Separating Behavioral and Physiological Mechanisms in Testosterone-Mediated Trade-Offs

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Abstract: Testosterone often mediates trade-offs between reproduction and other life-history traits, which are usually investigated using testosterone implants. However, this approach does not distinguish between the physiological and behavioral effects of testosterone. We studied a wild game bird, the red grouse Lagopus lagopus scoticus, and took a new approach to investigate mechanisms linking elevated testosterone to increased parasite intensity. We caught males in autumn, removed their parasites, implanted them with the antiandrogen flutamide in combination with an aromatase inhibitor (FA males) or with empty implants (control males), and challenged them with parasites. The FA treatment increased testosterone concentration and physiological stress, but without enhancing testosterone-dependent behaviors, because testosterone receptors were blocked. FA males ended up with more parasites than the control males the following autumn, an effect similar to that of a testosterone treatment reported elsewhere. However, and unlike the testosterone treatment, the FA treatment did not affect home range, pairing, or breeding success. The results supported a physiological mechanism (increased susceptibility) linking elevated testosterone and increased parasite intensity. The FA treatment provided a new way of investigating testosterone-mediated trade-offs whereby testosterone concentration was increased while the effects on behavior were blocked, resulting in physiological costs without phenotypic benefits.

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The steroid hormone testosterone plays a pivotal role in regulating the expression of territorial and sexual behaviors and secondary sexual traits of many species (e.g., Folstad and Karter 1992; Wingfield et al. 2001a). Males can benefit from elevated testosterone levels through increased aggressiveness, dominance status, or territory size and in terms of increased display rate or expression of sexual ornaments that attract females, facilitate mate choice, and increase mating success (e.g., Wingfield et al. 1987; Alatalo et al. 1996). However, having elevated testosterone is often costly, resulting in increased injury or predation risk, loss of condition, impaired immune function, or increased parasite intensity (e.g., Grosmann 1985; Folstad and Karter 1992; Hillgarth and Wingfield 1997; Wingfield et al. 2001b).

Studies investigating the costs and benefits of elevated testosterone have provided interesting insights into the role that this hormone might play in mediating trade-offs between reproduction and other life-history traits (e.g., Ketterson and Nolan 1992; Hillgarth and Wingfield 1997). One area of particular interest has been the role of testosterone in mediating trade-offs between sexual ornamentation, immune function, and parasites, which could ensure honesty of testosterone-dependent signals of individual quality, such as healthiness and parasite resistance (Folstad and Karter 1992; Casto et al. 2001; Getty 2002). Mounting an immune defense is usually costly (Sheldon and Verhulst 1996), so individuals may trade investment in parasite defense against investment in territorial or sexual activities or traits that require testosterone for their expression (Verhulst et al. 1999; Tella et al. 2002). Various studies have used testosterone implants to increase testosterone concentration and have reported an increase in parasite intensity (e.g., Saino et al. 1995; Hughes and Randolph 2001; Mougeot et al. 2004). A limitation to this approach, however, is that it is difficult to separate the physiological (increased susceptibility) from the behavioral
calls (Mougeot et al. 2005). The experiment was designed to investigate the effect of testosterone and aggressiveness on infection intensity by a nematode parasite, the caecal threadworm *Trichostrongylus tenuis*. Male grouse were either sham implanted as a control (C males) or implanted with flutamide and ATD (FA males) while controlling for exposure to *T. tenuis* parasites at the start of the experiment (parasites were removed, and birds were challenged with a standard dose of infective larvae). In a similar experiment, elevated testosterone caused by testosterone implants resulted in increased *T. tenuis* intensity, but it was unclear whether the effect was via increased exposure or susceptibility (Seivwright 2004; L. J. Seivwright, S. Redpath, F. Mougeot, F. Leckie, and P. J. Hudson, unpublished manuscript). If the mechanism through which testosterone mediates increased parasite intensity is mainly behavioral, the FA treatment should not increase parasite intensity because the testosterone receptors were blocked. In contrast, if the mechanism is mainly physiological (physiological effects of elevated testosterone resulting in increased susceptibility), then the FA treatment should result in increased parasite intensity, like a testosterone implant treatment. The latter also predicts physiological stress and reduced immunity associated with elevated testosterone, and we tested this possibility by comparing blood parameters (leukocyte counts, relative albumin, and globulin concentrations) in FA and C males. In red grouse, globulin concentration increases with *T. tenuis* intensity, and heterophile numbers increase in response to damage caused by establishing *T. tenuis* worms (Wilson and Wilson 1978; Wilson 1983). We also compared the survival, home range, pairing, and breeding success of FA and C males. Male red grouse implanted with testosterone typically benefit by having bigger sexual ornaments (combs), being more aggressive, holding larger territories, being more attractive to females, and achieving a better breeding success (Moss et al. 1979, 1994; Mougeot et al. 2004). Since there is a lack of phenotypic effects of the FA treatment on sexual ornament size and territorial behavior (Mougeot et al. 2005), we expected FA males not to benefit from their short-term elevation of testosterone levels and to have similar home range (territory size), pairing success, or breeding success to those of control males.

**Methods**

**Experimental Protocol**

We conducted the experiment on three sites in northeast Scotland during 2000–2001 (Edinglassie, Invermark, and Invercauld estates) and on three sites in Northern England during 2002–2003 (Feldom and Catterick in North Yorkshire and Moorhouse National Nature Reserve in Cumbria). In September–October, we caught 112 male grouse
on these sites by dazzling and netting them at night. Males were individually ringed, marked with wing tags, and fitted with a radio collar (TW3-necklace radio tags, Biotrack). At first capture, males were randomly assigned to one of two treatments: control (C males; \( n = 57 \); 28 males in 2000 and 29 males in 2002) or treated with flutamide and ATD (FA males; \( n = 55 \); 31 males in 2000 and 24 in 2002). All birds were implanted with two silastic tubes (each one 20 mm long, 0.62 mm inner and 0.95 mm outer diameter) sealed with glue at both ends. C males were given two empty implants and FA males one implant filled with flutamide (\( \alpha,\alpha,\alpha\)-trifluoro-2-methyl-4'-nitro-m-propionotoluidid) and another filled with ATD (1,4,6-androstatriene-3,17-dione; Sigma Aldrich, Poole, Dorset). Implants were inserted between skin and breast muscles on the flank under local anesthesia. We previously determined the length of the tubing during trials on captive grouse so that implants would last for a maximum of 3 months (S. M. Redpath and F. Mougeot, unpublished data). Examination of a sample of recaptured males confirmed that the implants were empty in the spring, 6 months after implanting. Before release, we took a blood sample, collected caecal droppings to estimate *Trichostrongylus tenuis* parasite intensity, and orally dosed all males with 1 mL of anthelmintic (Nilverm Gold) to remove their *T. tenuis* worms (Hudson 1986a).

We recaptured males about 1 month after implanting (in October–November), took another blood sample, and orally challenged them with approximately 3,000 *T. tenuis* parasite larvae that had been cultivated in the lab (see Shaw 1988a, 1988b for details on the larval culture and storage methods). We caught males again the following spring (March 19–April 30) and the next autumn (September 4–25), and we collected caecal droppings to estimate parasite intensity (Seivwright et al. 2004) before releasing them. Hereafter, we refer to “autumn \( t \)” as the capture events in the autumn of the first year of the experiment (when males were implanted and challenged with parasite larvae) and “spring \( t + 1 \)” and “autumn \( t + 1 \)” as the capture events in the spring and autumn of the following year.

**Study Parasite, Counts, and Culture for Challenges**

*Trichostrongylus tenuis* is a significant parasite of red grouse. This gut nematode has a direct life style and no alternative hosts within the same habitat. Eggs laid by adult worms are voided onto the moor in caecal droppings, where they develop into infective larvae and are ingested by grouse when feeding on heather *Calluna vulgaris* (Hudson 1986b). We estimated *T. tenuis* intensity using either caecal egg counts (from caecal samples collected from catured males) or direct worm counts (from guts of males that were shot by hunters on our study sites). Details regarding the parasite egg and worm counting methods are given in Moss et al. (1990) and Seivwright et al. (2004). Caecal egg counts provide reliable estimates of worm burdens and were used to calculate worm intensity (Seivwright et al. 2004). We used the number of worms per grouse as a measure of parasite intensity for all analyses. Males were sampled only once in each season. In spring, parasite intensity can rapidly increase (Moss et al. 1993; Hudson and Dobson 1997), so we tested for overall differences in parasite intensity between treatment groups (using all the males sampled in spring) and for treatment effect on changes in parasite intensity between early spring (males sampled in March) and late spring (males sampled in April).

We used the remains of fecal samples collected on first capture to cultivate *T. tenuis* parasite infective larvae for the subsequent challenges. In outline, fecal samples were washed thoroughly over a 125 \( \mu \)m sieve (Endecotts, Morden, London) to remove coarse fibrous material. The fecal residue containing the eggs was collected with a 25 \( \mu \)m sieve using distilled water, placed in petri dishes, and incubated at a constant temperature of 20°C. These fecal cultures were mixed and watered daily to maintain optimal moisture conditions for the hatching of *T. tenuis* eggs (Shaw 1988b). After 2 weeks, the fecal contents were filtered, and the larvae extracted and concentrated using a modified Baerman apparatus. Infective larvae were stored in distillate water for up to a week in a refrigerator at 4°C. On the day before the challenges, the solution containing the infective larvae was mixed, and larvae concentration was measured by counting live larvae in subsamples. Individual doses containing approximately 3,000 *T. tenuis* infective larvae were then extracted and stored until given orally to males. Further details on the methods for cultivating, storing, and counting *T. tenuis* larvae are given by Wilson and Wilson (1978), Shaw (1988b), and Seivwright (2004).

**Blood Sampling**

In autumn, before implanting and 1 month after implanting, we collected 0.5–1.5 mL of blood from a sample of males by taking a pinprick sample from the brachial vein. A drop of blood was smeared on a microscopic slide, air dried, fixed in absolute methanol, and later stained with Wright-Giemsa. Plasma samples were obtained by centrifuging blood for 10 min at 4,000 rpm and were stored at −80°C until analyzed. Samples collected in autumn 2001 in Scotland were used for testosterone assays, and those collected in 2002 in England were used for protein assays and leukocyte counts.
Testosterone Assays

Testosterone concentrations were measured using a direct double antibody radioimmunoassay. Duplicate 20 µL plasma samples were assayed. The standards, serially diluted in charcoal-stripped chicken serum, were assayed in triplicate. Both unknown samples and standards were heated to 80°C for 2 min to denature binding proteins. The primary antibody (8680-1419 Biogenesis, Poole) was used at a dilution of 1 : 3,500, and the tracer was [1,2,6,7-3H] testosterone (Amersham Pharmacia Biotech, Bucks). After 24-h incubation, the second antibody (donkey antirabbit) was added, and bound and free hormones were separated after a further 24 h by centrifugation at 5,000 g. The sensitivity of the assay was 0.06 ng mL⁻¹, with intra- and interassay coefficients of variance of 8.2% and 12.4%, respectively. Cross-reactivity with other steroids was less than 1%.

Plasma Protein Assays

Relative measures of gamma globulin and albumin concentrations were assayed by densitometric analysis following electrophoretic separation of plasma proteins on agarose gels (SAS-MX High Resolution Agarose Electrophoresis Kit; Helaina Biosystems Europe). One microliter of plasma was diluted 1 : 5 in Barbital buffer (pH 8.6), and then 2 µL of this diluted sample were applied to the agarose gel. A standard of diluted chicken serum (Sigma) was also applied in one lane of the gel. Electrophoreses were carried out at 250 V for 25 min. After electrophoresis, gels were stained, and densitometric analysis was performed using Gelworks 1D Advanced computer image analysis software. Relative albumin and gamma globulin concentrations (A : G ratio) were measured as the proportion of the densitometric profile they occupied. Plasma protein concentrations provide insights into individual physiological status and immunocompetence. Decreases in albumin concentrations are a common symptom of pathological state, and immunoglobulin levels typically increase in response to infections but decrease during stress and immunosuppression (Kawai 1973; Ots et al. 1998).

Heterophile and Lymphocyte Counts

We used blood smears for counting leukocyte types under a microscope ( × 40). A total of 30–50 white blood cells were identified to estimate the number of heterophile relative to that of lymphocyte (H : L ratio). This ratio is widely used as an index of physiological stress, with increases in H : L ratio being associated with increased stress and reduced immunity (Siegel 1985; Ots et al. 1998).

Survival, Home Range, and Pairing and Breeding Success

Males were radio-tracked and checked every 2 months to see if they were alive. We lost the radio signal for seven males (because either the radio failed or the birds emigrated or died away from the study sites) and so excluded them from analyses of survival probability.

On two sites in England (Catterick and Moorhouse), we radio-tracked males more often (about every month and weekly in spring) to compare the home range of FA and C males. Each time a male was found, we recorded its location to the nearest 5–7 m with a GPS (e-Trex personal navigator, Garmin). We obtained on average 10.4 (± 2.5) locations per male. The number of locations per male did not differ between treatment groups (Generalized Linear Models [GLM]: F = 0.36, df = 1, 30, P = .64) and did not affect home range estimates. We used the Home Range extension of ArcView 3.2 and Kernel contour ranges (using 90% or 70% of the locations) to estimate home range of experimental males. Kernel analyses are location density estimators or probability distributions of finding the animal (Kenward 2001). The information provided by this method is more biologically meaningful when evaluating frequency of occurrence of a bird in space. It also provides more reliable estimations of home range when using few locations, as Kernel contours typically require less than half as many locations to reach a maximum size (Kenward 2001).

In spring, we assessed the paired status of surviving males (paired vs. not paired) by checking them weekly during the day or at night (pairs stay and roost together before laying; Cramp and Simmons 1980) until laying started in late April. We caught the females in spring by locating males at night, lamping and netting their mate. All females were marked and dosed with 1 mL of anthelmintic (Nilverm Gold) to remove their T. tenuis worms. This allowed standardizing for this source of variation when comparing breeding success between treatment groups and for possible differences in exposure of experimental males due to possible differences in parasite intensity of their mate. Breeding success was measured as brood size at fledging in late July. We then located males and counted their young with the aid of trained pointer dogs (Jenkins et al. 1963; Hudson 1986b).

Statistical Analyses

We analyzed data using GLM (Genmod procedure) or Generalized Linear Mixed Models (Glimmix and Mixed procedures) implemented in SAS, version 8.01 (SAS 2001). All mixed models included site as a random effect to account for variation among moors. In the experiment, different sites were used in different years, and variation in
all study parameters between years was negligible compared with that between sites. Analyses controlling for year led to similar statistical results and identical conclusions, so we chose to present the results of the analyses without the year effect for simplification. When testing for an effect of treatment on changes over time (time × treatment interaction) in study parameters, models also included individual males as a random effect to account for repeated measures and variation within individuals. Testosterone concentrations and A : G ratios were log-transformed for normalization and fitted to models using a normal error distribution. To analyze variation in H : L ratios, counts of heterophiles were fitted with a Poisson error distribution with (the log of) counts of lymphocytes included as an offset in the models (SAS 2001). Trichostrongylus tenuis worm intensity data were overdispersed and fitted to models with a negative binomial error distribution. Home range estimates were log-transformed and fitted to models with a normal error distribution. Number of young (brood size) was fitted to models with a Poisson error distribution, survival probability, and pairing status (paired or not) with a binomial error distribution. All data are expressed as means ± SD, and all tests are two-tailed.

Results

Effect of Treatment on Testosterone Concentration and Blood Parameters

Temporal changes in testosterone concentration during autumn between first capture and recapture 1 month later differed significantly between treatment groups (mixed model with site and individual as random effects; time: $F = 40.6$, df = 1, 22, $P < .001$; treatment: $F = 22.5$, df = 1, 22, $P < .001$; time × treatment: $F = 29.6$, df = 1, 22, $P < .001$). Before treatment, testosterone concentration did not differ between treatment groups (mixed model: $F = 0.05$, df = 1, 37, $P = .95$). One month after implanting, FA males had about seven times more testosterone than in C males ($F = 23.67$, df = 1, 26, $P < .001$; fig. 1a).

We also found significant differences between treatment groups in changes over time in albumin to globulin (A : G) ratio (mixed model; time: $F = 6.4$, df = 1, 43, $P < .05$; treatment: $F = 4.1$, df = 1, 43, $P < .05$; time × treatment: $F = 4.2$, df = 1, 43, $P < .05$; fig. 1b) and heterophile to lymphocyte (H : L) ratio (mixed model; time: $F = 7.0$, df = 1, 48, $P < .05$; treatment: $F = 0.01$, df = 1, 48, $P = .87$; time × treatment: $F = 15.55$, df = 1, 48, $P < .001$; fig. 1c). The A : G ratios increased over time in both treatment groups, but the increase was proportionally greater in FA males than in C males (fig. 1b). In contrast, H : L ratios decreased from capture to recapture but decreased less in FA than in C males (fig. 1c).

Effect of Treatment on Parasite Intensity

In autumn $t$ (before implanting and parasite removal), parasite intensity did not differ between treatment groups ($F = 0.13$, df = 1, 101, $P = .71$; fig. 2a). Six months later, in spring $t + 1$, Trichostrongylus tenuis intensity did not

![Figure 1: Mean (±SE) plasma testosterone concentration (a; ng mL$^{-1}$), hetereophile to lymphocyte ratio (b), and albumin to globulin ratio (c) according to treatment (white bars = control, sham-implanted males; black bars = males implanted with flutamide and aromatase inhibitor) and time (autumn, before implanting and one month later). Sample size above bars refers to number of males.]
Figure 2: Differences between treatment groups in (a) parasite intensity (*Trichostrongylus tenuis* worms per grouse; geometric means/SE) and (b) survival (percentage alive) during the course of the experiment. Treatment: *white bars* = control, sham-implanted males; *black bars* = males implanted with flutamide and aromatase inhibitor. Sample size above bars refers to number of males. Males were dosed with anthelmintic (parasite removal) and implanted in the first autumn (autumn 1), challenged with approximately 3,000 *T. tenuis* larvae 1 month later, and resampled 6 months (spring 1) and 12 month later (autumn 1 + 1).

Differ between treatment groups (mixed model; \( F = 0.02, df = 1, 70, P = .87 \); geometric means of 330 worms, \( n = 37 \), and of 191 worms, \( n = 40 \), for C and FA males, respectively; fig. 2a). However, changes over time in parasite intensity differed between treatment groups. During spring, parasite intensity increased in FA males, but this pattern was not apparent in C males (mixed model; time: \( F = 1.25, df = 1, 68, P = .26 \); treatment: \( F = 6.05, df = 1, 68, P < .05 \); time x treatment: \( F = 5.95, df = 1, 68, P < .05 \)). The C males sampled in March had on average (geometric means) 530 worms (\( n = 16 \)), and FA males had 158 worms (\( n = 18 \)). The C males sampled in
April had on average 242 worms (n = 21), and FA males had 249 worms (n = 22).

In autumn t + 1, *T. tenuis* intensity differed between treatment groups (mixed model: F = 4.75, df = 1,42, P < .05). The FA males had almost twice as many worms as C males (geometric means of 1,977 and 1,087 for FA and C males, respectively; fig. 2a). Changes over time in worm intensity from spring t + 1 to autumn t + 1 differed between treatment groups, with a greater increase in FA than in C males (mixed model; time × treatment interaction: F = 6.41, df = 1,85, P < .05; fig. 2a). Variation in *T. tenuis* intensity in autumn t + 1 was also significantly explained by initial parasite intensity (parasite intensity before parasite removal and challenge) and by the interaction between initial parasite intensity and treatment (mixed model; treatment: F = 5.50, df = 1,36, P < .05; initial parasite intensity: F = 16.53, df = 1,36, P < .001; interaction: F = 3.66, df = 1,36, P = .06). Parasite intensity in autumn t + 1 positively correlated with that in autumn t, before any treatment (fig. 3). In FA males, however, final parasite intensities were higher than those of C males, and differences between treatment groups were greater for grouse with fewer parasites before the experiment (fig. 3).

**Effect of Treatment on Survival, Pairing Success, Breeding Success, and Home Range**

The percentage survival of males did not differ between treatment groups during the course of the experiment (mixed model with site as a random effect; autumn t, 1 month after implanting: F = 0.82, df = 1,103, P = .37; spring t + 1: F = 0.15, df = 1,102, P = .70; autumn t + 1: F = 0.32, df = 1,99, P = .57; fig. 2b). Among males alive in spring, pairing success was similar for C and FA males (64.1%, n = 40, and 64.9%, n = 37, of C and FA males paired and bred, respectively; mixed model: F = 0.00, df = 1,74, P = .95). Breeding success (number of young fledged per male in July) also did not differ between treatment groups (mixed model: F = 0.04, df = 1,59, P = .84; means ± SD of 2.15 ± 2.76, n = 32 and 2.02 ± 2.61 young per male, n = 34 for C and FA males, respectively).

Home range estimates (kernel contours using 90% or 70% of locations) did not significantly differ between FA and C males (mixed models; Kernel 90%: F = 0.56, df = 1,30, P = .46; Kernel 70%: F = 0.21, df = 1,30, P = .65). Kernel 70% home range averaged 30.31 ± 31.67 ha in C males (n = 19) and 25.49 ± 28.34 ha in FA males (n = 14). Parasite intensity at the end of the experiment was not related to home range (mixed model; Kernel 70%: F = 0.01, df = 1,17, P = .90; Kernel 90%: F = 0.00, df = 1,17, P = .97).

**Discussion**

Our initial hypothesis was that if the mechanism linking elevated testosterone to increased parasite intensity was via increased exposure (behavioral effects of testosterone), the FA treatment should not affect parasite intensity. Alternatively, if the mechanism was increased susceptibility (physiological effects of testosterone), then the FA treatment should cause increased parasite intensity. Our data supported the susceptibility mechanism. Treating male red grouse with flutamide and ATD in autumn caused a short-term increase in testosterone concentration and increased physiological stress when males were challenged with infective parasite larvae. The FA treatment also resulted in increased parasite intensity in the autumn of the following year. However, this treatment did not affect subsequent survival, pairing success, breeding success, or home range size. Below we discuss these findings and their implications for our understanding of the mechanisms involved in testosterone-mediated trade-offs, in particular those linking elevated testosterone and increased parasite intensity.

**Effect of Treatment on Parasite Intensity**

Figure 3: Relationship between initial parasite intensity (autumn t, before implant, parasite removal, and challenge) and parasite intensity in the next year (autumn t + 1) in males treated with flutamide and aromatase inhibitor (solid circles and solid line) and in control males (open circles and dashed line).
males had almost twice as many worms as the controls. This could be explained in terms of either increased host susceptibility or increased exposure of the grouse to infective stages.

The greater increase in parasite intensity observed in FA males than in C males might have been because FA males had a different behavior that exposed them to more infective larvae between spring and autumn. The FA males had a similar pairing success than did C males, and by dosing their females in spring (parasite removal), we standardized males for potential parasite transmission from their mate. Neither did FA males have bigger broods than C males, which could have contributed to increasing parasite transmission during summer. The FA males also had less testosterone than control males in spring (Mougeot et al. 2005) and were unlikely to have been more aggressive. The FA males also had a similar home range to that of C males throughout the course of the experiment, and final parasite intensity was not significantly related to home range, as expected if differences in autumn parasite intensity were mainly due to differences in exposure to infective larvae. We thus had no evidence suggesting that FA males behaved differently than C males when the implants were active or afterward (see also Mougeot et al. 2005) and in a way that would have increased their exposure to parasites.

In autumn, parasite transmission is reduced, and ingested larvae tend to arrest their development. In spring, when the weather conditions are favorable, these larvae de-arrest, and parasite intensity can increase rapidly as a result (Shaw 1988a; Moss et al. 1993; Hudson and Dobson 1997). An alternative explanation for the greater increase in parasite intensity observed in FA males might be that the treatment affected host susceptibility and the success of parasite challenge in the first autumn. As a result, FA males would have had more arrested larvae than C males, which developed into worms in the next spring. The re-emergence of arrested larvae accounts for the increased recruitment into the adult worm population usually observed in grouse during spring, but the timing of de-arrestment can differ between sites: February–March in a study by Hudson and Dobson (1997) and mid-April in another study by Moss et al. (1993). Therefore, we might not have detected differences in parasite intensity between treatment groups in spring because parasites were sampled before the peak in larvae de-arrestment. Parasite intensity increased in FA males during spring but not in C males, which was consistent with the idea that FA males might have had more de-arresting larvae than C males in spring.

When we challenged males with parasite larvae 1 month after implanting, FA males had about seven times more testosterone than C males. In autumn, testes are usually regressed, and endogenous testosterone is produced in the brain (Soma et al. 2000; Silverin et al. 2004). The observed increase in testosterone concentration was most likely a consequence of the FA treatment blocking negative feedback, thus causing elevated luteinizing hormone concentrations and increased endogenous testosterone production (Soma et al. 1999, 2000; Moore et al. 2004). Differences between treatment groups in H : L and A : G ratios at the time of the parasite challenge also supported the hypothesis that FA males were physiologically stressed and immunologically more susceptible. At the start of the experiment, males were dosed with an anthelmintic, which cleared them of their *Trichostrongylus tenuis* and other nematode parasites. In red grouse, globulin concentration increases with *T. tenuis* intensity, and heterophile numbers increase in response to bacterial invasion of the intestine after damage by establishing *T. tenuis* worms (Wilson and Wilson 1978; Wilson 1983). Thus, the overall decrease in H : L ratio and increase in A : G ratio observed in C males during autumn *t* (fig. 1) could be explained by the removal of parasites. One month after implanting, FA males had relatively higher A : G ratio and H : L ratios than C males. This is an indication that the FA treatment caused increased physiological stress and a relative decrease in gamma globulin concentration (Kawai 1973; Ots et al. 1998) and might have increased susceptibility to *T. tenuis* infective larvae.

In red grouse, there is little evidence of acquired immunity to *T. tenuis* infection (Shaw and Moss 1989; Hudson and Dobson 1997), but innate immunity is likely to be important. In captivity, grouse show a wide variation in innate susceptibility to the same dose of *T. tenuis* larvae, and in wild grouse, relative differences in parasite intensity among individuals within years tend to persist across years, so that relatively high or low parasite intensities appear to be characteristics of individual birds (Shaw and Moss 1989; Moss et al. 1993). We found that parasite intensity in autumn *t* + 1 was positively correlated with that in the previous autumn *t*, despite treatment with anthelmintic and reinfection with a constant number of infective larvae. However, FA-treated males ended with more parasites than would be expected from their initial parasite intensity when compared with control males. The relative differences between treatment groups between initial and final parasite intensity also tended to be greater for males with fewer worms at the start of the experiment (fig. 2). These findings are consistent with previous ones (Shaw and Moss 1989; Moss et al. 1993) and suggested that the treatment interacted with innate immunity and increased host susceptibility.

Side effects of the FA treatment, such as the elevated testosterone concentration at the time of the parasite challenge, might have interacted with immunity, for instance, by influencing complement production, cytokine produc-
tion, or simply the production of mucus, which is the main defense against nematode parasites such as *T. tenuis* (Watson et al. 1987; Onah and Nawa 2000). Recent evidence indicates that testosterone-induced immunosuppression does not occur through a direct pathway (Owen-Ashley et al. 2004). Some studies have suggested that immunosuppression might be caused by corticosterone, which increases as a side effect of elevated testosterone (Evans et al. 2000). Interestingly, other studies using FA treatment in birds have reported that this treatment caused not only an increase in testosterone concentration but also an increase in corticosterone concentration (e.g., Soma et al. 1999). It is thus possible that the FA treatment affected host immunity via effects on corticosterone. Another possible indirect pathway could involve the conversion of testosterone to estradiol by the enzyme aromatase (Owen-Ashley et al. 2004). In our experiment, the ATD should have prevented this conversion by blocking the enzyme aromatase activity, and the results would thus support a role for corticosterone. However, in red grouse, the phenotypic effects of the FA treatment (lack of significant reduction of aggressive behavior) might still be consistent with a role for estradiol in the regulation of territorial behavior (see Mougeot et al. 2005). The large increase in testosterone caused by the treatment could still have resulted in significant estradiol production (for instance, if the effects of ATD had run out before that of flutamide), and it would have been useful to measure estradiol concentration to be more conclusive. These possible roles for corticosterone or estradiol on host susceptibility could be further investigated in future studies.

**Effects of Combined Flutamide and ATD Treatment Compared to Those of Testosterone Treatment**

In a similar experiment, male red grouse were given testosterone implants in autumn, challenged with 3,000 *T. tenuis* larvae 1 month later, and their parasite intensity monitored subsequently, as we did in this study, until the next autumn (Seivwright 2004; L. J. Seivwright, S. Redpath, F. Mougeot, F. Leckie, and P. J. Hudson, unpublished manuscript). Testosterone implant caused an eightfold increase in testosterone concentration (Mougeot et al. 2005), and testosterone-implanted males had a testosterone concentration similar to that observed in FA males when they were challenged with parasite larvae. Testosterone-implanted males also ended with about twice the *T. tenuis* parasites than controls in the following autumn (Seivwright 2004; L. J. Seivwright, S. Redpath, F. Mougeot, F. Leckie, and P. J. Hudson, unpublished manuscript). Thus, the outcomes of the FA and testosterone treatments, in terms of their short-term effects on testosterone concentration and delayed effects on parasite intensity, were virtually identical. A main difference, however, was that, unlike in testosterone implanted males, the combined flutamide and ATD treatment increased testosterone concentration without enhancing aggressiveness or sexual ornament size during autumn (Mougeot et al. 2005) and without increasing home range. The comparison of both sets of experimental results reinforces the idea that the mechanism linking elevated testosterone and increased parasite intensity was most likely physiological (side effects associated with elevated testosterone or corticosterone that influenced host susceptibility and the effectiveness of the parasite challenge) rather than behavioral (increased exposure via increased aggressiveness and territory size or via interactions and contact with other infected individuals).

Pairing and breeding success did not differ between FA and C males. This is in contrast with males treated with testosterone, which had a better pairing success and achieved a better breeding success than controls, probably by attracting females of higher quality and in some cases by pairing with more than one female (Moss et al. 1994; Redpath et al., forthcoming). Male red grouse implanted with testosterone typically benefit by having bigger sexual ornaments, being more aggressive, and holding larger territories (Moss et al. 1979, 1994), which enhances pairing and breeding success. The lack of fitness benefits in FA males, in terms of pairing or breeding success, is consistent with the lack of effect of the FA treatment on sexual ornamentation or territorial behavior (Mougeot et al. 2005) and on home range or territory size. During the course of the experiment, we found that FA males survived as well as controls. This also contrasts with the effect of autumn testosterone implants, which tended to suffer increased overwinter mortality (14% higher than in controls), probably mainly due to increased raptor predation (Redpath et al., forthcoming). Increased predation by diurnal predators in testosterone-treated males is most likely a consequence of behavioral modifications caused by testosterone, such as increased territorial behavior and conspicuousness. If so, such effects should not be observed in FA males. Accordingly, we found that FA males survived as well as controls despite having more testosterone during autumn. Our results are consistent with a trade-off between testosterone and predation risk mediated via behavioral effects of testosterone.

In conclusion, the FA treatment provided interesting and valuable insights into the mechanisms mediating trade-offs between testosterone and parasites and between testosterone and predation in red grouse. The results support a physiological mechanism linking elevated testosterone concentration and increased parasite intensity and are consistent with a behavioral mechanism linking testosterone and predation risk. This treatment could provide
new ways of investigating testosterone-mediated trade-offs in other model species.

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Literature Cited


Mechanisms Linking Testosterone and Parasite Infection

The effects of testosterone on survival and productivity in red grouse Lagopus lagopus scoticus. Animal Behaviour.


SAS. 2001. SAS/STAT user’s guide, version 8.01. SAS Institute, Cary, NC.


