious diseases. *Ecology Letters* **9**: 467–484.
- Amundsen T. 2000. Why are female birds ornamented? *Trends in Ecology and Evolution* **15**: 149–155.
- Andersson M. 1994. *Sexual selection*. Princeton, NJ: Princeton University Press.
- Bortolotti GR, Mougeot F, Martinez-Padilla J, Webster LMI, Piertney SB. 2009. Physiological stress mediates the honesty of social signals. *PLoS ONE* **4**: e4983.
- 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? *Proceedings of the Royal Society of London Series B, Biological Sciences* **271**: 771–783.
- Cotton S, Fowler K, Pomiankowski A. 2004b. Condition dependence of sexual ornament size and variation in the stalked fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). *Evolution* **58**: 1038–1046.
- Cramp S, Simmons KEL. 1980. *The birds of the western Palearctic*, Vol. 2. Oxford: Oxford University Press.
- Delahay RT, Speakman JR, Moss R. 1995. The energetic consequences of parasitism - effects of a developing infection of *Trichostrongylus-tenuis* (nematoda) on red grouse (*Lagopus-lagopus-scoticus*) energy-balance, body-weight and condition. *Parasitol.* **110**: 473–482.
- Endler JA. 1995. Multiple-trait coevolution and environmental gradients in guppies. 1995. *Trends in Ecology and Evolution* **10**: 22–29.
- Evans AE, Mougeot F, Redpath SM, Leckie F. 2007. Alternative methods for estimating breeding density in an uplandgame bird, the red grouse *Lagopus lagopus scoticus*. *Wildlife Biology* **13**: 130–139.
- Evans ME. 1991. The size of adornments of male scarlet-tufted malachite sunbirds varies with environmental conditions, as predicted by handicap theories. *Animal Behaviour* **42**: 797–803.
- Fitze P, Tschirren B, Richner H. 2003. Carotenoid-based colour expression is determined early in nestling life. *Oecologia* **137**: 148–152.
- Fowler HJ, Kilsby CG. 2002. Precipitation and the North Atlantic Oscillation: a study of climatic variability in northern England. *International Journal of Climatology* **22**: 843–866.
- 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.
- Gustafsson L, Qvarnström A, Sheldon BS. 1995. Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. *Nature* **375**: 311–313.
- Hancock MH. 2008. An exceptional *Calluna vulgaris* winter die-back event, Abernethy Forest, Scottish Highlands. *Plant Ecology and Diversity* **1**: 89–103.
- Hegyí G, Rosivall B, Szölloosi E, Hargitai R, Eens M, Török J. 2008. Phenotypic plasticity in a conspicuous

- female plumage trait: information content and mating patterns. *Animal Behaviour* **75**: 977–989.
- Hegyí G, Török J, Garamszegi LZ, Rosivall B, Szölloosi E, Hargítai R. 2007.** Dynamics of multiple signals in relation to climatic conditions. *Evolutionary Ecology Research* **9**: 905–920.
- Hudson PJ, Dobson AP, Newborn D. 1998.** Prevention of population cycles by parasite removal. *Science* **282**: 2256–2258.
- Hudson PJ, Dobson AP, Newborn D. 2002.** Parasitic worms and population cycles of red grouse. In: Berryman AA, ed. *Population cycles: the case for trophic interactions*. Oxford: Oxford University Press, 109–130.
- Jirotkul M. 1999.** Population density influences male–male competition in guppies. *Animal Behaviour* **58**: 1169–1175.
- Karubian J, Lindsay WR, Schwabl H, Webster MS. 2011.** Bill coloration, a flexible signal in a tropical passerine bird, is regulated by social environment and androgens. *Animal Behaviour* **81**: 795–800.
- LeBas NR. 2006.** Female finery is not for males. *Trends in Ecology and Evolution* **21**: 170–173.
- Loiseau C, Iezhova T, Valkiunas G, Chasar A, Hutchinson A, Buermann W, Smith TB, Sehgal RNM. 2010.** Spatial variation of haemosporidian parasite infection in African rainforest bird species. *Journal of Parasitology* **96**: 21–29.
- MacColl ADC, Pieltney SB, Moss R, Lambin X. 2000.** Spatial arrangement of kin affects recruitment success in young male red grouse. *Oikos* **90**: 261–270.
- Martínez-Padilla J, Mougeot F, Webster LMI, Pérez-Rodríguez L, Pieltney SB. 2010.** Testing the interactive effects of testosterone and parasites on carotenoid-based ornamentation in a wild bird. *Journal of Evolutionary Biology* **23**: 902–913.
- Martínez-Padilla J, Vergara P, Pérez-Rodríguez L, Mougeot F, Casas F, Ludwig SC, Haines JA, Zeineddine M, Redpath SM. 2011.** Condition- and parasite-dependent expression of a male-like train in female bird. *Biology Letters* **7**: 364–367.
- Matthiopoulos J, Halley JM, Moss R. 2005.** Socially induced red grouse population cycles need abrupt transitions between tolerance and aggression. *Ecology* **86**: 1883–1893.
- McGraw KJ, Dale J, Mackillop EA. 2003.** Social environment during molt and the expression of melanin-based plumage pigmentation in male house sparrows *Passer domesticus*. *Behavioral Ecology and Sociobiology* **53**: 116–122.
- Møller AP, Christe P, Lux E. 1999.** Parasitism, host immune function, and sexual selection. *The Quarterly Review of Biology* **74**: 3–20.
- Morales J, Velando A, Torres R. 2009.** Fecundity compromises attractiveness when pigments are scarce. *Behavioral Ecology* **20**: 117–123.
- Moss R, Kolb HH, Marquiss M, Watson A, Treca B, Watt D, Glennie W. 1979.** Aggressiveness and dominance in captive cock red grouse. *Aggressive Behaviour* **5**: 58–84.
- Moss R, Watson A. 2001.** Population cycles in birds of the grouse family (Tetraonidae). *Advances in Ecological Research* **32**: 53–111.
- Moss R, Watson A, Parr R. 1996.** Experimental prevention of a population cycle in red grouse. *Ecology* **77**: 1512–1530.
- Moss R, Watson A, Trenholm IB, Parr R. 1993.** Caecal threadworms *Trichostrongylus tenuis* in red grouse *Lagopus lagopus scoticus*: effects of weather and host density upon estimated worm burdens. *Parasitology* **107**: 199–209.
- Mougeot F, Irvine JR, Seivwright L, Redpath SM, Pieltney SB. 2004.** Testosterone, immunocompetence, and honest signalling in male red grouse. *Behavioral Ecology* **15**: 930–937.
- Mougeot F, Martínez-Padilla J, Blount JD, Pérez-Rodríguez L, Webster LMI, Pieltney SB. 2010.** Oxidative stress and the effect of parasites on a carotenoid-based ornament. *Journal of Experimental Biology* **213**: 400–407.
- Mougeot F, Pieltney SB, Leckie F, Evans S, Moss R, Redpath SM, Hudson PJ. 2005b.** Experimentally increased aggressiveness affects population kin structure and subsequent recruitment in red grouse *Lagopus lagopus scoticus*. *Journal of Animal Ecology* **74**: 488–497.
- Mougeot F, Redpath SM, Leckie F. 2005a.** Ultra-violet reflectance of male and female red grouse, *Lagopus lagopus scoticus*, sexual ornaments reflects nematode parasite intensity. *Journal of Avian Biology* **36**: 203–209.
- Mougeot F, Redpath SM, Leckie F, Hudson PJ. 2003.** The effect of aggressiveness on the population dynamics of a territorial bird. *Nature* **421**: 737–739.
- Mougeot F, Redpath SM, Pieltney SB. 2006.** Elevated spring testosterone increases parasite intensity in male red grouse. *Behavioral Ecology* **17**: 127–135.
- Murphy SJ, Washington R. 2001.** United Kingdom and Ireland precipitation variability and the north Atlantic sea-level pressure field. *International Journal of Climatology* **21**: 939–959.
- Myrsterud A, Meisingset E, Langvatn R, Yoccoz NG, Stenseth NC. 2005.** Climate-dependent allocation of resources to secondary sexual traits in red deer. *Oikos* **111**: 245–252.
- Myrsterud A, Stenseth NC, Yoccoz NG, Langvatn R, Steinheim G. 2001.** Nonlinear effects of large-scale climatic variability on wild and domestic herbivores. *Nature* **410**: 1096–1099.
- Pieltney SB, Lambin X, MacColl ADC, Lock K, Bacon PJ, Dallas JF, Leckie F, Mougeot F, Racey PA, Redpath SM, Moss R. 2008.** Temporal changes in kin structure through a population cycle in a territorial bird, the red grouse *Lagopus lagopus scoticus*. *Molecular Ecology* **17**: 2544–2551.
- Przybylo R, Sheldon BC, Merilä J. 2000.** Climatic effects on breeding and morphology: evidence for phenotypic plasticity. *The Journal of Animal Ecology* **69**: 395–403.
- Redpath SM, Mougeot F, Leckie F, Elston DA, Hudson PJ. 2006a.** Testing the role of parasites in driving the cyclic population dynamics of a gamebird. *Ecology Letters* **9**: 410–418.

- Redpath S, Mougeot F, Leckie F, Evans S. 2006b.** The effects of autumn testosterone on survival and productivity in red grouse *Lagopus lagopus scoticus*. *Animal Behaviour* **71**: 1297–1305.
- Savory CJ. 1983.** Selection of heather age and chemical composition by red grouse in relation to physiological state, season and time of day. *Ornis Scandinavica* **14**: 135–143.
- Seivwright LJ, Redpath SM, Mougeot F, Watt L, Hudson PJ. 2004.** Faecal egg counts provide a reliable measure of *Trichostrongylus tenuis* intensities in free-living red grouse *Lagopus lagopus scoticus*. *Journal of Helminthology* **78**: 69–76.
- Stenseth NC, Ottersen G, Hurrell JW, Mysterud A, Lima M, Chan K-S, Yoccoz NG, Ådlandsvik B. 2003.** Studying climate effects on ecology through the use of climate indices: the North Atlantic oscillation, El Niño southern oscillation and beyond. *Proceedings of the Royal Society of London Series B, Biological Sciences* **270**: 2087–2096.
- Svensson PA, Pélabon C, Blount JD, Forsgren E, Bjerkeng B, Amundsen T. 2009.** Temporal variability in a multicomponent trait: nuptial coloration of female two-spotted gobies. *Behavioral Ecology* **20**: 346–353.
- Trivedi MR, Browne MK, Berry PM, Dawson TP, Morecroft MD. 2007.** Projecting climate change impacts on mountain snow cover in central Scotland from historical patterns. *Arctic, Antarctic, and Alpine Research* **39**: 488–499.
- Vanpé C, Gaillard J-M, Kjellander P, Mysterud A, Magnien P, Delorme D, Van Laere G, Klein F, Liberg O, Hewison AJM. 2007.** Antler size provides an honest signal of male phenotypic quality in Roe Deer. *American Naturalist* **169**: 481–493.
- Vergara P, Martínez-Padilla J, Mougeot F, Leckie F, Redpath SM. 2012a.** Environmental heterogeneity influences the reliability of secondary sexual traits as condition indicators. *Journal of Evolutionary Biology* **25**: 20–28.
- Vergara P, Martínez-Padilla J, Redpath SM, Mougeot F. 2011.** The ornament-condition relationship varies with parasite abundance at population level in a female bird. *Die Naturwissenschaften* **98**: 897–902.
- Vergara P, Mougeot F, Martínez-Padilla J, Leckie F, Redpath SM. 2012b.** The condition-dependence of a secondary sexual trait is stronger under high parasite infection level. *Behavioral Ecology* **23**: 502–511.
- 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. *British Birds* **57**: 137–170.
- Watson A, Miller GR. 1971.** Territory size and aggression in a fluctuating red grouse population. *Journal of Animal Ecology* **40**: 367–383.
- Watson A, Moss R. 2008.** *Grouse*. London: HarperCollins.
- Watson A, Moss R, Parr R. 1984.** Effects of food enrichment on number and spacing behaviour of red grouse. *Journal of Animal Ecology* **53**: 663–678.
- Wingfield JC, Hegner RE, Dufty AM, Ball GF. 1990.** The ‘Challenge hypothesis’: theoretical implications for patterns of testosterone secretion, mating system, and breeding strategies. *The American Naturalist* **136**: 829–846.
- Wright D, Kerje S, Brändström H, Schütz K, Kindmark A, Andersson L, Jensen P, Pizzari T. 2007.** The genetic architecture of a female sexual ornament. *Evolution* **62**: 86–98.
- Zahavi A. 1975.** Mate selection – a selection for a handicap. *Journal Theoretical Biology* **53**: 205–214.