

Environmental heterogeneity influences the reliability of secondary sexual traits as condition indicators

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Keywords:

condition dependence;
genotype × environment interactions;
honest signalling;
indicator model;
Lagopus lagopus scoticus;
sexual selection.

Abstract

Numerous studies have shown positive associations between ornaments and condition, as predicted by indicator models of sexual selection. However, this idea is continuously challenged by opposite results, which reveal our lack of full understanding of how sexual selection works. Environmental heterogeneity may explain such inconsistencies, but valid field tests of this idea are currently lacking. We first analysed the relationship between condition and ornament expression from nine populations over 7 years in a wild bird, the red grouse *Lagopus lagopus scoticus*. We then manipulated male aggressiveness at the population level by means of testosterone implants in a replicated field experiment. We found that the relationship between condition and ornamentation varied greatly between environments and became stronger when environmental conditions (ECs) were worse or when aggressiveness in the population was experimentally increased. Some ornaments may therefore reliably advertise a better condition only in adverse ECs. Considering environmental heterogeneity can help reconcile conflicting findings regarding the reliability of ornaments as indicators of condition and will help our understanding of sexual selection processes.

Introduction

Indicator models of sexual selection predict that secondary sexual ornaments are reliable signals of phenotypic or genetic quality (Zahavi, 1975; Iwasa *et al.*, 1991; Iwasa & Pomiankowski, 1999). Although this theory is widely accepted (Andersson, 1994), empirical evidence supporting the basic assumption that sexual ornament expression positively correlates with measures of condition or fitness components is often not found (Cornwallis & Uller, 2009). Even within a single species, positive relationships between ornament expression and a measure of condition or fitness have been found under some circumstances but not in others (Cockburn *et al.*, 2008; Dunn *et al.*, 2010; Freeman-Gallant *et al.*, 2010; Tolle & Wagner, 2011).

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Environmental heterogeneity may enhance or constrain the relative differences between low- and high-quality (understood as a proxy of fitness) individuals (David *et al.*, 2000; Cotton *et al.*, 2004; Cothran & Jeyasingh, 2010). Under the stronger selection pressures that operate under severe environmental conditions (ECs), only high-quality individuals should be able to display exaggerated ornaments without reducing their fitness. Under favourable ECs, both low- and high-quality individuals could invest equally in these displays without compromising fitness (David *et al.*, 2000; Cotton *et al.*, 2004; Cothran & Jeyasingh, 2010). If true, the ornament–condition relationship (i.e. its condition dependence) should vary with ECs and should be stronger and steeper under more severe conditions (Candolin, 2000a; Fargallo *et al.*, 2007; Cothran & Jeyasingh, 2010). In addition, changes in the relationships between sexual ornaments and measures of condition or fitness across time and space can also occur when viability selection associated with investment in

ornamentation varies among environments (Candolin & Heuschele, 2008; Robinson *et al.*, 2008). Alternatively, the changing association between ornamentation and individual condition may arise when alternative genotypes are adapted to different ECs (i.e. genotype \times environment interactions, Higginson & Reader, 2009; Ingleby *et al.*, 2010). Therefore, environmental heterogeneity may explain the changing associations between sexual ornaments and other phenotypic (morphological, physiological or behavioural) traits. To date, it is well established that ECs may regulate the expression of secondary sexual traits (e.g. Griffith *et al.*, 1999), and a number of studies have shown how environmental heterogeneity may promote different average ornament expression between populations or years (Hill *et al.*, 2002; Dunn *et al.*, 2010). However, the above-described studies testing whether environmental heterogeneity affects the ornament–condition relationship in wild animals are scarce, incomplete (i.e. often considering few populations or years and therefore a limited range of ECs) and rarely corroborated by field experiments.

Tetraonid birds such as the red grouse *Lagopus lagopus scoticus* display supra-orbital combs, an ornament whose size functions in intra- and intersexual contexts: in spring, males with bigger combs are dominant, more aggressive, hold larger territory and are preferred by females as they more often pair with more than one female (Moss *et al.*, 1979; Redpath *et al.*, 2006). This dual function of comb size (male–male competition and female choice, respectively) was also shown in the closely related rock ptarmigan *Lagopus mutus* in which both male territory characteristics and comb size are determinants of mate success and polygyny (Brodsky, 1988; Bart & Earnst, 1999). As predicted by sexual selection theory, comb size in red grouse positively correlates with several indexes of body condition and fitness (Mougeot & Redpath, 2004; Mougeot *et al.*, 2004, 2006), with some evidence indicating that these relationships differ among years and populations (Moss *et al.*, 1996; Martínez-Padilla *et al.*, 2010). Body mass is commonly used as a proxy for condition (defined as the pool of resources available for allocation to fitness-related traits) in birds: it is largely affected by nutritional and health status and constrained by many environmental stressors (Fargallo *et al.*, 2007; Dunn *et al.*, 2010). In red grouse, body mass is a good proxy of condition: it is much more variable than size, strongly affected by environmental stressors and also related to several indexes of health status or physiological condition (Mougeot *et al.*, 2006, 2010). To test the ornament–condition relationship, we have therefore focused on the comb area–body mass relationship. Here we specifically tested the hypothesis that the strength of the ornament–condition relationship increases as EC declines. This species experiences great temporal and spatial variation in a wide range of environmental stressors such as parasites, competitors or predators (Hudson, 1986; Mougeot *et al.*,

2003, 2005a) and is therefore particularly suited to explore how the ornament–condition relationship varies in heterogeneous environments. We studied the relationship between ornament size (measured as comb area) and body mass (as a proxy of condition) in nine populations over 7 years, covering a wide range of ECs.

To experimentally test whether environmental heterogeneity modifies the ornament–condition relationship, we manipulated the aggressiveness at population level in three grouse moors, as a part of a study that aimed to reveal the effect of aggressiveness on population dynamics (Mougeot *et al.*, 2003). Among the various environmental factors that may affect red grouse, the level of aggressiveness is of crucial importance (Moss *et al.*, 1994; Mougeot *et al.*, 2003, 2005a). In addition, it has been recently suggested that the level of intrasexual competitiveness in a population may play a key role in the resolution of the trade-offs underlying the condition dependence of sexual ornamentation in red grouse (Martínez-Padilla *et al.*, 2010). Therefore, we focused the experimental approach based on the manipulation of aggressiveness in the population. In each of three populations, a proportion of males were treated with testosterone (Testosterone-area) or given empty implants (Control-area) (Mougeot *et al.*, 2003, 2005a). Previous studies confirmed that this manipulation increased aggressiveness and competitiveness, reduced recruitment and subsequent density, and also modified kin structure (fewer kin groups and related individuals with the male populations) for up to 1.5 years in the Testosterone-areas as compared with the Control-areas (Mougeot *et al.*, 2003, 2005a). All these consequences of the manipulation of aggressiveness suggested worse ECs at the population level in the Testosterone-areas. We first verified this, expecting the manipulation of aggressiveness to have modified ECs and more specifically to have reduced average body mass at population level on the Testosterone-areas as compared with the Control-areas. We then compared the slope of the relationship between comb area and body mass of untreated males (i.e. that had not been implanted) living in a Testosterone-area with individuals living in Control-area. If aggressiveness in the population affected the ornament–condition relationship by modifying the relative differences between low- and high-quality individuals (Martínez-Padilla *et al.*, 2010), we expected a steeper positive relationship between ornament size and body mass where aggressiveness had been increased.

Material and methods

Correlative data

Between 2000 and 2011, we captured in spring a total of 571 male red grouse from nine UK populations (Catterick, Geltsdale and Moorhouse, in Northern England; and Edinglassie, Glen Dye, Glen Muick,

Invermark, Invercauld and Millden, in Scotland; see Martínez-Padilla *et al.*, 2011 for more details). Not all populations were sampled each year, but data were collected from a total of 17 site-years (see Supporting Information). We ringed each male caught and determined age (i.e. young, < 1-year old or adult, > 1-year old) from plumage and measured body mass (g), and the maximum length and width of flattened combs to calculate comb area (comb length \times width, mm²) as a index of ornament size, as in previous studies on red grouse (Mougeot & Redpath, 2004; Mougeot *et al.*, 2004, 2006).

Proxy measure of Environmental Condition

In an attempt to summarize the variety of ECs to which individuals were exposed, we used the average body mass of both males and females within a given population in a given year (hereafter referred as to ECs index) (see Martínez-Padilla *et al.*, 2011 for details about female data). A lower average body mass in a given population and year was taken to indicate tougher overall conditions for individuals, regardless of the stressors involved (e.g. parasites, food, competitors), given that they all negatively impact on grouse body mass (Delahay & Moss, 1996; Mougeot *et al.*, 2006, 2010). Our ECs index was calculated as the average body mass per population and year after taking into account the variation in body mass related to sex, age and day of capture. To do this, we used the residuals from a GLM that included body mass as a response (dependent) variable and the following explanatory variables: sex, age (as factors) and day of capture (covariate) as well as the interaction between day of capture and sex (in spring, prior to laying, females, but not males, may gain body mass; see also supplementary material). These residual body mass data were then averaged for each population and year to obtain our ECs index (lower average indicative of tougher conditions). Nevertheless, none of the results or conclusions change if we use a much simpler approach that consists in using the average male body mass per population and year (instead of the corrected average body mass values) as a measure of ECs (see Results).

Population-level manipulation of aggressiveness

In three populations (Moorhouse, Edinglassie and Glenn Dye), two 1-km² areas separated by a 0.5-km wide buffer area were randomly assigned a treatment (Testosterone or Control). In the first autumn (autumn *t*; i.e. 2000 for Moorhouse and 2001 for Edinglassie and Glenn Dye), 31–33% of all males present in each area were implanted either with implants that were filled with testosterone (Testosterone-areas) or empty (Control-areas). Males with territories on these areas were subsequently caught in the two subsequent springs (spring *t* + 1 and spring *t* + 2) after the initial manipulation in autumn *t* (Mou-

geot *et al.*, 2003, 2005a) to measure comb area and body mass (as previously). Comb area and body mass were not measured in all individuals; thus, sample sizes may differ between models. To avoid potential effect of increased levels of exogenous testosterone on comb area and body mass (Mougeot *et al.*, 2006), we used for analyses only data from males that had not been implanted in autumn *t* within T-areas. Seventeen males were sampled more than one time (14 in two different time periods and 3 in three). To avoid pseudoreplication, only one, randomly chosen, data point per individual was considered in the analyses. Due to logistic limitations (restriction due to a foot and mouth disease outbreak, see Davies, 2001), no male could be captured in Moorhouse during spring *t* + 1. In addition, very few untreated individuals were present during spring *t* + 1, particularly adults (because 96% and 86% of all adult males present in the study areas at autumn *t* were implanted). Thus, samples sizes were smaller at that time (spring *t* + 1) than in the subsequent spring (*t* + 2) as no manipulation was performed in the spring *t* + 1 cohort of individuals.

Statistical analyses

We used SAS 9.0 (SAS Institute Inc., Cary, NC, USA) and General Linear Mixed Models (GLMM). For correlative analyses, comb area was the response variable, with body mass, EC index and their interaction included as fixed effects (to test whether the relationship comb area–body mass changed with EC index). Age-class was also included as a two-level fixed factor, and we tested their potential interactions with body mass and ECs index. Because we had individuals from the same site and year, we included both these factors as random variables in the models. Capture date (i.e. Julian day) was also included as a covariate to correct for different sampling dates. We used the Satterthwaite method to calculate degrees of freedom. In some rare cases (< 5%), the same male was recaptured in different years. To avoid problems of pseudoreplication, only one data point per individual was included, always considering the first capture of each individual. The number of males per site and year was not related to ECs index ($F_{1,11.3} = 0.11$, estimate = 0.03 ± 0.09 , $P = 0.742$). To verify that unequal sample sizes between site-year were not an issue, we repeated the analysis with a subsample of nine (that is our minimum sample size) randomly selected individuals per site and year. To check that there was no bias because of the differences in the scaling of each variable, which may also make the relationship between comb area and body mass nonlinear (e.g. plateauing effect on comb area as body mass increases), we repeated the analysis using the square root transformed comb area and the cube root transformed body mass. Finally, to avoid potential problems associated with the collinearity of variables (body mass being negatively correlated with ECs index, $F_{1,567} = 29.52$, estimate = 1.13 ± 0.20 , $P < 0.001$), we

used relative (i.e. centred) body mass (individual male body mass minus average male body mass per population and year) instead of body mass (relative body mass being not significantly related to ECs index, $F_{1,568} = 0.01$, estimate = 0.02 ± 0.20 , $P = 0.917$). In addition, we analysed whether the coefficients of variation ($CV = \sigma/\mu$) of comb area and individual body mass per site and year were related to the ECs index (including year and site as random factors in our models).

We also analysed the data from the population-level experiment of aggressiveness using GLMMs. We first tested whether body mass (response variable) differed between treatment areas (Control- or Testosterone-area) before the manipulation (autumn t) and after the experimental increase in testosterone levels and aggressiveness (i.e. in the two subsequent springs) in separate models. In this second model, we tested for a potential delayed effect by using data from both springs (spring $t + 1$ and spring $t + 2$) and testing for a potential 'Year' effect, and interaction between Year and Treatment area. Age (adult and young) was included as a fixed factor, and Population and the population \times treatment area interaction were included as random effects to account for the experimental design. Secondly, we analysed whether the experimental manipulation of aggressiveness changed the relationship between comb area and body mass. As in previous models, we analysed before or after the manipulation separately. For these analyses, comb area was the response variable, with age, body mass, treatment area, and the body mass \times treatment area interaction included as explanatory variables. Year and the year \times treatment area interaction were also included as explanatory terms in the model analysing data after the manipulation, as described earlier. Population and the population \times treatment area interaction were also included as random effects. We also explored whether the effect of the experimental manipulation of aggressiveness on the comb area–body mass relationship differed between age-classes (testing for a body mass \times treatment area \times age interaction).

Results

Comb area and body mass were overall positively correlated ($F_{1,561} = 63.66$, estimate = 0.41 ± 0.05 , $P < 0.001$), and this relationship did not differ significantly between age-classes (body mass \times age $F_{1,557} = 2.94$, $P = 0.086$). However, the strength of the comb area–body mass relationship varied considerably and changed according to the EC index: the slope of this relationship was steeper as the EC index decreased (Table 1, Fig. 1). Age and the rest of studied interactions were not significant (Table 1). Using the average male body mass per population and year (instead of the corrected average body mass values) as a much simpler measure of ECs results did not change, as the individual body mass \times average male body mass interaction was

Table 1 Effect of environmental conditions (ECs) on the relationship between ornamentation and body mass. Results of the General Linear Mixed Models analysing the comb area–body mass relationship under different ECs. The ECs index was calculated as the average standardized body mass for each population and year (i.e. mean of residuals of a GLM of body mass on age, sex, day of capture and sex \times day of capture; see Materials and methods). Year (estimate = 532.4 ± 396.1 , $Z = 1.34$, $P = 0.089$) and site (estimate = 754.7 ± 555.1 , $Z = 1.36$, $P = 0.087$) were included as random factors in the model. Estimates in age-class interactions correspond to young levels. The full model is shown, but when removing the nonsignificant interactions from the model, the significance of the body mass \times ECs index interaction was not noticeably modified ($F_{1,559} = 7.54$, estimate = -0.016 ± 0.006 , $P = 0.006$). Significant P -values are given in bold.

Dependent variable: Comb area				
Explanatory variables:	d.f.	F	Estimate \pm SEM	P
Body mass	1, 550	65.21	0.53 ± 0.08	< 0.001
Environmental conditions index (EC)	1, 560	8.55	11.45 ± 7.09	0.003
Body mass \times EC	1, 559	7.79	-0.010 ± 0.009	0.005
Day of capture	1, 102	13.66	1.08 ± 0.29	< 0.001
Age	1, 551	2.38	123.0 ± 79.7	0.123
Body mass \times age	1, 551	2.43	-0.16 ± 0.10	0.119
EC \times age	1, 549	0.28	4.87 ± 9.18	0.595
Body mass \times EC \times age	1, 550	0.35	-0.007 ± 0.012	0.557

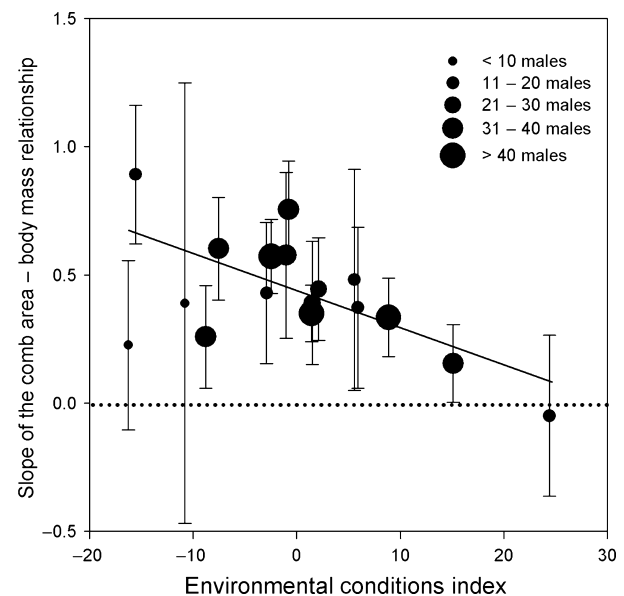


Fig. 1 Changes in the relationship between ornamentation and body mass according to environmental conditions (ECs) index. The slopes (\pm SEM) of the comb area (mm^2)–body mass (g) relationship are plotted according the ECs index. The ECs index was calculated as the average standardized body mass for each population and year (i.e. mean of residuals of a GLM of body mass on age, sex, day of capture and sex \times day of capture; see Materials and Methods).

significant ($F_{1,560} = 9.67$, estimate = -0.011 ± 0.003 , $P = 0.002$), thus showing consistency and robustness in this relationship. Using a reduced but balanced data set of nine males per site and year, transformed variables (square root comb area and cubic root body mass) or the relative body mass (individual male body mass minus average male body mass per population and year), results did not change (see Supporting Information). Coefficients of variation of both comb area ($F_{1,14} = 2.64$, estimate = 0.0008 ± 0.0005 , $P = 0.126$) and body mass ($F_{1,14.5} = 2.13$, estimate = 0.0004 ± 0.0002 , $P = 0.165$) were not correlated with the EC index.

We further tested how population-level manipulations of aggressiveness affected the average male body mass and comb area–body mass relationship considering only nontreated males within the experimental populations (i.e. nonimplanted). Body mass and the relationship between comb area and body mass did not differ between Testosterone- and Control-areas before the manipulation (Table 2). However, untreated grouse from the Testosterone-areas had lower body mass after the experimental manipulation than birds from the Control-areas (Table 2, LSMEANS \pm SE: Testosterone-area = 711 ± 7 , Control-area = 739 ± 7), this difference being greater for adult than for young males (Table 2; LSMEANS \pm SE: Adults Testosterone-area = 710 ± 9 , Control-area = 754 ± 9 ; Young Testosterone-area = 711 ± 8 , Control-area = 725 ± 8). These relationships did not differ between years (year \times treatment area, $F_{1,207} = 2.63$, $P = 0.106$; year \times treatment area \times age $F_{1,204} = 0.29$, $P = 0.589$). However, further analyses considering each spring separately indicated that differences in body mass between treatment areas were significant only in spring $t + 2$ (Table 2,

Fig. 2). The comb area–body mass relationship also differed between areas after the manipulation, the slope being greater on Testosterone-areas than on Control-areas (Table 2; Testosterone-area = 0.52 ± 0.11 ; Control-area = 0.02 ± 0.12) in both young and old males (body mass \times treatment area \times age, $P = 0.570$) and irrespective of the year (body mass \times treatment area \times year $P = 0.890$). As for the analyses of body mass, when considering separately the spring $t + 1$ and $t + 2$, the difference in the slopes between Testosterone- and Control-areas was only significant in spring $t + 2$, but not in spring $t + 1$, where sample sizes were small (Table 2, Fig. 2). Using the square root transformed comb area and cube root transformed body mass values, the ornament–condition relationship also differed between areas after the manipulation (reduced model: $F_{1,143} = 5.90$, $P = 0.0164$).

Discussion

Ornament expression (comb area) positively correlated with a measure of condition (body mass), as predicted by indicators models of sexual selection. However, the strength of the relationship varied considerably between populations and years and changed according to an index of ECs (average body mass of both males and females within the population), the relationship being steeper as this index decreased, indicative of worsened ECs. A reduction in our EC index was not associated with an increase in body mass variance, indicating that the improved positive relationship was not simply due to a greater variance in body mass. The population-level experiment confirmed correlative data and provides, to

Table 2 Population-level experimental effects of increased aggressiveness on individual body mass and comb area–body mass relationships. Results of the General Linear Mixed Models analysing differences between Control-areas or Testosterone-areas on body mass (g) and comb area (mm^2) before the experimental manipulation (autumn t) or in the two subsequent springs (spring $t + 1$ and spring $t + 2$). Population (three levels) and treatment area \times population interaction were included as random effects. No captures were performed in Moorhouse during spring $t + 1$ (see Materials and Methods). Year (spring $t + 1$ and spring $t + 2$) was also included in models analysing variation in body mass (Year, $F_{1,207} = 6.96$, $P = 0.009$) and comb area (Year, $F_{1,142} = 3.49$, $P = 0.063$) when considering both springs in the same model. Coefficients of variation (raw data): Spring $t + 1$: Comb area; Control-area = 0.161, Testosterone-area = 0.118; Body mass: Control-area = 0.038, Testosterone-area = 0.077. Spring $t + 2$: Comb area; Control-area = 0.149, Testosterone-area = 0.146; Body mass: Control-area = 0.062, Testosterone-area = 0.058. Significant P -values are given in bold.

Variables		Before manipulation			After manipulation								
		Autumn t			Springs $t + 1$ and $t + 2$			Spring $t + 1$			Spring $t + 2$		
Dependent	Explanatory	d.f.	F	P	d.f.	F	P	d.f.	F	P	d.f.	F	P
Body mass	Treatment (T)	1, 4	0.43	0.548	1, 4	14.72	0.018	1, 2	0.20	0.700	1, 4	23.09	0.008
	Age (A)	1, 222	39.67	< 0.001	1, 207	5.08	0.025	1, 23	0.58	0.455	1, 180	22.37	0.013
	T \times A	1, 221	1.06	0.304	1, 207	6.18	0.013	1, 22	1.04	0.318	1, 180	3.93	0.049
Comb area	Body mass (BM)	1, 220	36.94	< 0.001	1, 143	15.08	< 0.001	1, 21	7.12	0.014	1, 115	7.74	0.006
	T	1, 2	0.72	0.485	1, 2	5.27	0.148	1, 1	0.09	0.814	1, 2	3.57	0.199
	A	1, 219	0.63	0.428	1, 141	0.37	0.543	1, 21	4.63	0.043	1, 114	0.20	0.657
	BM \times T	1, 217	0.01	0.864	1, 143	6.29	0.013	1, 19	0.81	0.378	1, 115	4.30	0.040
	BM \times A	1, 218	2.69	0.102	1, 139	0.02	0.893	1, 20	1.79	0.195	1, 113	1.52	0.219
	T \times A	1, 216	1.41	0.236	1, 140	2.31	0.131	1, 21	8.89	0.007	1, 112	0.04	0.851
	BM \times T \times A	1, 215	1.92	0.167	1, 138	0.32	0.570	1, 18	1.16	0.295	1, 111	0.95	0.332

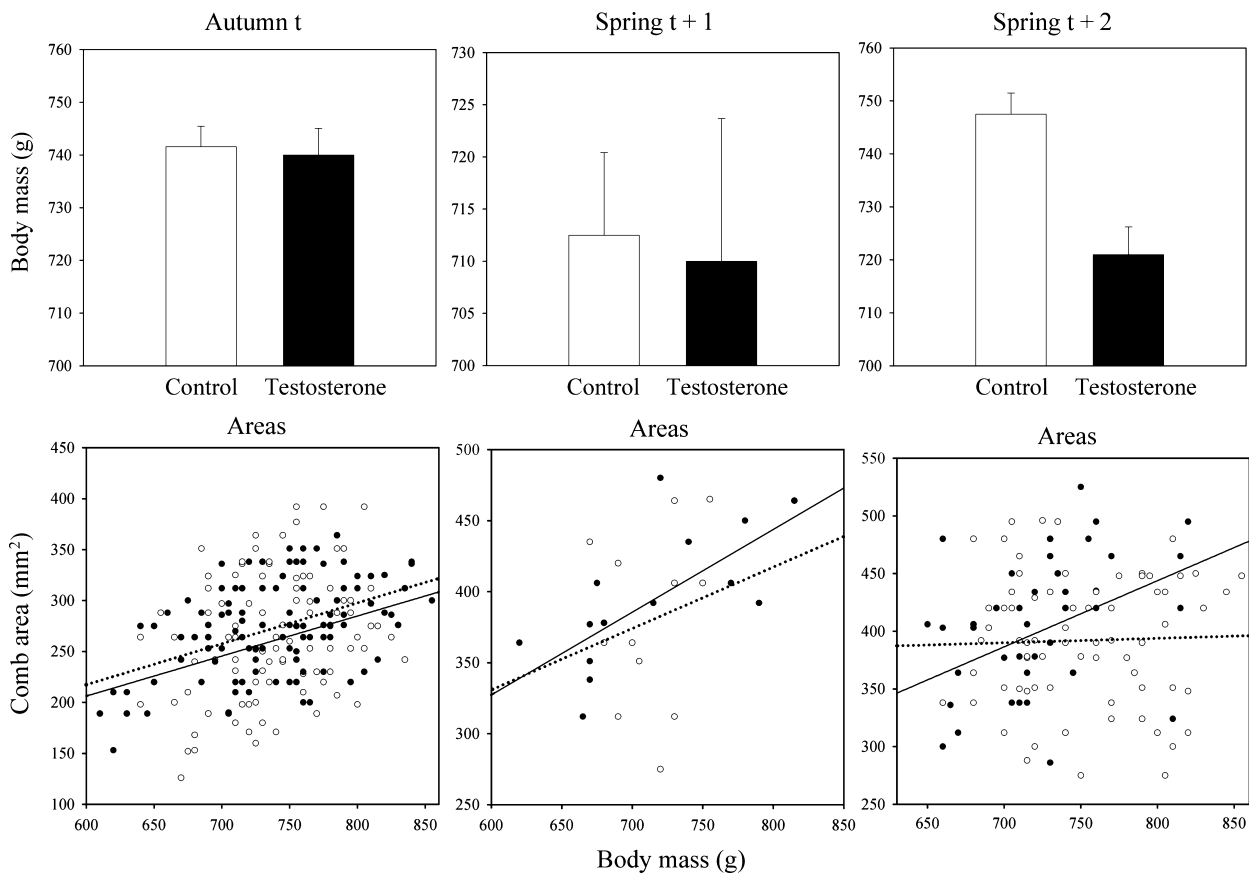


Fig. 2 Population-level experimental effects of increased aggressiveness on individual body mass (upper row) and on the relationship between ornamentation and body mass (lower row). Differences before (autumn t) and after (spring $t + 1$ and spring $t + 2$) the manipulation in body mass (g) (upper row, bars and whiskers represent means and s.e.m, respectively) and in the comb area–body mass relationship (lower row) between individuals living in Control- (open symbols and dotted lines; autumn t $n = 115$, spring $t + 1$ $n = 12$, spring $t + 2$ $n = 76$) and Testosterone-areas (solid symbols and lines; only those males not implanted with testosterone, autumn t $n = 112$, spring $t + 1$ $n = 16$, spring $t + 2$, $n = 47$, see Materials and Methods).

the best of our knowledge, the first experimental demonstration in the wild that increased aggressiveness at the population level strengthens the ornament–condition relationship.

Is the condition dependence of sexual traits shaped by environmental conditions?

Environmental heterogeneity is expected to create variation in the condition dependence of sexual ornaments (Cotton *et al.*, 2004; Cothran & Jeyasingh, 2010). This variation arises when the resolutions of trade-offs, such as investing limited resources either in self-maintenance or in the production of costly ornaments, differ between contrasted environments (Gustafsson *et al.*, 1995; Robinson *et al.*, 2008). The condition dependence of comb size has been largely demonstrated in red grouse (Mougeot *et al.*, 2004, 2006; Martínez-Padilla *et al.*, 2010), but here we showed that variation in the average body mass within the population modifies this relationship. This

change in the comb area–body mass relationship is in agreement with increased costs for condition-dependent sexual ornament expression under adverse environments, which may increase the relative differences between low- and high-quality individuals as ECs worsen (Cotton *et al.*, 2004; Cothran & Jeyasingh, 2010). We cannot, however, rule out other potential explanations for the changing ornament–condition relationship between population and years. If individuals adjust their investment in secondary sexual traits according to their survival prospects, individuals with low chances of survival may increase their investment in ornamentation under adverse environments. Individuals with higher survival probabilities could save their resources for more benign environments, with likely better breeding outcomes (e.g. Candolin, 1999). In addition, changes in the relationships between sexual ornaments and measures of condition across time and space can occur when alternative genotypes are adapted to different ECs (i.e. genotype \times environment interactions, Higginson &

Reader, 2009; Ingleby *et al.*, 2010). Nevertheless, as condition is supposed to be also genetically determined, increased costs for condition-dependent sexual ornament expression under adverse environments could be a particular case of the genotype \times environment interactions [e.g. a reaction norm where the rank of the genotypes is maintained (i.e. high-condition individuals always more ornamented) but not the scale of variation (i.e. differences in comb size) across environments]. Finally, if viability selection associated with investment in ornamentation changes among environmental contexts, different relationships between sexual traits and condition can be found across time and space (Robinson *et al.*, 2008). Further studies, considering the potential role of selection processes rather than phenotypic plasticity, and also taking into account the genetic component of the studied trait, are needed to distinguish between these possibilities.

Aggressiveness, environmental conditions and the condition dependence of sexual traits

Previous studies have suggested that environmental factors such as social context (Moss *et al.*, 1996; Martínez-Padilla *et al.*, 2010), weather conditions (Cockburn *et al.*, 2008; Sirkiä *et al.*, 2010), food (Candolin, 2000a; Cotton *et al.*, 2004; Cothran & Jeyasingh, 2010) or parasites (Dunn *et al.*, 2010; Vergara *et al.*, 2011; P. Vergara, F. Mougeot, J. Martínez-Padilla, F. Leckie & S. Redpath, unpublished) may modify the relationship between ornament expression and condition or fitness. With our correlative data, we tested whether tough or favourable ECs, in a wide sense, affected the relationship between comb area and body mass. However, such an approach does not allow us to specify the environmental factors behind the changing ornament–condition relationship. We chose a measure of ECs (i.e. male and female body mass at population level) that summarizes the effect of several environmental factors such as nematode infestation (Mougeot *et al.*, 2010), food intake (Delahay & Moss, 1996), or levels of male aggressiveness (this study) affecting the grouse population. As different stressors affect a given population simultaneously, we have a limited capacity to disentangle the relative importance of each environmental factor. However, we experimentally modified male aggressiveness in several populations (Mougeot *et al.*, 2003, 2005a) and showed a delayed effect on the ornament–condition relationship (Fig. 2), this relationship being steeper in the Testosterone-areas. Previous evidence, such as lower recruitment rates in the Testosterone-areas than in the Control-areas, also agrees with the idea that our experiment successfully affected the competitiveness within populations, Testosterone-areas being more adverse environments than Control-areas (Mougeot *et al.*, 2003, 2005a). The experimental results therefore support a role for the social, competitive, environment in modifying the ornament–condition rela-

tionship (Moss *et al.*, 1996; Martínez-Padilla *et al.*, 2010). Previous studies suggested a likely role of the socially imposed costs in the maintenance of the honesty of sexual traits (Candolin, 2000b). It has been shown that increased levels of male–male competition ensure the honesty of the signal because low-quality males or those in poor condition decreased signal expression to avoid agonistic encounters with superior males (Candolin, 2000b). Our findings agree with these suggestions, but longitudinal studies are needed to confirm such possibility. Interestingly, we showed that our manipulation of aggressiveness later affected average body mass at population level, the variable considered here as an index of ECs. This adds support to the adequacy of using average body mass at population level as an index of ECs. The lower body mass in the Testosterone-areas may be a direct or indirect result of increased aggressiveness in such areas. There was not evidence that the manipulation increased parasite infection levels (Mougeot *et al.*, 2005a), which could have contributed to reduce average body mass. To compete with testosterone-implanted individuals (for our analyses, we considered only males that had not been implanted with testosterone during the initial manipulation), non-implanted males must increase their aggressiveness (Mougeot *et al.*, 2005b), which may have negative consequences on their condition (Mougeot *et al.*, 2006; Fargallo *et al.*, 2007). Alternatively, those males implanted with testosterone may relegate other males to lower quality or smaller territories, where they are more likely to lose condition (Moss *et al.*, 1994). In both scenarios, nonimplanted individuals living in the Testosterone-areas are under more stressful conditions than individuals living in Control-areas, hence the steeper ornament–condition relationship (Candolin, 2000a; Fargallo *et al.*, 2007). Nevertheless, we must be cautious when interpreting the overall effect of our experimental manipulation of the social environment in both the body mass and the ornament–condition relationship. Although we found statistically significant effects in the models considering both spring $t + 1$ and spring $t + 2$, and that such effect did not differ between years (nonsignificant Year by Treatment interactions), the limited sample size in spring $t + 1$ prevent us to clearly confirm the general effect of the experiment. In fact, if analysed separately, the statistical effects were significant only in spring $t + 2$, when body mass was also significantly reduced on the Testosterone-areas. Further studies would be needed to clarify this point.

Signal unreliability under favourable environments: implications for sexual selection.

Our findings have broader implications for our understanding of sexual signalling and sexual selection, suggesting that ornaments are unreliable as predictors of condition when environments are favourable. Reliable signalling is a key concept to understand how male ornamentation has coevolved with female preferences,

by both Fisherian and viability (i.e. good genes) mechanisms (Greenfield & Rodriguez, 2004). However, signals may become unreliable indicators as a result of environmental heterogeneity (Greenfield & Rodriguez, 2004; Higginson & Reader, 2009). Signal unreliability may compromise the operation of the mechanisms of sexual selection, as it may promote females preference for the 'wrong' males. Our study provides powerful empirical evidence that environmental heterogeneity shapes signal reliability, thus highlighting the need of considering environmental heterogeneity to fully understand male sexual ornament expression and female preferences evolution (Cornwallis & Uller, 2009). Recent studies have shown that female preferences for males bearing ornaments that reliably indicate condition or better predict breeding success can fluctuate over time (Chaine & Lyon, 2008), providing a likely mechanism to explain the evolution of ornament-mate preference evolution. Our study helps to understand the changing relationship between sexual ornaments and condition across time and space, but further research corroborating the changing mate preferences between favourable and adverse environments is needed to confirm this idea. However, given that mate choice is a condition-dependent trait and that environmental stressors may differ between males and females, making general predictions regarding the direction of female preferences for the most ornamented males under changing environments remains difficult (Cotton *et al.*, 2006; Narraway *et al.*, 2010). Accordingly, stronger sexual selection for condition-dependent male traits has been suggested under both favourable (Cockburn *et al.*, 2008) and adverse conditions (Fisher & Rosenthal, 2006).

In conclusion, we have demonstrated that the relationship between ornament expression and body mass is altered by an index of ECs, with stronger ornament-condition relationships when ECs are worsened, such as when aggressiveness is increased. This provides a framework for better understanding and reconciling conflicting results in sexual selection studies of the validity of ornaments as indicators of condition, condition-dependent ornamentation, changing female preferences for male ornaments and also highlights the need to consider environmental heterogeneity as context to explain how male ornamentation has coevolved with female preferences.

Acknowledgments

We are grateful to the people who helped in the field, and owners and gamekeepers from Edinglasie, Glen Muick, Glen Dye, Invercauld, Invermarck and Millden states, to the British Army (Catterick Training estate, north Yorkshire), RSPB (Geltsdale reserve) and English Natural Heritage (Moorhouse) for allowing us to conduct the work on their grouse moors. We thank S.B. Piertney, J.A. Fargallo and G. Bortolotti for comments. Two anonymous reviewers provided valuable comments. PV was supported by an Intra-European Marie Curie Fellowship (European

Union Seventh Framework Programme, FP7/2007–2013, grant agreement no. 252499). FM was supported by a NERC advanced fellowship. We held all the necessary UK Home Office licences for conducting these procedures (PPL80/1437 and PPL60/3824). This study was funded by Natural Environment Research Council Grants NER/A/S/1999/00074, NE/D000602/1 and NE/D014352/1.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Results of the GLM analysing body mass variation in relation to sex, age and day of capture.

Table S2 Sample sizes of caught and measured males and females by site, year and age, and inter- and intra-population variation in body mass and the Environmental Conditions index (EC index).

Table S3 Effect of environmental conditions on the relationship between ornamentation and body mass correcting by differences in sample sizes between sites and years (a) between differences in scales (b) or avoiding collinearity between variables (c).

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Received 15 July 2011; revised 7 September 2011; accepted 19 September 2011