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Condition and androgen levels: are condition-dependent and testosterone-mediated traits two sides of the same coin?

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The immunocompetence handicap hypothesis posits that androgen-mediated signals honestly indicate the responsiveness of the immune system because of the immunosuppressive effect of androgens. However, androgen levels may also be related to nutritional status, and differences in body condition could be a more parsimonious explanation for variation in the expression of the signal. We maintained captive male red-legged partridges, *Alectoris rufa*, under regulated food shortage until they reached 85% of their initial body mass. Controls were provided with food ad libitum. After food shortage, experimental birds had lower androgen and higher corticosterone levels than controls. The condition dependence in androgen levels suggests that androgen-mediated signals could be indicating general nutritional state rather than immune function specifically. We propose that androgen-dependent signals may act as indicators of body condition or indicators of immune system quality, depending on the nutritional status of the individual.

Males of many animal species have elaborate morphological traits or behaviours (e.g. long feathers, large antlers, bright coloration, complex songs) that are considered to be a consequence of sexual selection (Andersson 1994). These special structures and displays may evolve because of intrasexual competition and mate choice. An increasing number of theoretical and empirical studies have focused on the role and origin of these traits. Many of them support the idea that secondary sex characters and courtship displays have evolved as information cues about the bearer's quality (reviewed in Andersson 1994) that can be evaluated by choosy females or opponents.

Of special interest is the question of what maintains the correlation between the expression of the trait or the level of display and the quality of the individual; in other words, what maintains the honesty of the signal? The handicap principle (Zahavi 1975) proposes that such traits and behaviours are costly to produce, and a similar level of expression imposes larger costs to low- than to high-quality individuals. For example, secondary sex characters are expressed more extravagantly in individuals in good body condition, which pay relatively less than low-quality ones for the same size trait, thus ensuring the reliability of the signal system. Such condition dependence in the expression of sexually selected traits and behaviours has been found in a variety of species (e.g. Zeh & Zeh 1988; Vehrencamp et al. 1989; Veiga & Puerta 1996).

A particular case of the handicap principle is the immunocompetence handicap hypothesis (ICHH, Folstad & Karter 1992), which proposes that the expression of many secondary sexual traits and behaviours in males is positively related to testosterone levels, which in turn negatively affect the immune system. Thus, the honesty of the signal is maintained by the 'double-edged sword' effect of testosterone: higher androgen levels enhance trait expression but depress immune defences, so only individuals with an immune system of high quality will be able to maintain elevated hormonal levels without compromising their own health.

The ICHH is based on three main assumptions: first, testosterone has a deleterious effect on the immune system; second, males with more elaborate traits or displays (or more aggressive behaviour in male–male contests) have higher mating success; and third, the degree

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of development of these secondary sexual traits, the display intensity or aggressiveness are correlated with testosterone levels. A considerable number of studies have contributed results compatible with, or supporting, the first, second and third assumptions, although it seems that empirical support for the ICHH is still weak (reviewed in Roberts et al. 2004).

However, body condition may be important for testosterone production (Wilson et al. 1979; Wingfield 1987; Duckworth et al. 2001) and it is possible that variation in body condition is the ultimate factor causing differences in the expression of the trait. Folstad & Karter (1992) suggested that androgen-dependent signal intensity is a plastic response, so it should be closely related to the current physiological state of the animal (e.g. level of parasite infection, health, nutritional status, that is, body condition, Brown 1996) which may influence the trade-off between androgen production and self-maintenance. Immune response may be affected by body condition as well (Lochmiller & Deerenberg 2000; Alonso-Álvarez & Tella 2001). Thus, if testosterone is immunosuppressive, individuals in poor body condition should have lower levels of testosterone to minimize the risks of disease or infection. In addition to immunological costs, testosterone-dependent behaviours may be costly in terms of energy (Vehrencamp et al. 1989; Marler & Ryan 1996), which reinforces the possible importance of body condition for testosterone production, as only individuals in good body condition could face such energetic costs.

Although in many cases variation in body condition may be the most parsimonious explanation for variation in the expression of both secondary sexual traits and testosterone levels, many correlational studies do not control for this variable and focus only on the relation between testosterone and trait expression (e.g. Saino & Møller 1994; Rintamäki et al. 2000; González et al. 2001). Furthermore, this hypothetical relation between androgen production and nutritional status may be mediated by other components of the endocrine system, such as circulating levels of corticosterone (Wingfield et al. 1990; Wingfield & Ramenofsky 1999). A negative interaction between corticosterone and androgens may be the physiological link between body condition and androgen-dependent trait expression or behavioural performance. According to this hypothesis, energetic stress will elevate circulating corticosterone, which, in turn, may reach a threshold level that inhibits androgen production (Wingfield & Kitaysky 2002) and impair the immune system (Møller 1995; Buchanan 2000).

In this study we experimentally assessed the causal relation between body condition and androgen production. We also analysed variation in corticosterone levels associated with food shortage to investigate the possible role of stress hormones as a mediator in this relation. Our study species was the red-legged partridge, *Alectoris rufa*. Among male galliforms, androgens are involved in the development of many secondary sex traits and behaviours important in mate choice and male–male interactions (red junglefowl, *Gallus gallus*: Ligon et al. 1990; Zuk et al. 1990; ring-necked pheasant, *Phasianus colchicus*:

Mateos & Carranza 1997; Papeschi et al. 2003; quail, *Coturnix japonica*: Hagelin & Ligon 2001; black grouse, *Tetrao tetrix*: Alatalo et al. 1996; red grouse, *Lagopus lagopus scoticus*: Mougeot et al. 2005; grey partridge, *Perdix perdix*: Fusani et al. 1994, 1997). During courtship and fighting, male red-legged partridges show intense vocal activity a behaviour known to be a sexually selected trait affected by testosterone levels in birds (e.g. Van Duyse et al. 2002) including galliforms (Fusani et al. 1994, 1997; Mougeot et al. 2005). Male red-legged partridges are also highly territorial during the breeding season and are frequently aggressive to intruding males (Cramp & Simmons 1980); this terrritorial behaviour is also influenced by androgen levels (Ramenofsky 1984; Wingfield et al. 1987).

METHODS

We did the experiment in the 'Dehesa de Galiana' experimental facilities of the Instituto de Investigación de Recursos Cinegéticos, Ciudad Real, central Spain, during March–April 2003, when male red-legged partridges are reaching their period of maximal testosterone production (Bottoni et al. 1993). The partridges used in the experiment were hatched in the previous year at our facility. They were kept from December to February in a communal pen of 50 males (8×10 m) and fed with a mixture of commercial pelleted food (20% protein, 4.5% fat, 3.7% cellulose) and wheat. In February, 39 males were individually housed in cages (1×0.5 m and 0.4 m high). The cages were visually isolated from each other, at ambient temperature and natural photoperiod, and received sunlight through the top. Shelter was provided both in the outdoor pen and in the individual cages. During individual isolation, caged birds were fed only the pelleted food mentioned above and water was provided ad libitum. At this time, individuals were randomly assigned either to an experimentally food-restricted group ($N = 20$) or to a control group fed ad libitum ($N = 19$). The daily food intake of each partridge from the food-restricted group was calculated before the experiment during the first 2 weeks of March. We considered average daily consumption during those 15 days to be the daily food intake of each individual. Just before the experiment started, and also upon completion, we weighed each bird with a Pesola spring balance (± 5 g) and measured the pectoral muscle thickness with an ultrasonic meter (USM22B, Krautkramer, Huerth, Germany). The latter measure has been used in several bird studies and is considered to be a more reliable estimate of body condition than residuals of body mass on body size (Sears 1988). Before the experiment we also collected 400 µl of blood from the brachial vein in a heparinized syringe. To avoid androgen and corticosterone levels being confounded by the stress of capture and sampling (Wingfield et al. 1997), we took blood samples within 3 min of catching the bird, and at approximately the same time of day (between 1000 and 1200 hours) to control for possible diel effects. Samples were kept cold and centrifuged within 8 h, and plasma was stored at $-20°$C until analysis. Left tarsus length and width and head width were also measured (± 0.01 mm).

Each bird from the food-restricted group was provided with 70% of its daily food intake during the first week and with 50% during the rest of the experiment. We weighed birds every other day during the first 2 weeks of the experiment, and daily afterwards. Food restriction was maintained until 1 day after each experimental bird had lost 15% of its initial body mass. When a bird of the food-restricted group was sampled again after the treatment, a randomly selected bird from the control group was also sampled, avoiding differences in sampling date between groups.

The food restriction period ranged from 15 to 34 days. None of the variables tested (initial and final body mass, pectoral muscle thickness, percentage of weight lost, initial and final levels of testosterone or corticosterone) was correlated with the length of the food restriction period. Furthermore, as sampling of both experimental and control birds was paired (see above), any potential effect of this source of variability was statistically controlled in the between-groups comparisons.

In some cases we could not obtain enough plasma for the analysis of both androgens and corticosterone, so sample sizes vary between analyses. Parametric statistics were used for all variables except for testosterone levels because they were not normally distributed even after transformation. Paired tests were used to analyse differences before and after treatments within groups. All tests are two tailed.

Plasma Hormone Assays

We measured plasma androgen and corticosterone levels by radioimmunoassay (RIA), described fully elsewhere (Bortolotti et al. 1996; Wayland et al. 2002). To eliminate possible interference from plasma steroid-binding proteins, we used reconstituted organic diethyl ether extracts of the plasma samples; extraction efficiency was consistently greater than 95% for both assays. In addition to cross-reacting with testosterone (100%), the antiserum used in the androgen RIA also shows a significant (23%) cross-reaction with 5α-dihydrotestosterone; therefore, these RIA results are best represented as androgen levels rather than testosterone (Bortolotti et al. 1996). In both the androgen and corticosterone RIAs, serial dilutions of red-legged partridge plasma extracts produced displacement curves parallel to the standard curves. Samples were analysed in duplicate in two separate androgen assays (intra- and interassay coefficients of variation of 3.5% and 8.0%, respectively) and one corticosterone assay (intra-assay coefficient of variation of 8.0%). The minimum detectable plasma androgen level was 0.084 ng/ml. Androgen values in these samples ranged from the midpoint of the standard curve (50% relative binding = 0.33 ng/ml) to below the minimum detection limit for the RIA. For statistical purposes, we assigned an androgen value of 0.084 ng/ml to those samples measuring below the detection limit (Bortolotti et al. 1996). The minimum detection limit of the corticosterone RIA was 0.16 ng/ml. All samples had corticosterone levels well above this value, and were diluted so as to be measured

near the midpoint (50% relative binding = 0.60 ng/ml) of the corticosterone standard curve.

Ethical Note

To minimize unnecessary suffering or pain to the birds we prioritized ethical considerations over scientific goals. All birds came from a captive population. They were individually isolated because we needed to control individual food intake and avoid social interactions that may alter circulating androgen levels which would mask the effect of the physiological factors investigated. Although the birds lost weight, their final body condition was not excessively poor, and was similar to that of poor-condition birds found on the farm (L. Pérez-Rodríguez, personal observations) or in the wild (F. Buenestado, personal communication). After the experiment, all birds were fed ad libitum and returned to the original communal pens when they recovered their initial weights. None of the birds showed any symptoms of disease during the subsequent 2-month monitoring period. The study was approved by the Agencia Nacional de Evaluación y prospectiva.

RESULTS

Control and experimental birds did not differ in any of the structural variables measured: tarsus length (t test: $t_{37} = 0.47$, $P = 0.87$), tarsus width ($t_{37} = 1.36$, $P = 0.17$) and head width ($t_{37} = 0.48$, $P = 0.62$). Before the experimental food restriction, there were no differences between groups in body weight ($t_{37} = 0.60$, $P = 0.54$) or pectoral muscle thickness ($t_{37} = 0.78$, $P = 0.43$), and both corticosterone and androgen levels were also similar between groups (corticosterone: t test: $t_{36} = 0.63$, $P = 0.30$; androgens: Mann–Whitney U test: $Z = 0.91$, $N_1 = 18$, $N_2 = 19$, $P = 0.36$).

After the food restriction period, we found a significant effect of the treatment on body weight ($t_{37} = 11.0$, $P < 0.001$, Fig. 1) and pectoral muscle thickness ($t_{37} = 6.10$, $P < 0.001$), showing that our experiment was effective in creating differences in body condition. Food-restricted
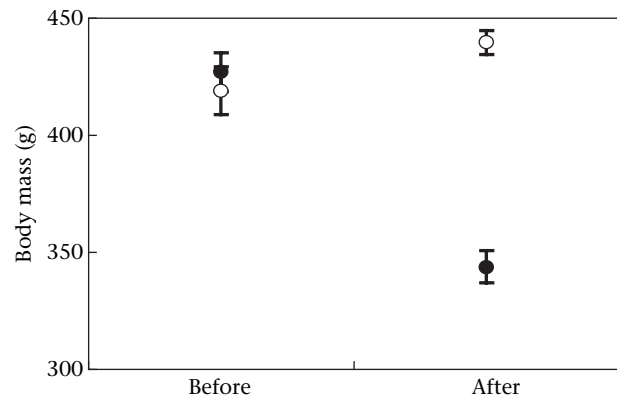

Figure 1. Mean ± SE body mass of control (○) and experimental (●) birds before and after food restriction.

birds lost a mean of 19.5% of their initial body mass because we maintained the restriction until 1 day after each bird reached a loss of 15% (see Methods). In the food-restricted group, both body mass and pectoral muscle thickness decreased (paired t tests: body mass: $t_{19} = 24.8$, $P < 0.001$; pectoral muscle: $t_{19} = 5.18$, $P < 0.001$), whereas these two variables increased significantly in the control group (body mass: $t_{18} = 2.47$, $P < 0.05$; pectoral muscle: $t_{18} = 5.26$, $P < 0.001$), perhaps because of the move from open pens to smaller cages where movements, and hence activity, were limited. Androgen levels differed between groups only after the food restriction (Mann–Whitney U test: $Z = 3.38$, $N_1 = 19$, $N_2 = 15$, $P < 0.001$; Fig. 2a). During the experiment, androgen concentrations increased significantly in the control group (Wilcoxon matched-pairs test: $Z = 2.48$, $N = 18$, $P < 0.05$) and were correlated with the number of days elapsed between samples before and after food restriction (Spearman correlation: $r_S = 0.47$, $N = 19$, $P < 0.05$). In contrast, androgen levels decreased in the food-restricted group ($Z = 2.36$, $N = 14$, $P < 0.05$), and all birds had androgen concentrations below the detection limit of our assays (0.084 ng/ml) at the end of the experiment. Corticosterone levels showed significant differences between groups after food restriction (t test: $t_{32} = 3.37$, $P < 0.01$; Fig. 2b), with food-restricted birds having higher hormone levels. The experimental group showed a significant increase in plasma corticosterone between the two samples (paired t test: $t_{18} = 2.34$, $P < 0.05$), whereas it
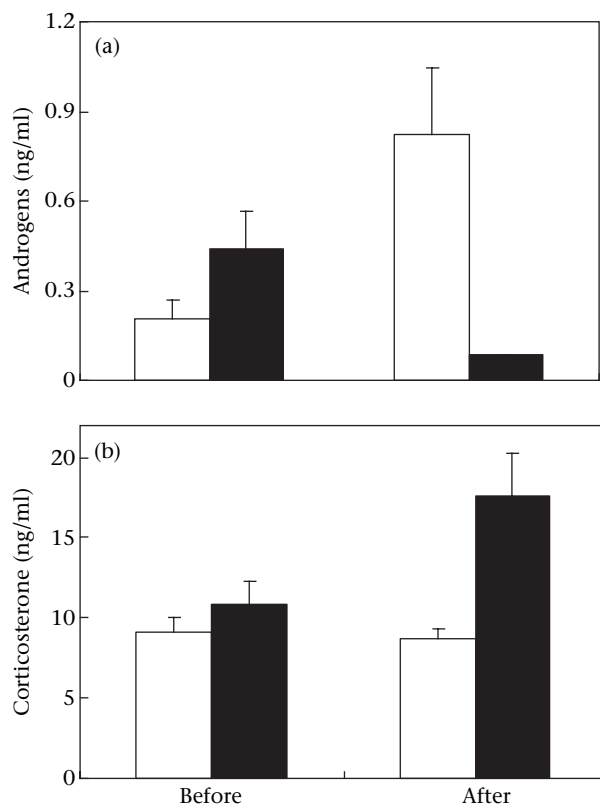
remained unchanged in the control group ($t_{18} = 0.24$, $P = 0.81$). Corticosterone levels after food restriction were not related to the length of the experiment, either in the experimental (Pearson correlation: $r_{13} = -0.26$, $P = 0.36$) or in the control ($r_{17} = 0.14$, $P = 0.46$) group.

DISCUSSION

In this study we have experimentally documented condition dependence of plasma androgen levels. Circulating androgens increased during the experiment in the control group fed ad libitum, following the expected pattern for seasonally breeding birds (Bottoni et al. 1993). In contrast, food-restricted birds suffered a significant decline in plasma androgens, parallel to experimentally induced body weight changes. To our knowledge, this test and associated results are novel within the literature of experimental manipulations of avian body condition, although a broad body of evidence from observational studies suggests the ubiquity of this relation in birds (e.g. Wingfield 1987; Duckworth et al. 2001; Chastel et al. 2005). Our treatment also affected the expression of eye lore coloration (L. Pérez-Rodríguez & J. Viñeula, unpublished data), a sexually dimorphic trait that seems to be involved in sexual selection processes in this species (Villafuerte & Negro 1998).

In contrast to this and the above-cited studies supporting the condition dependence of androgen levels, some other correlational studies have failed to find a relation between testosterone and body condition (e.g. Weatherhead et al. 1993; Foerster et al. 2002). There may be several explanations for these different results. For example, in the case of small passerines (e.g. Foerster et al. 2002) body condition is difficult to estimate from body mass scores, because diel changes in mass can be large (Peters 2000). Furthermore, condition dependence in androgen levels may exist below a threshold value of condition, and observational studies would reveal it only when the sampled variance comprises individuals below that threshold.

Despite the relevance of a condition–androgen link, the implications for honest signalling in the light of the handicap principle (Zahavi 1975), and more explicitly the ICHH (Folstad & Karter 1992), have received little attention, which contrasts with the popularity of studies relating androgen production to the development of sexual signals. The existence of a condition–androgen link suggests that energetic factors may ultimately constrain testosterone-mediated sexual selection, making natural variation in body condition a more basic explanation for individual differences in the development of sexually selected traits.

From a mechanistic point of view, this perspective implies that either (1) body condition affects androgen levels, which in turn determines trait expression, or (2) body condition independently affects both trait expression and androgens, the latter two being spuriously (i.e. noncausally) correlated. Both alternatives are consistent with a number of published studies. With regard to the first, body condition in male red junglefowl affects androgen levels (Ligon et al. 1990), which in turn regulate comb size

Figure 2. Mean + SE (a) plasma androgen and (b) corticosterone levels before and after food restriction. □: Control; ■: experimental group.

and colour, aggressiveness and courtship behaviour, all traits involved in sexual selection (Ligon et al. 1990; Zuk et al. 1990). Another example is male tungara frogs, *Physalaemus pustulosus*, which respond to supplemental food by increasing circulating testosterone and the likelihood of calling, a sexually selected behaviour (Marler & Ryan 1996). Body condition may also be affected by the increase in sexual behaviour (Vehrencamp et al. 1989; Marler & Ryan 1996) and basal metabolic rate (Buchanan et al. 2001) produced by androgens, so the direction may not be one-way. The second mechanistic path (i.e. a noncausal link between androgens and sexual traits) may be illustrated by feather development in barn swallows, *Hirundo rustica*. The elongated outermost tail feather is a sexually selected trait, and is correlated with testosterone levels at breeding (Saino & Møller 1994). However, these feathers grow several months in advance, when androgen production is normally basal among birds (Hillgarth & Wingfield 1997). Although the proximate control of testosterone on plumage trait expression has been demonstrated in some species (Evans et al. 2000; Peters et al. 2000) it does not seem to be a general rule (Kimball & Ligon 1999). In fact, Saino & Møller (1994) acknowledged that a third factor (i.e. body condition) could be related to both androgen production and feather development and cause a spurious result. Other studies have shown that stress during feather development predicts fitness and overall body condition during the rest of the year (Bortolotti et al. 2002). Accordingly, if birds in poor body condition at moult are also in poor condition at breeding, testosterone during the mating season may correlate spuriously with ornament expression as developed earlier.

Rather than questioning the validity of the ICHH, we suggest that body condition may be the ultimate factor regulating the development of sexually selected traits. The ICHH relies on the immunosuppressive effects of testosterone, which induces the trade-off between trait expression and resistance to disease and parasites. In fact, Folstad & Karter (1992) pointed out the importance of the individual's physiological state in the trade-off between testosterone-dependent trait expression and health. However, they focused primarily on parasite infection rather than body condition per se, although the hypothetical role of parasites may be mediated by their effect on body condition (Gulland 1995; Hudson & Dobson 1995). Body condition, however, affects the immune response (Alonso-Álvarez & Tella 2001 and references therein) because immune defences seem to be costly to maintain and activate (Lochmiller & Deerenberg 2000). In addition, the negative effect of poor body condition on androgen production may be a physiological way of protecting the immune system, which is potentially suppressed by the associated chronic corticosterone titres (Dhabhar & McEwen 1997; Sapolsky et al. 2000). In our study, corticosterone levels were also affected by food shortage, with food-restricted birds having higher plasma concentrations than controls only after food deprivation.

Another mainstay of the ICHH is to explain phenotypic variation by linking this to genetic variation that is generated by a rapidly changing selection pressure exerted by parasites via the handicap principle. As a result, individuals in a population can never converge on the optimal phenotype and variability is maintained, making possible the evolutionary persistence of the system. However, a condition-dependent signalling system would also be evolutionarily stable. Resources available for trait investment in each individual are the result of so many variables (endogenous and exogenous) and interactions between them that it seems difficult to reach a state of absence of variability. Genetic variability in immune traits is just one of these influencing variables and, other things being equal, such genetic variability could lead to differences between individuals in foraging abilities (e.g. because of the effect of illness) and be a source of variation in condition. Furthermore, environmental influences on condition (and further trait expression) are likely to be strong relative to the underlying genetic variation (Kurtz & Sauer 1999).

A nonlinear relation between body condition and immune response may reconcile the condition versus androgen constraints on trait development via immunosuppression. Alonso-Álvarez & Tella (2001) found a threshold value of condition above which improvements do not enhance immune response. A similar threshold relation may exist between condition and testosterone production (see above). Thus, it is possible that the immunosuppression handicap acts in the upper range of values of body condition, where immune function is determined mainly by the genetic quality of the individual rather than by its nutritional status. Only when individuals are free of the constraints imposed by nutritional status would androgen-mediated traits start working as real immunosuppressive handicaps signalling heritable quality of the immune system, as proposed by Folstad & Karter (1992).

When trying to test the ICHH in natural populations, researchers usually do not find any association between parasite loads and testosterone levels (Weatherhead et al. 1993; Saino & Møller 1994) or may even find a negative one (Peters 2000). However, rather than an inconsistency, this is exactly what the hypothesis predicts, as individuals are expected to self-regulate their hormonal levels according to their current health, the environment and their nutritional status. Experimental designs in which testosterone levels are modified regardless of differences in body condition seem to be the best way to test the ICHH without any potentially confounding factor.

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