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Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling?

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Sexually selected signals of individual dominance have profound effects on access to resources, mate choice and gene flow. However, why such signals should honestly reflect individual quality is poorly understood. Many such signals are known to develop under the influence of testosterone. We conducted an experiment in male house sparrows in which testosterone was manipulated independently during two periods: before the onset of the breeding season and prior to the autumn moult. We then measured the effects of these manipulations on basal metabolic rate and on the size of the chest bib, a sexually selected signal. The results demonstrate that testosterone simultaneously affects both signal development and basal metabolic rate in the house sparrow (*Passer domesticus*). This evidence, therefore, supports a novel conclusion: that testosterone-dependent signals act as honest indicators of male quality possibly because only high-quality individuals can sustain the energetic costs associated with signal development.

Keywords: testosterone; signalling; energetics; *Passer domesticus*

1. INTRODUCTION

'Badges of status' are found in a variety of species and allow dominant individuals access to resources whilst minimizing their aggressive interactions with subordinate individuals (Rohwer 1975; Dawkins & Krebs 1978; Rohwer & Rohwer 1978). Current signalling theory predicts that signals indicating individual quality (Andersson 1994) must be costly, in order to conserve the honesty of the signalling system and prevent poor-quality individuals from cheating (Pomiankowski *et al.* 1991; Zahavi & Zahavi 1997). To date, studies have suggested that badges of status remain honest through frequent testing of the signalling system (Møller 1987*a*; Johnstone & Norris 1993).

The chest bib of the house sparrow (*Passer domesticus*) determines territory acquisition and signals dominance (Møller 1987*a*,*b*, 1988). Larger-bibbed males have preferential access to resources, excluding smaller-bibbed males without the need for continued antagonistic interactions (Møller 1987*b*). Furthermore, bib size appears to be an age-related and a condition-dependent trait (Veiga 1993; Veiga & Puerta 1996). Previous work has found that changes in bib size during the autumn moult are affected by circulating levels of testosterone (Evans *et al.* 2000). This is also thought to have consequences for immune function, as the hormones associated with badge production can be immunosuppressive and could, therefore, render larger-badged males more susceptible to disease or pathogens (Gonzalez *et al.* 1999; Evans *et al.* 2000). It has recently been suggested that immunosuppression occurs in response to competition for limited energetic resources (Svensson *et al.* 1998), indicating that testosterone levels may have implications not only for immune function but also for resource allocation and energetic demands. As dominant individuals have often been found to have higher metabolic rates (Senar *et al.* 2000), signalling could have an associated energetic cost. If the elevations in testosterone levels necessary for the production of a large badge are also energetically costly then this could explain how such plumage signals act as honest indicators of male fitness. We therefore tested the hypothesis that high circulating testosterone levels incur an energetic cost by increasing metabolic rate.

One problem with examining the cost of testosterone-controlled signalling is that the timing of the cost is unclear. Testosterone levels peak during the breeding season (2–5.8 ng ml^{-1}) (Hegner & Wingfield 1986) but adult house sparrows moult and acquire their badges about six months later, by which time levels are much lower (0.1–0.3 ng ml^{-1}) (Hegner & Wingfield 1986; Evans *et al.* 2000). Hence, the importance of testosterone in controlling male plumage signals has been questioned (Owens & Short 1995). To complement our study of metabolism, therefore, we addressed a second hypothesis: variations in male testosterone levels during the autumn, when levels of the hormone are low, affect male plumage development directly in the subsequent moult.

2. METHODS

(a) *Experimental design*

In February and March of 1998 ($n = 32$) and 1999 ($n = 64$) wild-caught male sparrows were randomly assigned to one of four groups: 'high testosterone', 'low testosterone' and 'castrated' groups, which were all castrated, and an 'intact' group, which was sham operated and so continued to produce natural levels of testosterone throughout the season. The high-testosterone group received subcutaneous testosterone implants designed to provide circulating levels of testosterone that mimicked the upper naturally occurring level during the breeding season (Evans

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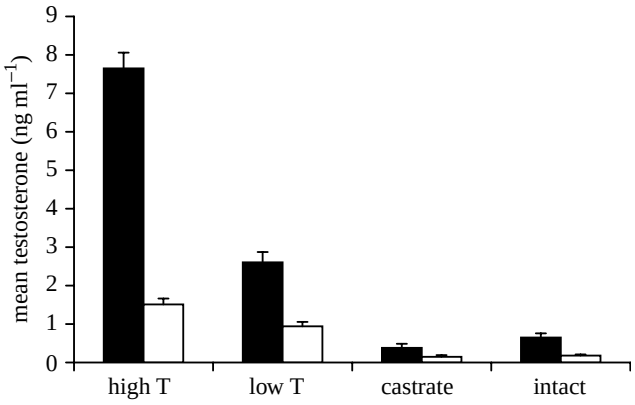

Figure 1. The mean + s.e.m. testosterone titres of the experimental groups. Filled bars represent breeding-season values and open bars represent post-breeding-season values.

et al. 2000). The low-testosterone group received smaller implants designed to mimic the lower range of naturally occurring levels of testosterone. Both the 'castrated' and the 'intact' males received empty implants. The birds were also allocated to one of two dietary treatments, with the high-quality-diet group receiving seed mixture (Haith's Wild Bird Seed, Cleethorpes, UK) and meal-worms *ad libitum*, whilst the low-quality-diet group also received seed mixture *ad libitum* but received meal-worms only once per week. Birds were housed in groups of four (one from each treatment group) per cage and remained under the same dietary treatment throughout the experiment.

In addition, in order to test the relative importance of testosterone on male bib size during the breeding and post-breeding periods, males from the high-testosterone, low-testosterone and castrated groups were randomly re-assigned to these groups in June. The re-assignment was conducted in such a way that birds in any breeding-season experimental group could be allocated to any of the post-breeding-season groups. The intact birds remained in the same group throughout the year. In June, during the re-assignment, the breeding-season implants were removed and replaced with implants producing testosterone at levels mimicking the natural variation in post-breeding production of testosterone (figure 1). The castrated group again received an empty implant. The intact birds were given new empty implants, to control for the stress of the implant change, but remained in the intact group throughout the season. Breeding levels of testosterone were calculated as the mean values of blood samples taken in March and May, whilst post-breeding values were calculated as the mean values of blood samples taken in August and October. Intact birds were blood sampled at the same time. Over the season there were, therefore, nine testosterone-manipulation groups: high to high ($n=4$), high to low ($n=7$), high to castrated ($n=8$), low to high ($n=8$), low to low ($n=7$), low to castrated ($n=5$), castrated to high ($n=9$), castrated to low ($n=8$) and castrated to castrated ($n=8$). A small number of birds died between the measures of energy metabolism and moult completion and as a result the sample sizes for the bib size analysis are slightly reduced.

The dominance hierarchy of the birds in each cage was assessed through observations during both the breeding and the post-breeding periods. Each cage was observed for a 30 min observation period (mean, four observations), during which time any agonistic interactions were noted. A dominance score was constructed by noting the success or failure of these interactions, and where the rank was unclear a split rank was awarded.

(b) *Hormone assays*

For the hormone assays, 100 µl of blood were collected through a puncture of the brachial vein, centrifuged and the plasma stored at −20 °C until assay. The total androgen concentrations were measured in the plasma samples by direct radioimmunoassay (Parkinson & Follett 1995) using anti-testosterone antiserum (code 8680-6004, Biogenesis, Bournemouth, UK) and [^{125}I]-testosterone label (code 07-189126, ICN, Basingstoke, UK). The antiserum not only detects testosterone but also cross-reacts with other androgens present in the blood. However, this cross-reactivity is low and therefore this assay represents a reliable surrogate measure of absolute testosterone levels. The assay was run with 50% binding at 2.8 ng ml^{-1} and a detection limit of 0.35 ng ml^{-1}. After confirming that house sparrow plasma diluted parallel to the standard curve, experimental samples were measured in either 10 µl or 5 µl duplicate volumes. The interassay coefficient of variation was 15.5%. Blood samples were also taken once during each of the experimental periods for determination of corticosterone production (Parkinson & Follett 1995). We took 100 µl of blood at 0 min, 10 min and 30 min after capture to test for corticosterone production during the stress response to restraint (Wingfield *et al*. 1992). Corticosterone concentrations were measured after extraction of 20 µl aliquots of plasma in diethyl ether, by radioimmunoassay (Wingfield *et al*. 1992) using anti-corticosterone antiserum (code B21-42, Endocrine Sciences, Tarzana, CA) and [1,2,6,7-3H]-corticosterone label (Amersham, Little Chalfont, UK). The extraction efficiency of the assay was 80–90%. The assay was run with 50% binding at 3.3 nmol l^{-1} and the detection limit (for 7.3 µl aliquots of extracted plasma) was 4 nmol l^{-1}. The interassay coefficient of variation was 9.2%.

(c) *Bib size*

House sparrows moult into their winter plumage after the breeding season, around September (Summers-Smith 1988). The effect of the testosterone manipulations on bib moult during this time was assessed by measuring bib size both immediately before the onset of moult and after the completion of moult. Bib area was measured by tracing the outline onto an acetate sheet. The mass of the resulting area was converted into an area by reference to an area of known size and mass. Our calculations indicated that this technique had a repeatability of 87% ($F_{81,108}=16.58$) (Lessells & Boag 1987).

(d) *Energetics*

The energy metabolism of house sparrows was investigated under basal conditions (Kleiber 1961). Accordingly, we refer to our measurements of energy metabolism as basal metabolic rates (BMRs), although comparable measurements in other studies have been called resting metabolic rates. The effects of testosterone manipulations on metabolism were measured before the onset of moult (during July and August) using open flow respirometry (Bryant & Furness 1995). Sparrows were placed individually in 4.4 l respirometry chambers overnight, with a minimum of 4 h between the last opportunity to eat and the first measurements being taken, including at least 3 h within the chamber. We assume that all birds would have been in a post-absorptive state, although food held in the crop at the time of capture might, in some cases, have allowed residual food processing to continue into the sampling period. However, since measurements of three or four birds were made each night, with treatment groups being selected for measurement in a random order, our results are unlikely to be confounded by variation in

Table 1. *The effect of post-breeding testosterone manipulations on basal metabolic rate for the high-testosterone, low-testosterone and castrated groups (first column) and for the intact group (second column)*

	high-testosterone, low-testosterone and castrated groups ($n = 64$)	intact group ($n = 18$)
breeding-season experimental group	$F_{2,53} = 0.01$	—
post-breeding-season experimental group	$F_{2,53} = 3.34^{*}$	—
mean breeding testosterone level		$F_{1,9} = 2.47$
mean post-breeding testosterone level	$F_{1,53} = 6.92^{*}$	$F_{1,9} = 3.95$
breeding basal corticosterone level		$F_{1,9} = 6.63^{*}$
post-breeding basal corticosterone level		$F_{1,9} = 2.46$
post-breeding peak corticosterone level	$F_{1,53} = 1.14$	
mean post-breeding testosterone level × post-breeding peak corticosterone level	$F_{1,53} = 3.82$	
year	$F_{1,53} = 23.06^{**}$	$F_{1,9} = 0.13$
diet	$F_{1,53} = 3.17$	$F_{1,9} = 2.16$
year × mean breeding testosterone level		$F_{1,9} = 6.12^{*}$
year × mean post-breeding testosterone level		$F_{1,9} = 6.19^{*}$

$^{*}p < 0.05$; $^{**}p < 0.01$.

absorptive state. Equally, systematic overnight variation, which typically involves a metabolic 'low point' in the middle of the night, would be unlikely to bias our results. Both these assumptions are consistent with our observation that measurement order had no effect on BMR. The metabolism chambers were maintained in darkness at 25 °C (i.e. within the thermoneutral zone; Kendeigh *et al.* 1977). Air (CO_2 free) was drawn through the chambers at 0.5 l min^{-1} and dried and analysed for oxygen and carbon dioxide concentrations using a VG quadrupole mass spectrometer (VG Quadrupoles, Manchester, UK). Sampling periods of 1–3 h were used for each bird to derive BMRs, during which time data were logged at 5 min intervals, while gas concentrations remained steady, indicating a constant rate of gas exchange. Gas volumes were converted to energetic equivalents using the Brouwer equation (Brouwer 1957). Subsequently, energy expenditure was expressed in units of kJ d^{-1}. The activity of one of the birds sampled each night was monitored using a Doppler radar device (MacLeod & Jewitt 1985). This confirmed that the birds were invariably in a quiescent state overnight.

(e) *Stepwise analysis*

The results were analysed using MINITAB version 10.5 for the Macintosh (Minitab Inc., State College, PA, USA). The effects of the manipulations were analysed in two parts by constructing general linear model nested ANOVA with, first, BMR, and second, change in bib size as the dependent variable. In each case, breeding and post-breeding experimental groups were entered into the model as categorical independent variables and the mean breeding and post-breeding testosterone titres were treated as continuous independent variables. Pre-moult bib size, mass, wing length, year, dominance score, dietary group, and basal and peak corticosterone production were entered as continuous independent variables, as well as a variety of interactions. Separate models were constructed to determine the effects within, first, the high-testosterone, low-testosterone and castrated groups, and second, the intact birds (the intact birds remained in the same group throughout the experiment and therefore could not be analysed with the same model as the birds that changed manipulation groups during the experiment). As testosterone levels in the high-testosterone, low-testosterone and castrated groups were manipulated independently during the breeding and post-breeding periods, these results are considerably more powerful than those from the intact group for interpreting the effects of the seasonal variations in testosterone levels on both BMR and bib moult. The ANOVA models were reduced to their simplest forms by eliminating any variables that failed to explain significant variation in the dependent variable. This was conducted in a stepwise manner with the factors explaining least variation being removed until all the remaining factors explained significant variation (Zar 1984). The model residuals were checked for normality and homoscedasticity at each step. The bib-change data were square-root transformed in order to achieve normality of the residuals.

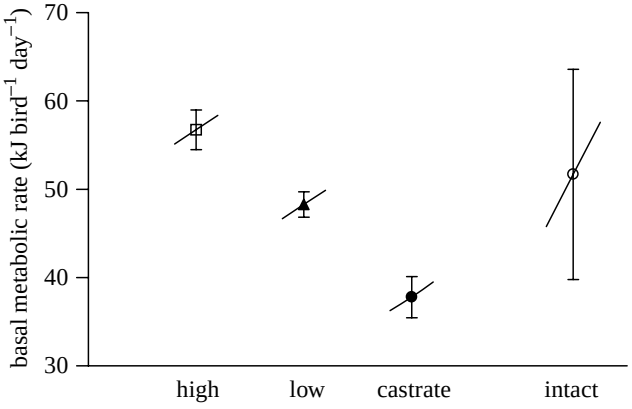

Figure 2. The mean ± s.e.m. basal metabolic rates (kJ per bird per day) for birds in the post-breeding and intact groups. The diagonal lines indicate the directions and strengths of the significant relationship between testosterone and basal metabolic rate in the castrated, low-testosterone and high-testosterone groups ($F_{1,53} = 6.92$, $p = 0.011$) and the marginally non-significant positive relationship in the intact group ($F_{1,9} = 3.95$, $p = 0.078$).

(f) *Simple analysis*

To confirm the results of the stepwise reduction models, a simpler model was constructed containing only factors believed to be essential prior to analysis, to test the effect of the experimental treatments on BMR and bib size. The factors included for the experimental groups were: breeding and post-breeding experimental groups; year; pre-moult bib size; mean breeding and

Table 2. *The effect of post-breeding testosterone manipulations on the change in bib size during the autumn moult for the high-testosterone, low-testosterone and castrated groups (first column) and for the intact group (second column)*

	high-testosterone, low-testosterone and castrated groups ($n=38$)	intact group ($n=13$)
breeding-season experimental group	$F_{2,28}=2.04$	—
post-breeding-season experimental group	$F_{2,28}=0.5$	—
mean breeding testosterone level		$F_{1,3}=31.81^{*}$
mean post-breeding testosterone level	$F_{1,28}=11.10^{**}$	$F_{1,3}=335.49^{**}$
diet	$F_{1,28}=0.21$	$F_{1,3}=18.69^{*}$
pre-moult bib size	$F_{1,28}=16.26^{**}$	$F_{1,3}=41.27^{**}$
mean post-breeding testosterone level × post-breeding experimental group	$F_{2,28}=4.69^{*}$	
mean breeding testosterone level × diet		$F_{1,3}=10.55^{*}$
wing		$F_{1,3}=62.71^{**}$
mass		$F_{1,3}=36.04^{**}$
mass × pre-moult bib size		$F_{1,3}=49.28^{**}$
year		$F_{1,3}=26.38^{*}$

$^{*}p<0.05$; $^{**}p<0.01$.

post-breeding testosterone titres; dominance score; dietary group; an interaction between breeding-season group and breeding-season testosterone titre; and an interaction between post-breeding-season group and post-breeding-season testosterone titre. For the simple analyses for the intact groups the factors included were: year; pre-moult bib size; mean breeding and post-breeding testosterone titres; dominance score; and dietary group.

3. RESULTS

(a) *Basal metabolic rate*

The final stepwise elimination model explaining individual variation in BMR contained significant effects of post-breeding experimental group, year, mean post-breeding testosterone level and an interaction ($p=0.056$) between post-breeding testosterone level and post-breeding-peak-corticosterone level (table 1). The interaction suggests that while both testosterone and peak corticosterone production have significant positive effects on BMR, at high levels of corticosterone production the relationship between testosterone and BMR becomes less positive. The breeding-period experimental group and the dietary group were constrained into the analysis but neither significantly affected BMR; removal of these factors did not affect the significance of the other variables. The final model confirms that testosterone had a significant positive effect on BMR, both between and within experimental groups (figure 2). There was no detectable effect of body mass or condition on BMR: mass, wing length and an interaction between the two were all found to be non-significant (mass, $F_{1,47}=0.13$, $p=0.745$; wing length, $F_{1,47}=0.11$, $p=0.721$; mass wing length, $F_{1,47}=0.08$, $p=0.772$). However, there was a significant positive effect of post-breeding testosterone titre on individual mass ($F_{1,52}=5.38$, $p=0.024$) in a model controlling for non-significant effects of breeding-period experimental group, post-breeding-period experimental group, breeding-season testosterone titre, dietary treatment and wing length.

The intact group was analysed separately and there were significant interactions of both breeding season and post-breeding-season-testosterone levels with year (table 1). The effect of post-breeding testosterone level alone was also positive, but marginally non-significant ($F_{1,9}=3.95$, $p=0.078$). The positive relationship between testosterone levels and BMR, both within and between groups, provides support for our first hypothesis that testosterone, directly or indirectly, causes an increase in energetic demands.

Within the simplified model determining the effects of the experimental manipulations on BMR, the only significant factors were post-breeding testosterone level ($F_{1,44}=4.55$, $p=0.039$) and year ($F_{1,44}=12.19$, $p=0.001$), confirming the positive effect of testosterone on BMR. The simplified model analysing the effect of testosterone within the intact group showed no significant effects of any of the model variables.

The activity levels monitored during the respirometry recordings did not register any locomotor activity (i.e. movements other than breathing activity), nor did activity registrations differ significantly between the post-breeding manipulation groups ($F_{3,30}=2.22$, $p=0.106$) or with the post-breeding level of testosterone ($F_{1,29}=0.09$, $p=0.77$). As the birds were held at a chamber temperature within the thermoneutral zone and in a post-absorptive state, and were, therefore, without a heat increment of feeding, this indicates that the observed metabolic responses indeed related to a basal state and were unaffected by movements or behavioural differences within the chambers.

(b) *Bib size*

Analysis of the change in bib size during the autumn moult revealed that the significant factors affecting the plumage signal were original bib size, post-breeding testosterone level, and an interaction between post-breeding experimental group and post-breeding testosterone level (table 2). As in the BMR analysis, breeding-period experimental group and dietary group were constrained into the model but neither factor significantly affected the change in bib size; removal of these factors did not affect the significance of the other variables. In the intact group, the final model indicated significant effects of breeding and post-breeding testosterone levels,

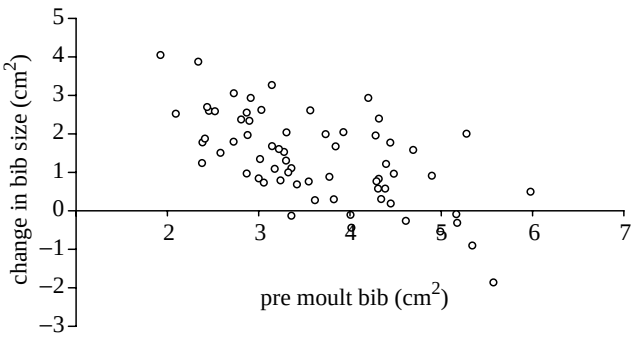

Figure 3. The relationship between the change in bib size during the autumn moult (cm^2) and the pre-moult bib size (cm^2).

pre-moult bib size, diet, wing length, mass, year, and interactions between diet and breeding testosterone level and between pre-moult bib size and mass (table 2).

In the simplified model determining the effects of the experimental manipulations on bib size the only significant factors were pre-moult bib size ($F_{1,23}=18.22$, $p=0.00$), post-breeding testosterone level ($F_{1,23}=7.98$, $p=0.01$) and an interaction between post-breeding experimental group and post-breeding testosterone level ($F_{2,23}=4.62$, $p=0.021$). In the intact group, the simplified analysis confirmed significant effects of breeding ($F_{1,7}=21.80$, $p=0.003$) and post-breeding ($F_{1,7}=10.10$, $p=0.019$) testosterone levels and pre-moult bib size ($F_{1,7}=19.40$, $p=0.005$).

The factors determining the change in bib size were, therefore, original bib size and circulating testosterone levels during the moult. Smaller-bibbed males showed substantially larger absolute increases in bib size over the autumn moult (figure 3), whilst males with higher circulating testosterone levels also showed larger increases in bib area during this time (figure 4). This was also demonstrated by a positive relationship between the change in bib size during the moult and testosterone level within each of the experimental groups. This result provides support for our second hypothesis: that although testosterone levels are relatively low during the autumn, variation in testosterone production at this time can affect the size of the badge produced during the moult.

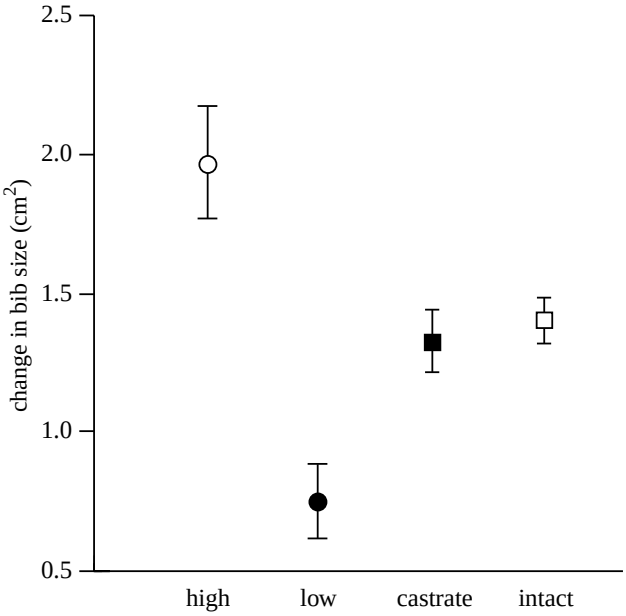

Figure 4. The mean ± s.e.m. change in bib size (cm^2) during the autumn moult for each of the post-breeding experimental groups and the intact birds.

4. DISCUSSION

Many sexual behaviours and sexually dimorphic traits are known to develop under the influence of testosterone, and several potential associated costs have previously been suggested (Andersson 1994). Testosterone has been hypothesized to decrease developmental growth (Ros 1999), suppress immune function (Folstad & Karter 1992) and increase stress levels (Braude *et al.* 1999) and the risk of mortality (Marler & Moore 1988) or injury (Wingfield *et al.* 1990), as well as being associated with sexual behaviours that involve significant energetic investment (Vehrencamp *et al.* 1989).

Our results are consistent with a number of possible hypotheses involving the direct or indirect effects of testosterone on energetic costs. The results show that, both within and between experimental groups, increases in testosterone levels were associated with increases in BMR. Furthermore, as the measurements were made at rest, in isolation, and were assumed to reflect a post-absorptive state, neither testosterone-induced variation in locomotor behaviour nor testosterone-induced differences in feeding intensity, nor its effects on the heat increment of feeding, could be directly responsible for these differences. The results are therefore unlikely to be a product of behavioural differences between the groups, and demonstrate that elevated levels of testosterone within naturally occurring ranges, directly or indirectly, cause an increase in BMR. Changes in both body temperature and BMR have been previously reported in Japanese quail (*Coturnix coturnix japonica*) in relation to testosterone production (Hänssler & Prinzinger 1979). Furthermore, it has recently been demonstrated that testosterone directly affects muscle metabolism *in vitro* (Tsai & Sapolsky 1996), suggesting that relatively small changes in circulating testosterone levels *in vivo* may have important consequences for metabolic processes.

In contrast, Wikelski *et al.* (1999) found that increases in the testosterone levels of white-crowned sparrows (*Zonotrichia leucophrys gambelii*) were associated with decreases in resting metabolic rate, measured, as for our study, during the overnight period. Wikelski *et al.* (1999) suggested that as males with increased testosterone levels were found to increase their daytime activity (measured as perch hopping) it was likely that these males compensated by reducing their resting metabolic rate at night (Deerenberg *et al.* 1998). In contrast, the birds in our study with the highest breeding-season levels of testosterone were found to have the lowest levels of daytime activity, measured as flights across the cage ($F_{1,54}=10.72$, $p=0.002$; Buchanan *et al.* 2001). These differences in activity may, in part, be due to the housing conditions; the birds in our study were housed in groups, where the dominance hierarchies were maintained throughout the

measurement period, whereas the birds in the study by Wikelski *et al.* (1999) were housed singly. We suggest, therefore, that our results may contrast with those of Wikelski *et al.* (1999) as a consequence of differences in daytime activity patterns, due ultimately to differences in housing conditions.

In support of previous results (Evans *et al.* 2000), we have demonstrated that bib size development during the autumn moult is influenced by circulating levels of testosterone. Furthermore, we have shown that the subtle differences in testosterone levels during the post-breeding period are more important in determining bib size than levels during the breeding period, which are many times greater. Of crucial importance is the finding that the energetic cost coincides with the time in the season when the bib is under control from testosterone production, reinforcing the conclusion that increases in bib size are associated with energetic costs. It is worth noting, however, that in the unmanipulated intact birds, and probably, therefore, in free-living birds, levels of testosterone in the spring were positively correlated with levels in the autumn ($F_{1,17}=4.88$, $p=0.041$), suggesting that some cost may also be incurred during the breeding season.

Our results demonstrate that experimental increases in testosterone levels were associated with increases in BMR, suggesting a causal relationship. Whether the effect was direct or indirect, however, is not clear. We found no effect of mass or body size, nor, by inference, of body condition, on BMR. Yet we did show that testosterone positively affected body mass. This would be consistent with testosterone simultaneously affecting both energy expenditure and mass, but where the effect on metabolism is overriding and masks the residual effect of mass or size. It is possible that testosterone induces other physiological changes, such as immunosuppression (Evans *et al.* 2000), biochemical changes at the cellular level, fat storage, muscle mass growth, growth of other lean tissues or cellular repair, which contribute to increased energetic costs. As stated above, because the measures of BMR were made at rest and in a post-absorptive state, the observed differences are more likely to be due to differences in physiology than behaviour. But it is also possible that testosterone causes behavioural changes (Hunt *et al.* 1999; Lynn *et al.* 2000), which could, in turn, affect energy expenditure in the wild via an effect on costly behaviours that were rare or absent among our captives.

The fitness consequences of a raised BMR are not always clear, particularly as they might apply to birds in the wild. Under thermoneutral conditions, an elevation in BMR can be viewed as an energetic cost. This is because our results show that, in house sparrows, night-time costs related to testosterone are additive. Clearly, overnight survival may be impaired as a result, especially when food is scarce, fat reserves are low or other adverse factors prevail. Wherever other normally additive energy costs occur below the thermoneutral zone, however, be they thermostatic or related to activity and other factors, the possibility of concurrent metabolic compensation arises, with any additional heat output from an elevated BMR potentially subsidizing thermoregulatory expenditure. In these circumstances, an energetic cost due to testosterone may not be detectable unless compensation does not occur (Schuchmann 1979; Poppitt *et al.* 1994). Similarly, where compensation is sequential, so that daytime reductions offset night-time rises, there may be no increase in daily energy expenditure. Nevertheless, in both cases metabolic compensation normally involves a trade-off of metabolic components, and so under these conditions it is reasonable to infer a consequent loss of fitness due to the reduction of investment in a metabolic component. A possible exception is when the trade-off simply involves a reduction in thermoregulatory demands that matches the elevation in BMR. Even so, since BMR is considered to be a minimum level of metabolism, when there is pressure for energy economies related to activity or some other functions, an elevated BMR due to testosterone could limit overall energy savings, even where some level of compensation is achieved. Overall, therefore, we suggest that while a rise in BMR could sometimes be neutral in its effect on fitness, there are sound reasons to suppose that it will often imply a fitness cost.

Dietary treatment was not found to affect either BMR or bib production in the experimentally manipulated groups. But the power of the test examining the importance of these dietary manipulations for BMR was extremely low (less than 10%) and the minimum discriminable difference was 3.21 kJ per bird per day (the mean BMR of all birds was 42.78 kJ per bird per day). However, the power for testing the effect of dietary treatment on bib development (Cohen 1988) was more than 95%, so we can confirm that there was no influence of dietary manipulation on bib size development. In contrast, in the intact group, males fed the high-quality diet produced significantly larger increases in bib size than did males fed the low-quality diet, suggesting that diet may well be important when males are able to differentially allocate the resources available to them and modify their own energetic expenditure. This result is in line with the recent finding that environmental variation, including the quality of parental care, is more important for initial bib development in fledgling sparrows than genetic control of bib size (Griffith *et al.* 1999).

Our study demonstrates, we believe for the first time, that sexual signals subject to endocrine control can have associated energetic costs. Our study does not demonstrate that the increases in BMR associated with increases in bib size represent a biologically meaningful energetic cost, but it does raise the possibility that such a cost exists. Such energetic costs suggest an alternative route by which sexually selected signals of male quality can act as honest indicators. If signals require testosterone for their production, and testosterone causes an increase in BMR that is biologically meaningful in terms of energetic expenditure, then only high-quality males may be able to bear the cost of producing an elaborate display or ornament. The energetic resources required to produce testosterone-controlled sexual traits could, therefore, reinforce the honesty of the signal, and explain why such plumage traits can act as honest indicators of male quality.

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