

# Testosterone, immunocompetence, and honest sexual signaling in male red grouse

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The expression of sexual ornaments has been suggested to reliably indicate individual quality, such as the ability to cope with parasites and diseases. The Immunocompetence Handicap Hypothesis (IHH) states that testosterone-dependent ornaments honestly signal such quality because of physiological costs associated with testosterone, such as impaired immune function. We tested predictions of the IHH both correlatively and experimentally in red grouse *Lagopus lagopus scoticus*. Male grouse exhibit supra-orbital red combs whose size is testosterone-dependent. We found that comb size was not correlated to infection intensity by two parasites (coccidia and the nematode *Trichostrongylus tenuis*), but it was significantly positively correlated with condition and T-cell-mediated immunity (the ability to mount a primary inflammatory response). We manipulated testosterone by means of implants and re-caught males after a month to investigate the effects on comb size, condition, immunity, and parasite load. Males implanted with testosterone had increased comb size, lost more condition, and had lower T-cell-mediated immunity than control males. Increased testosterone also resulted in a significant increase in coccidia infection intensity but had no effect on *T. tenuis* burden. The results are consistent with predictions of the IHH and suggest that comb size honestly indicates immunocompetence and males' ability to cope with certain parasites. Females could thus benefit from choosing mates based on the expression of this sexual trait. *Key words*: Immunocompetence Handicap Hypothesis, parasite, red grouse, sexual ornament, T-cell-mediated immunity, testosterone. [*Behav Ecol* 15:930–937 (2004)]

In many species, males exhibit ornaments that probably evolved through intra- or inter-sexual selection. These sexual traits assume the dual function of dissuading other males competing for the same resources and facilitating mate choice, allowing females to identify the fittest males on the basis of their ornaments (Andersson, 1994). Hamilton and Zuk (1982) suggested that female preferences for males with the most exaggerated sexual traits evolved because ornament expression signals the genetic ability of males to resist parasite infections. Females could benefit by choosing a mate with fewer parasites, or whose ability to cope with parasites will be passed onto offspring (Andersson, 1994; Hamilton and Zuk, 1982; Møller, 1990; Møller et al., 1999; Zuk, 1992).

The role of parasites in sexual selection has been particularly well studied in birds. Many studies have looked for negative relationships between sexual signals and parasite loads. However, the results are inconsistent, with studies reporting either negative relationships, no relationships, or even positive relationships between signals and parasites (see Folstad and Karter, 1992; Getty, 2002; Møller et al., 1999). Moreover, the lack of relationship between sexual signal and infection intensity by a particular parasite does not, however, exclude the possibility that either the ornament expression relates to the ability to cope with the detrimental effects of the parasite rather than its abundance itself, or that other parasites act as a constraint on the level of signaling.

Measures of host immune responses may therefore provide more accurate indications of the role of parasite-mediated sexual selection than estimates of the intensity of infection (Møller et al., 1998b, 1999; Møller and Saino, 1994; Zuk,

1996). Indeed, several studies have shown that sexual signaling relates to immunocompetence (the ability of an individual to produce an immune response to pathogens), providing evidence that sexual signals can be used by females as cues to this aspect of male quality (Duffy and Ball, 2002; Folstad and Karter, 1992; Møller et al., 1999; Møller and Saino, 1994; Saino et al., 1997; Zuk, 1996; Zuk et al., 1995). The relationship between signals and immunocompetence also appears to be stronger than that between signals and parasites (Møller et al., 1999).

Signaling theory predicts that signals of individual quality should be costly in order to ensure the honesty of the signaling system and prevent poor quality individuals from cheating (Zahavi and Zahavi, 1997). Elevated testosterone levels are usually required for the expression of sexual traits involved in male-male competition and female choice, but they might also be associated with physiological costs, such as impaired immune function (Folstad and Karter, 1992; Grosman, 1985; Hillgarth and Wingfield, 1997). According to the Immunocompetence Handicap Hypothesis (IHH), testosterone-dependent traits or behaviors would be particularly useful as honest signals of health, because of the immunosuppressive effects of elevated testosterone (Folstad and Karter, 1992). On the one hand, elevated testosterone levels increase the expression of ornaments relevant in an intra-sexual or a mate choice context, but, on the other hand, they could reduce immune function and increase susceptibility to parasite infections (Folstad and Karter, 1992). It has been suggested that this 'double-edged sword' effect of testosterone would prevent cheating, as males have to trade the benefits against the costs of elevated testosterone and optimize their level of signaling accordingly. Although correlative studies may provide insights about the individual qualities underlined by sexual traits, they do not establish causality. Moreover, optimal signaling theory is unable to predict the relationship between

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signal intensity and parasite loads (Getty, 2002). The IHH is based on trade-offs within individuals (between testosterone, levels of signaling, immunocompetence, and parasites), and experimental studies are therefore the most appropriate to investigate these trade-offs. Testosterone levels can be manipulated by the means of implants, allowing workers to modify the level of signaling of males and investigate effects on immunocompetence and parasite infections (e.g., Casto et al., 2001; Duckworth et al., 2001; Saino et al., 1995) or on fitness components such as viability, mating, and breeding success (e.g., Alatalo et al., 1996; Moss et al., 1994).

In this study, we investigated whether sexual ornamentation relates to condition, parasite burdens, and/or immunocompetence, and we used an experimental approach to test predictions of the IHH in male red grouse *Lagopus lagopus scoticus*. Grouse combs are brightly colored ornaments that function in both male-male competition and female choice (Alatalo et al., 1996; Bart and Earnst, 1999; Moss et al., 1979; Rintamaki et al., 2000). Their size is testosterone-dependent (Moss et al., 1979; Rintamaki et al., 2000), so these ornaments might therefore be particularly useful as honest signals of health. We first looked at the relationships between comb size, condition, intensity of infection by two parasites (a gut nematode, *Trichostrongylus tenuis* and coccidia *Isospora ssp.*), and immunocompetence (T-cell-mediated immunity, the ability to mount a primary inflammatory immune response). If comb size signals male quality, we expected males with bigger combs (and more testosterone) to be those in better condition, with a greater T-cell-mediated immunity and fewer parasites. Second, we manipulated testosterone levels of males to test two main assumptions of the IHH, namely that testosterone enhances sexual ornamentation and impairs immune function. A previous study highlighted that males with more testosterone can be more immunocompetent despite the immunosuppressive effects of testosterone (Peters, 2000). Predictions in the experimental situation were thus different from those in the natural situation. If signaling is honest, changing the optimal level of sexual signaling by increasing testosterone should be associated with immunosuppressive effects and should therefore be costly. Thus, we expected increased testosterone levels to result in a reduction of T-cell-mediated immunity, a loss of condition, and an increase in parasite intensity.

## METHODS

### Study species

The red grouse is a medium sized *Tetraonid* bird characteristic of the heather moorland habitats of the United Kingdom (Cramp and Simmons, 1980). This species is territorial and mostly monogamous (Cramp and Simmons, 1980). Male territorial behavior is particularly important in the autumn, during territory establishment, and in early spring. Pairing starts in autumn and continues throughout the winter until early spring, when breeding starts (Cramp and Simmons, 1980; Watson, 1985). As in other grouse species, red grouse combs are larger and brighter in males, and their size is testosterone-dependent (Moss et al., 1979). These sexual ornaments are known to play an important role in male-male competition; male red grouse with big combs are dominant and more aggressive (Moss et al., 1979, 1994) and are more likely to obtain a breeding territory than others (MacColl et al., 2000). Comb size also functions in mate choice, females preferring males with the biggest combs (Bart and Earnst, 1999; Brodsky, 1988; Rintamaki et al., 2000). In willow grouse *Lagopus lagopus lagopus*, both territory size and comb size were found to be equally important for mate choice (Bart and Earnst, 1999).

### Captures, measurements, and testosterone manipulations

In September–November 2001, we caught male grouse at night by dazzling and netting them (Hudson, 1986b) on two sites located in the northeast of Scotland (Glen Dye and Edinglassie estates, Aberdeenshire). We aged males (young vs. old, i.e., >1 year old) from the shape and color of their second and third primaries (tips pointed and mottled in young, round and plain in old birds) and the texture of their claws (smooth in young, with a growth ridge in old birds; Cramp and Simmons, 1980). At the time of first capture, we ringed all males, weighed them with a 1-kg balance, to the nearest 5 g, and measured the following morphological features: length and width of flattened combs, wing length (with a ruler, to the nearest 1 mm), and pectoral angle (with a protractor, to the nearest degree), as a measure of the extent of breast muscles on either sides of the breast bone. The same person (F.M.) did all the measurements, and each was taken twice on a sample of 30 males to assess measurement errors. Repeatability values were calculated according to Lessells and Boag (1987) and were high and significant for all the above morphological features (all repeatability values > 0.93; all  $p < .0001$ ). We calculated a condition index (weight corrected for size) as the residuals from the relationship between  $\log_{10}$ -transformed body weight and  $\log_{10}$ -transformed wing length ( $F_{1,108} = 52.31$ ;  $p < .0001$ ).

We randomly assigned males to one of three treatments: no implant, sham implants, or testosterone implants. Implanted males were each given two silastic tubes (each 20 mm long, 0.62 mm inner and 0.95 mm outer diam) that were either empty (sham implanted males) or filled with testosterone propionate (testosterone-treated males) and sealed with glue at both ends. Implants were inserted in the chest, between the skin and breast muscles, under local anesthesia. The length of the tubing was determined previously during laboratory trials on captive grouse so that the testosterone implants lasted for 2–3 months. We fitted each male with a necklace radio-tag (TW3-necklace radio-tags, Biotrack) to facilitate subsequent recapture, kept them overnight in an individual pen to collect fecal samples for parasite counts, and released them the following morning.

We recaptured most males about a month ( $32 \pm 8$  days) after the first capture. However, some males were found killed by predators or could not be located, possibly because the radio failed, and they were not re-sampled. Thus, sample size differed between capture and recapture. At recapture, we re-measured comb size, pectoral angle, and body weight, collected another fecal sample for parasite load estimates, and measured T-cell-mediated immunity.

When analyzing the data, we found no significant difference between unmanipulated males and sham-implanted males in any of the study parameters or relationships. Therefore, to simplify the presentation of the results, we pooled the data from these two groups, hereafter referred to as control males (as opposed to testosterone-implanted males).

### Assessment of T-cell-mediated immunity

We measured T-cell-mediated immunity (CMI) by challenging the immune system through the subcutaneous injection of an innocuous plant lectin (phytohaemagglutinin, or PHA). The PHA test follows a long-established protocol developed in poultry science (Goto et al., 1978) and is routinely applied in avian studies (e.g., Smith et al., 1999). The injection of PHA produces a prominent perivascular accumulation of T-lymphocytes followed by macrophage infiltration (Goto et al., 1978), and the intensity of the CMI response (swelling) indicates an individual's ability to mount a primary inflammatory response. Each male was injected with 0.1 ml of

physiological saline solution (PBS) at a marked site on the right wing web (control) and with 0.2 mg of PHA in 0.1 ml of PBS (challenge) on the left wing. We measured the web thickness at each injection site four times prior to injection and 24 h ( $\pm 10$  min.) after injection, using a pressure-sensitive dial thickness gauge (to the nearest 0.01 mm). Wing web thickness measures were highly and significantly repeatable ( $R = .83$ ;  $p < .01$ ). CMI response was calculated as the change at 24 h in average thickness of the left wing web (PHA test) minus that of the right wing web (control). For this procedure, males were kept in pens provided with food (heather) and water. After 24 h, we removed the radio tags and released all males. Sample size for immune challenges included all the males that we re-caught one month after first capture. At the same time, we also caught other males (unmanipulated) on which we conducted measurement, parasite counts, and immune challenges, in order to increase sample size.

### Parasite counts

Red grouse are host to a relatively small number of parasites. One of their main parasites is a gut nematode, the caecal threadworm *T. tenuis*, which has a direct lifestyle with no alternative hosts within the same habitat (Hudson, 1986a). It is known to have important negative effects on the energetics, breeding success, and survival of grouse (Delahay et al., 1995; Hudson, 1986a; Hudson et al., 1992; Shaw and Moss, 1990). Red grouse are also frequently infected by coccidia *Isospora ssp.* (Fantham, 1911). Patterns of infections and effects on red grouse are little known, but this parasite can have important adverse effects on avian host, in terms of reduced growth and condition (Fehlberg and Pohlmeier, 1991). For this study, we focused on these two parasites for which infection intensity is relatively easy to measure on live birds.

We used fecal egg counts to measure intensity of infection by *T. tenuis* worms and coccidia. Samples collected in the field were kept in the lab at 4°C and were analyzed within two weeks of collection. For each, a subsample of 0.2 g was diluted into 5 ml of distilled water, mixed, and placed in a MacMaster slide under a  $\times 40$  microscope, where *T. tenuis* and coccidia eggs were counted. Parasite infection intensity was estimated as number of eggs per g. Fecal egg counts were shown to reliably estimate the number of *T. tenuis* worms in grouse both in spring (Moss et al., 1990) and autumn (Seiwright et al., 2004). Infection rates by coccidia and the rate of oocyst shedding can show important diurnal and seasonal variations (Brawner and Hill, 1999; Hudman et al., 2000; Svoboda, 1992). Diurnal variation was minimized by collecting all fecal samples in the early morning, and seasonal variations in parasite infections over the study period were taken into account by controlling for sampling date.

### Statistical analyses

We used SAS 8.01 for the statistical analyses (SAS, 2001). We used Generalized Linear Models to investigate natural correlates of comb size, condition, or cell-mediated immunity. Dependent variables were fitted with a normal distribution and identity link function (comb size, condition index, and pectoral angle) or with a Poisson distribution and log link function (coccidia and *T. tenuis* egg counts). For the correlation analyses, coccidia and *T. tenuis* loads were  $\log_{10}$ -transformed as explanatory variables. We first tested for differences between study sites, age groups, and variation according to sampling date, and we controlled for these effects when necessary.

For the experiment, we used Generalized Linear Models to test for differences in study parameters between treatment

groups prior to and (one month) after implanting with testosterone. Because most parameters varied between capture and recapture, we also tested whether changes over time differed between treatment groups (testing for a time\*treatment interaction). The data set was unbalanced (i.e., not all individuals were measured before and after treatment and not all parameters were measured at a given time, especially parasite loads). For these analyses, we used Generalized Linear Mixed Models (Glimmix, SAS) with individual males included as random effects (to account for the repeated measures on individual males and the unbalanced data set). Models included site, age, time (first capture vs. recapture), treatment (control vs. Testosterone-treated males), and the time\*treatment interaction as fixed effects. All data are expressed as mean  $\pm$  SD and all tests are two-tailed.

## RESULTS

### Comb size, condition, and parasites burdens

We investigated the relationships between comb size, condition, and parasites, or between condition and parasites, using the measures made at the time of first capture (i.e., prior to treatment).

Comb size variation was not significantly explained by site ( $F_{1,52} = 0.37$ ;  $p = .54$ ), age ( $F_{1,52} = 0.79$ ;  $p = .34$ ), sampling date ( $F_{1,51} = 1.55$ ;  $p = .22$ ), *T. tenuis* load ( $F_{1,40} = 0.35$ ;  $p = .56$ ) or coccidia load ( $F_{1,40} = 0.86$ ;  $p = .36$ ), but it was significantly explained by both measures of condition; males with bigger combs had more breast muscles (pectoral angle:  $F_{1,52} = 4.78$ ;  $p < .05$ ) and were heavier relative to their size (condition index:  $F_{1,52} = 7.06$ ;  $p < .05$ ). These relationships between comb size and condition were still significant after controlling for site, age, and date (pectoral angle:  $F_{1,49} = 7.09$ ;  $p < .05$ ; condition index:  $F_{1,49} = 6.75$ ;  $p < .05$ ).

Pectoral angle did not differ between study sites ( $F_{1,52} = 0.32$ ;  $p = .57$ ) but significantly decreased during autumn ( $F_{1,52} = 9.94$ ;  $p < .01$ ) and differed between age groups after controlling for date ( $F_{1,51} = 6.31$ ;  $p < .05$ ), with old males having a greater pectoral angle (more breast muscles) than young males. However, both old and young males were similarly losing breast muscles during early autumn (nonsignificant date\*age interaction:  $F_{1,50} = 2.44$ ;  $p = .13$ ). After controlling for sampling date and age, pectoral angle was not related to *T. tenuis* load (partial  $F_{1,38} = 0.62$ ;  $p = .44$ ) or coccidia load (partial  $F_{1,38} = 0.16$ ;  $p = .69$ ). Condition index did not differ between study sites ( $F_{1,52} = 0.24$ ;  $p = .63$ ) or age groups ( $F_{1,52} = 2.39$ ;  $p = .13$ ) and was not significantly related to sampling date ( $F_{1,51} = 0.09$ ;  $p = .76$ ), *T. tenuis* load ( $F_{1,39} = 1.62$ ;  $p = .21$ ), or coccidia load ( $F_{1,39} = 1.43$ ;  $p = .24$ ).

### Effects of testosterone on comb size, condition, and parasites

Before treatment, none of study parameters (comb size, pectoral angle, condition index, *T. tenuis* and coccidia load) differed between treatment groups (control vs. testosterone males; Generalized Linear Models controlling for site, sampling date, and age; all  $F$ -values  $< 2.01$ ; all  $p > .05$ ; data in Table 1). One month after treatment, we found significant differences between T-implanted and control males in comb size ( $F_{1,51} = 43.45$ ;  $p < .001$ ), pectoral angle ( $F_{1,51} = 17.95$ ;  $p < .001$ ), condition index ( $F_{1,51} = 8.57$ ;  $p < .01$ ), and coccidia load ( $F_{1,39} = 7.32$ ;  $p < .01$ ), but we found no difference in *T. tenuis* load ( $F_{1,39} = 0.75$ ;  $p = .39$ ; data in Table 1).

We further tested whether changes over time in study parameters (between first capture and recapture) differed between treatment groups. Comb size increased in both

**Table 1**  
Changes over time (between capture and recapture) in study parameters according to treatment

Parameters	Time <sup>a</sup>	Treatment	
		Control	Testosterone-treated
Comb area (mm <sup>2</sup> )	t <sub>0</sub>	273.3 ± 46.9 (35)	286.8 ± 38.6 (21)
	t <sub>1</sub>	280.4 ± 47.8 (39)	401.5 ± 60.9 (18)
Pectoral angle (°)	t <sub>0</sub>	43.4 ± 2.5 (35)	45.5 ± 3.8 (21)
	t <sub>1</sub>	42.8 ± 4.0 (39)	39.7 ± 2.7 (18)
Condition index	t <sub>0</sub>	-0.001 ± 0.043 (35)	0.039 ± 0.053 (21)
	t <sub>1</sub>	-0.012 ± 0.044 (39)	-0.005 ± 0.055 (18)
Coccidia (egg/g)	t <sub>0</sub>	1180 $\times$ 4.8 (26)	1524 $\times$ 2.9 (16)
	t <sub>1</sub>	1254 $\times$ 4.6 (30)	4486 $\times$ 3.7 (14)
<i>T. tenuis</i> (egg/g)	t <sub>0</sub>	2342 $\times$ 3.3 (26)	1808 $\times$ 6.1 (16)
	t <sub>1</sub>	1527 $\times$ 11.0 (30)	1669 $\times$ 8.7 (14)

<sup>a</sup> t<sub>0</sub>: initial capture; t<sub>1</sub>: recapture, ~1 month later. Sample size, in parentheses, refers to number of individual males. Comb area, pectoral angle, and condition index are expressed as arithmetic means ± SD. Coccidia and *T. tenuis* egg counts are expressed as geometric means  $\times$  SD.

groups, but it increased significantly more in testosterone-implanted than in control males (Table 2; significant time\* treatment interaction; Figure 1). Both measures of condition (pectoral angle and condition index) also changed over time; and, in relation to treatment, males lost condition between capture and recapture, and testosterone-treated males lost significantly more breast muscles (pectoral angle) and body weight (condition index) than control males (Tables 1 and 2). Experimentally increased testosterone levels thus resulted in enhanced sexual ornamentation but also in a greater loss of condition.

*T. tenuis* burdens decreased between capture and recapture (Tables 1 and 2), this decrease being similar in testosterone-treated and control males (nonsignificant time\*treatment

**Table 2**  
Results of the Generalized Mixed Models (Glimmix procedure, SAS 2001) testing for an effect of treatment (testosterone-treated vs. control) on changes over time in study parameters

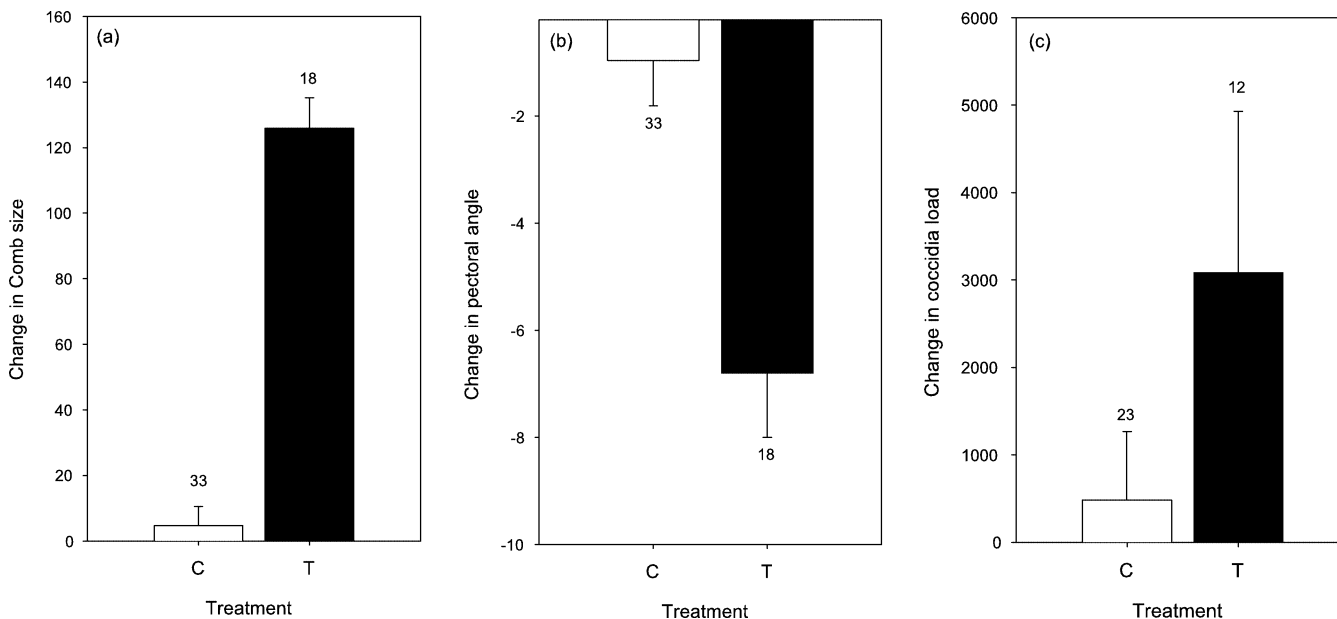
Parameters	df	Time		Treatment		Time*Treatment	
		F	p	F	p	F	p
Comb size (mm <sup>2</sup> )	1,47	95.81	***	16.21	***	99.30	***
Pectoral angle (°)	1,47	24.87	***	4.48	*	31.37	***
Condition index	1,47	32.32	***	2.85	ns	13.41	***
Coccidia (egg./g)	1,39	42.8	***	1.28	ns	7.65	**
<i>T. tenuis</i> (egg./g)	1,39	19.81	***	0.08	ns	0.20	ns

Models included study site and age as a fixed effect and individual males as random effects. Dependent variables were fitted with a normal distribution and an identity link function, except for coccidia and *T. tenuis* egg counts, which were fitted with a Poisson distribution and a log link function. (ns: *p* > .05; \* *p* > .05; \*\* *p* < .01; \*\*\* *p* < .001).

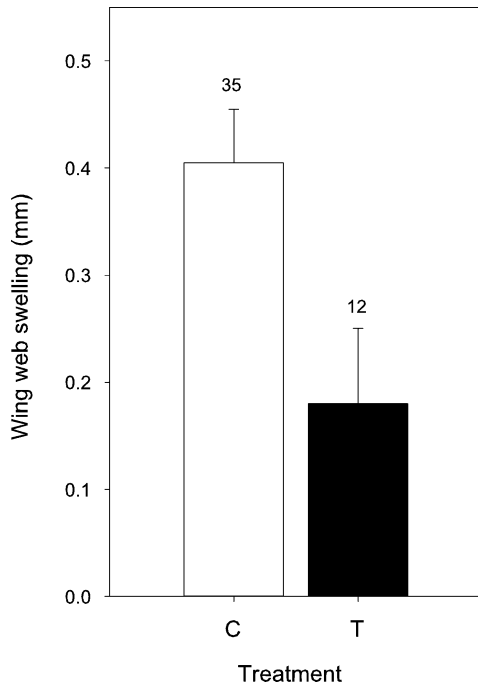
interaction; Table 2). In contrast, coccidia load increased between capture and recapture (Table 2). This increase was greater in testosterone-treated than in control males (Tables 1 and 2; Figure 1). Increased testosterone levels thus had no significant effect on changes in *T. tenuis* load but resulted in increased coccidia infection intensity.

**T-cell-mediated immunity, comb size, condition, and parasite burdens**

We investigated the natural correlates of T-cell-mediated immunity in the control males only (we excluded testosterone-treated males in these analyses, whose responses were affected by the treatment; see below). Variation in wing web swelling was not significantly explained by study site (GLM: *F*<sub>1,33</sub> = 2.45; *p* = .13), sampling date (*F*<sub>1,33</sub> = 0.30; *p* = .59), age (*F*<sub>1,33</sub> = 3.03; *p* = .10), or *T. tenuis* load (*F*<sub>1,28</sub> = 0.01; *p* = .93), but it was



**Figure 1**  
Mean (± SED) changes over time (between capture and recapture, ~1 month later) in (a) comb size, (b) pectoral angle (extent of breast muscles), and (c) coccidia load according to treatment (C: control males; T: testosterone-implanted males). Sample size on each bar refers to number of individuals in each group.



**Figure 2**  
Mean ( $\pm$  SED) wing web swelling according to treatment (C: control males; T: testosterone-implanted males). Sample size, above bars, refers to number of individuals in each group.

significantly explained by coccidia load ( $F_{1,28} = 5.38$ ;  $p < .05$ ), pectoral angle ( $F_{1,33} = 29.53$ ;  $p < .001$ ), condition index ( $F_{1,33} = 4.62$ ;  $p < .05$ ), and comb size ( $F_{1,32} = 20.08$ ;  $p < .001$ ). Males with greater T-cell-mediated immunity had fewer coccidia, more breast muscles, were heavier relative to their size, and had bigger combs (Figure 3).

#### Effect of testosterone on T-cell-mediated immunity

In males caught one month after treatment, wing web swelling 24 h post-challenge was significantly lower in testosterone-

treated than in control males (Figure 2;  $F_{1,45} = 6.59$ ;  $p < .05$ ). Differences in wing web swelling between treatment groups were significant after controlling for site, age, comb size, pectoral angle, and condition index ( $F_{1,40} = 5.57$ ;  $p < .05$ ). Experimentally increased testosterone levels thus resulted in reduced T-cell-mediated immunity.

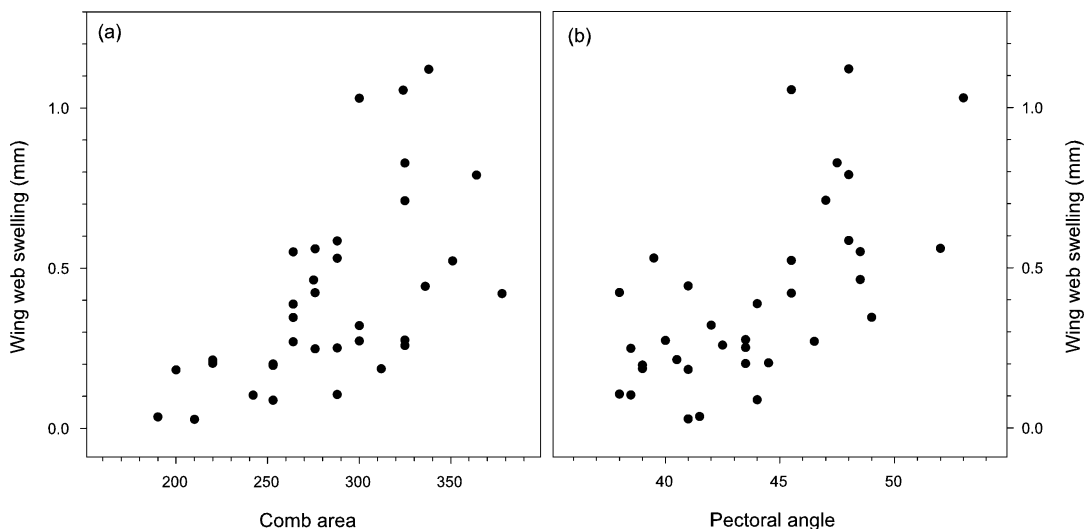
#### DISCUSSION

In red grouse, we found that males with the biggest combs were in better condition and had greater cell-mediated immunity but did not have fewer *T. tenuis* and coccidia parasites. We also found that experimentally increased testosterone enhanced comb size but reduced condition and cell-mediated immunity and increased coccidia infection intensity. Below, we discuss these findings in line with predictions from the Immunocompetence Handicap Hypothesis.

#### Relationships between comb size, condition, immunity, and parasite loads

We found that comb size during autumn was positively correlated with condition (males with bigger combs had more breast muscles). Levels of sexual signaling are usually expected to be condition-dependent, so that only individuals in prime condition are able to produce the most exaggerated ornaments without compromising their ability to cope with the costs imposed by the production and maintenance of the signal (Andersson, 1994). Parasites and diseases usually negatively affect condition (Møller et al., 1998a; Wilson et al., in press), so males with big combs might also be expected to have fewer parasites. However, we did not find that bigger combs were associated with fewer *T. tenuis* worms or fewer coccidia. Other studies nevertheless, have showed that infection by intestinal nematodes and coccidia can reduce levels of sexual signaling, such as comb size (Zuk et al., 1990a,b) or carotenoid-based color intensity (Brawner et al., 2000; Hill and Brawner, 1998).

We also found little evidence for a good condition to be associated with fewer of these parasites. *T. tenuis* is a main parasite of grouse, but previous work has shown that red grouse infected with *T. tenuis* worms lose more weight and condition than uninfected controls (Shaw and Moss, 1990;



**Figure 3**  
Relationships (a) between wing web swelling and comb area, and (b) between wing web swelling and pectoral angle (extent of breast muscles) in control males.

Wilson and Wilson, 1978). The *T. tenuis* infection levels found in this study were relatively low and decreasing, and might not have been sufficient to have noticeable detrimental effects on male condition or immune function. It is also possible that parasite transmission, which is low for *T. tenuis* in autumn, is more important than the parasite burden itself, as most damage is caused when ingested larvae develop into worms and bury in the caecal guts (Delahay et al., 1995). Similarly, coccidia infections can significantly reduce weight gain (Conway et al., 1995), although such an effect was not detected in this study. These results nevertheless suggested that comb size was unlikely to reliably indicate *T. tenuis* or coccidia infection levels, and thus they suggested that females were unlikely to prefer males with bigger combs because they have fewer of these parasites.

Our study shows that cell-mediated immunity, as measured by the response to the PHA skin test, was positively correlated with comb size and condition. Various aspects of the immune system are condition-dependent, because of the costs of raising an immune response, and a poor condition might thus result in a weak immune responsiveness (Møller et al., 1998a; Saino and Møller, 1996; Sheldon and Verhulst, 1996). Accordingly, we found that cell-mediated immunity was positively correlated with condition, with responses to the PHA test being positively correlated to both the relative weight and the amount of breast muscle of males. The relationship between sexual ornamentation and immunocompetence might thus be mediated by condition. Male red grouse in better condition and with greater cell-mediated immunity were those with bigger combs, and they were likely to be those of higher phenotypic/genetic quality. Females might therefore prefer males with bigger combs for their higher immunocompetence, which might underline a better ability to cope with parasite infections.

#### Effects of testosterone on comb size, cell-mediated immunity, and condition

Previous studies conducted on red grouse in spring showed that increased testosterone caused an increase in comb size (Moss et al., 1979), so we expected a similar effect in autumn. Indeed, we found that the testosterone implants caused a significant increase in comb size, up to the size that is usually exhibited by males in spring (and therefore within the range of natural variation). We also found that males implanted with testosterone had lower cell-mediated immunity (lower responsiveness to the PHA skin test) and lost more condition (body weight and breast muscles) than control and unmanipulated males.

Male red grouse implanted with testosterone typically call and display more often, are more aggressive, and expand their territory to the detriment of others (Moss et al., 1979; Watson and Parr, 1981). All these activities are energetically costly, which might explain why testosterone-implanted males lost condition. Because cell-mediated immunity was found to be condition-dependent, the testosterone treatment might have indirectly affected immunity, via its effects on condition. However, differences in immune response between treatment groups were still significant after controlling for condition, suggesting a more direct, physiological effect of testosterone on immunity. While many studies have showed that testosterone enhances the size or intensity of sexual ornaments (e.g., Hillgarth and Wingfield, 1997; Rintamaki et al., 2000; Zuk et al., 1995), direct experimental evidence for immunosuppressive effects of testosterone is still limited. Some studies found evidence for testosterone to be immunosuppressive (Casto et al., 2001; Peters, 2000), but others found little (Ros et al., 1997) or no support (Hasselquist et al., 1999) for this

prediction, which is central to the IHH. Our study provides experimental evidence for elevated testosterone levels to be associated with both enhanced sexual ornamentation and reduced immunocompetence. The experimental results were also consistent with the correlative results in showing that testosterone, comb size, condition, and immunocompetence are all closely interrelated in male red grouse.

Males with bigger combs therefore had greater cell-mediated immunity, despite having more testosterone and concomitant immunosuppression. These findings are similar to those from another study; in superb fairy-wrens *Malurus cyaneus*, testosterone treatment decreased immune responsiveness, but wild males with more testosterone were found to be more immunocompetent (Peters, 2000). This association between immunosuppressive testosterone and immune responsiveness may arise if males with big combs enhance their immunity to compensate for and counter the impact of immunosuppression by testosterone, or if both testosterone and immunocompetence correlate with male quality and condition. In autumn, male red grouse extensively engage in territorial displays to establish territories and in courtships, as females start assessing potential mates. Autumn territorial behavior is crucial for male red grouse, as most males failing to maintain or establish a territory usually die over the winter or will not reproduce (Watson, 1985). These activities require elevated testosterone, are energetically costly, and potentially stressful, as they usually involve fights and associated risks of injuries. Autumn territorial and display behaviors might thus require enhanced immune responsiveness, as a compensatory measure. Our results suggest males with the biggest combs are those better able to enhance immune responsiveness and cope with immunosuppressive testosterone, possibly because they are also in better condition and of higher phenotypic/genetic quality than others.

#### Testosterone and parasite infections

Because increased testosterone reduced immunocompetence, it might also have had indirect effects on parasite infection levels. A number of studies have shown that increased testosterone levels can cause significant increase in parasite infection intensities (Duckworth et al., 2001; Hughes and Randolph, 2001; Saino et al., 1995; Zuk et al., 1995). We found that the testosterone treatment affected levels of coccidia infection of males. Infection intensities increased during the study, but the increase in coccidia load was significantly greater in testosterone-implanted than in other males. The increased infection levels in testosterone-treated males might reflect a greater exposure or a reduced ability to control infection by coccidia. In control birds, cell-mediated immunity was found to correlate negatively with coccidia infection levels. The greater coccidia infection levels observed in testosterone males might thus have been caused by physiological effects associated with the testosterone treatment, which may have impaired the ability of males to control the establishment success or the reproductive rate of coccidia. In another study conducted on male house finches *Carpodacus mexicanus*, experimentally increased testosterone was found to accelerate the rate of coccidia infection, also suggesting that testosterone may have direct effects on resistance to this parasite (Duckworth et al., 2001). Elevated testosterone, however, does not always result in increased coccidia infection (e.g., Hudman et al., 2000). In contrast, we found no effect of testosterone on *T. tenuis* intensity. In this case, a possible explanation lies in the life history of *T. tenuis*. In autumn, ingested larvae become hypobiotic and delay development until the next spring, when they mature into adult worms and begin to produce eggs (Shaw, 1988). The adult worms that die in autumn are not

replaced, as shown in this study by a decrease in worm numbers throughout the autumn. It is thus possible that over the short time-scale of our experiment, the testosterone treatment had no effect on the adult worms because transmission was low, but it affected the arrestment of parasite larvae, which would affect parasite loads only in the following spring, when these larvae de-arrest. Further work is therefore needed to fully assess the effect that testosterone might have on this parasite.

### Comb size as an honest signal of male quality

Male red grouse with bigger combs, and more testosterone, benefit in terms of increased recruitment probability during autumn (MacColl et al., 2000) and increased territory size (Moss et al., 1994). They may also benefit from pairing earlier, attracting more than one female or a better quality female, and were shown to achieve a higher breeding success (Moss et al., 1994). Our results show that high testosterone levels are also costly, in terms of reduced immunocompetence, condition, and ability to control coccidia infection. Male red grouse therefore have to trade the benefits of elevated testosterone against the associated costs. Our experimental results, supported by correlative data, provide conclusive evidence to support the Immunocompetence Handicap Hypothesis (Folstad and Karter 1992). In male red grouse, the ability to mount an immune response is condition-dependent and testosterone-dependent, and it is signaled by comb size. Our findings suggest that males with the biggest combs are better able to cope with immunosuppressive testosterone and to control infection by certain parasites. Comb size is thus likely to reliably indicate the phenotypic and/or genetic quality of males, and females could benefit from choosing a mate based on this sexual trait.

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