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Ectoparasites of house sparrows (*Passer domesticus*): an experimental test of the immunocompetence handicap hypothesis and a new model

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Abstract Conspicuous secondary sexual traits may have evolved as handicap-revealing signals or as badges of status. We present results of an experiment using males of the sexually dimorphic house sparrow (*Passer domesticus*), that support the idea that the male-specific bib can be both a handicap-revealing signal and a reliable badge indicating the physical condition of the bird. In a test of the immunocompetence handicap hypothesis, wild-caught adult male house sparrows were studied in captivity. Birds implanted with elevated doses of testosterone were more dominant, had higher circulating levels of both testosterone and corticosterone and they also harboured relatively larger ectoparasite loads. Higher parasite loads were also associated with individuals showing lower immunocompetence and larger changes in bib size. A new model for immunocompetence effects in sexual selection is introduced, integrating actions that the hypothalamopituitary axis exerts on gonads, adrenals and the thyroid gland. The "integrated immunocompetence model" synthesizes both the "handicap" (i.e. survival-decreasing) and "badge of status" (i.e. survival-enhancing) models for evolution of secondary sexual traits.

Key words Ectoparasites · House sparrows · Sexual selection · Immunocompetence · Steroid hormones

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Introduction

The theory of parasite-mediated sexual selection has undergone considerable development since Hamilton and Zuk (1982) published their influential work (Andersson 1994). The idea that host-parasite cycles can maintain sufficient genetic variability on which sexual selection may act has inspired numerous empirical and theoretical studies (reviewed in Clayton 1991a; Zuk 1992; Andersson 1994; Johnstone 1995). A recent meta-analysis by Hamilton and Poulin (1997) supports the Hamilton-Zuk hypothesis, but only for passerine-endoparasite systems. Other works also provide support for the Hamilton-Zuk hypothesis, with both ectoparasite and endoparasite loads correlating with condition of secondary sexual traits in several sexually dimorphic avian species (e.g. Møller 1991; Pruett-Jones et al. 1991; Saino and Møller 1994; Buchholz 1995).

Many bird species develop conspicuous sex-specific secondary sexual traits (e.g. plumage, wattles, combs, spurs). These structures are believed to act as "status-signalling" devices (Rohwer 1975, 1977) or "badges of status" (Dawkins and Krebs 1978) in the contexts of intra-sexual competition and mate choice. Badges of status are expected to be honest signals of the quality of the transmitter as a potential sexual partner (Zahavi 1975) or adversary (Rohwer 1975, 1977). Cheaters can usually be controlled through the intrinsic cost of the signal (Zahavi 1975) or additional strategies aimed at testing the honesty of the signals (e.g. aggressiveness: Møller 1988; Owens and Hartley 1991). But even when signals are not costly, or cheating does not involve a direct cost to the cheater, honest signalling may be favoured due to costs paid by the offspring: e.g. offspring of low-quality partners may be more subject to diseases and therefore less likely to survive (Wedekind 1994).

In a recent important development, Folstad and Karter (1992) have proposed a specific handicap mechanism ensuring the honesty of secondary sexual traits as signals in the context of sexual selection. They suggested that testosterone (T) promoting the development of second-

ary sexual traits in males (or females in those species with sex role reversal: Höhn 1970), also has immunosuppressive effects. Thus, males displaying highly conspicuous ornaments are potentially more prone to parasitic infections, and only males which are capable of either preventing or overcoming the infection would be able to sustain such an elevated level of immunosuppression, thus establishing a direct test of the honesty of their signals as high-quality males. Females would then be able to choose the male signalling at the highest level without fear of pairing with a low-quality cheat.

Although the immunocompetence handicap hypothesis (ICHH; Folstad and Karter 1992) has been criticized (Owens and Short 1995), especially on the grounds that the expression of conspicuous plumage in males is not always controlled by T, there is empirical support for some of its predictions (Weatherhead et al. 1993; Dufva and Allander 1995; Zuk et al. 1995; Saino and Møller 1996; Salvador et al. 1996; Skarstein and Folstad 1996; see Hillgarth and Wingfield 1997a, 1997b for recent reviews). Other research, however, does not support the hypothesis (Saino and Møller 1994; Møller et al. 1996; Saino et al. 1997a; Klein and Nelson 1998; see also Hews and Moore 1997 for general criticisms). Møller (1995) suggested that the ICHH should probably be modified, with corticosterone (B) rather than T being the main immunosuppressive steroid associated with increased ectoparasitism in males (see also Saino and Møller 1994; Hillgarth and Wingfield 1997b).

House sparrows (*Passer domesticus*) are sexually dimorphic passerines, the males having a conspicuous patch of black feathers (bib) on the throat and breast, which they use in intra-sexual competition as a badge of dominance status (Møller 1987b, 1987c, 1988; Veiga 1993, 1996; Solberg and Ringsby 1997) and in communal and individual displays directed to females (Møller 1987a).

In a previous test of the ICHH in house sparrows, Møller et al. (1996) found a negative correlation between bib size and volume of the bursa of Fabricius, the avian organ where B-lymphocyte maturation occurs. They also found a positive association between intensity of infection by ischnoceran feather louse species and relative volume of the bursa, but no correlation between the relative volume of the bursa and relative testis volume. Based on the assumption that testis volume is positively correlated with circulating levels of T, Møller et al. (1996) rejected the ICHH for house sparrows. Unfortunately, a positive correlation between testis size and circulating levels of T cannot be assumed (e.g. Wingfield 1984a; Poiani and Fletcher 1994).

Evans et al. (2000) also tested the ICHH in house sparrows and found a positive correlation between T and B in plasma of males. Although B had an immunosuppressive effect, T had a facilitating effect on the immune system. The latter result was explained by Evans et al. (2000) through the effect T may have on dominance and therefore selective access to food: dominant individuals with high T levels may be better able to maintain an effective immune system due to their preferential access to food. Birds with high circulating levels of T also tended to moult into larger bibs.

There is thus evidence both for and against the ICHH in the house sparrow. The aim of this work was to expand on previous studies carried out on house sparrows to explore the relationships between T, B, behavioural variables (dominance, grooming) and ectoparasites. In particular, the following predictions of the ICHH were tested:

(a) Plasma levels of T and B are positively correlated with ectoparasite loads after controlling for the effect of grooming.
(b) High dominance status is associated with larger ectoparasite loads.
(c) Larger ectoparasite loads are associated with a less competent immune system and larger bib size.

Methods

The male house sparrows used in this study were a subset of the birds used in Evans et al.'s (2000) work. Wild adult birds were mist-netted in March 1996 in farms in the Stirling area (Scotland, 56°07' N, 3°57' W). A total of 32 birds were initially trapped and enclosed in outdoor aviaries (n=8 cages), four birds per cage (2.5×1×2 m height). Whenever possible, birds originating from the same farm were housed separately to minimize possible effects of previous acquaintance. Food in the form of mixed seeds and water were provided ad libitum. Two nest boxes and several perches in the form of tree branches were also provided in each cage. The initial number of birds decreased throughout the experiment (from March to July) as some individuals died (n=5 at different stages, and n=3 immediately after castration).

Behavioural observations

Dominance hierarchies

Observations were carried out ad libitum (sensu Altmann 1974) from a hide for variable periods of time. Birds were observed at different stages of variable duration during the experiment in the following sequence: stage I (10 days duration) before implanting them with T-filled silastic tubes (40 min per cage: 200 min in the afternoon and 120 min in the morning taking all eight cages together); stage II (88 days) after birds had been implanted (120 min per cage: 600 min in the afternoon and 360 min in the morning taking all cages together); stage III (12 days) after birds had been deparasitized (60 min per cage: 300 min in the afternoon and 180 min in the morning taking all cages together); stage IV (13 days) after birds had been experimentally infested with haematophagous mites (120 min per cage: 480 min in the afternoon and 480 min in the morning taking all cages together). Dominance hierarchies within each cage were determined on the basis of frequency and direction of aggressive behaviours (e.g. pecking) over perching sites. A previous study suggests that dominance hierarchies do not differ over perching site or food in house sparrows (Solberg and Ringsby 1997). Birds were given a score of 1 (most dominant) to 3 (least dominant), because a few cages had lost one bird by the time of the observation and because dominance positions three and four were not always easily distinguishable.

Grooming

We also recorded total time spent grooming for each bird. We follow Clayton (1991b) in defining avian grooming as preening (using the bill) plus scratching (using feet).

Ectoparasites

Nest boxes were clean at the start of the experiment. Nest boxes were sealed (i.e. no bird could enter) in the last third of stage II, and remained so until the end of this experiment, to prevent dominant birds from using them exclusively (Veiga 1993; Gwinner and Gwinner 1994). In this way, nest-box-related ectoparasite infestation (Møller and Erritzøe 1996) and temperature stress (Barnard et al. 1996) were homogeneous among birds during the period of observation, blood sampling and experimental infestation with mites (see below). Nest boxes were not sealed earlier in order to give the birds the chance to withstand the cold temperatures between March and May, especially at night (aviaries were set outdoors). We feared that a prolonged hypothermic stress might have unduly jeopardized birds' survival.

Dust-ruffling

All birds were deparasitized, in the afternoon, at the end of stages II and IV using a dust-ruffling method (Clayton and Walther 1997). About 0.4 g of Kil-Pest powder (0.1% pyrethrins and 0.8% piperonyl butoxide) were delivered in puffs under the wings, tail and on the crown. The powder was subsequently distributed homogeneously all over the body for about 1 min. Although these pesticides are not harmful to vertebrates, we took care not to contact the eyes of the birds. The bird was then left resting in a bag for 10 min to allow the pesticide to produce its effect. Feathers were then ruffled homogeneously all over the body and ectoparasites dropping off the birds were collected in a tray underneath. Ruffling extended for a minimum of 4 min per bird or until no further parasites dropped off for 1 min after the minimum period. The maximum total ruffling period was 6 min. Ectoparasites were also collected from both the bird bag and the resting bag. The bird bag was checked a final time upon release of the bird onto the aviary to estimate efficiency of the removal method.

Experimental mites

Ectoparasites were not sampled from the birds before the beginning of stage II (i.e. before application of T implants), in order to avoid depletion of ectoparasite populations in the birds. Although we have no reason to believe that numbers of ectoparasites in the treatment groups (see below) were not homogeneous before T implantation, we experimentally infested birds with haematophagous mites to determine whether the treatment effect mirrored the patterns found for natural ectoparasites.

Haematophagous mites of the genus *Dermanyssus* (Acarina: Dermanyssidae) are common ectoparasites of house sparrows (McGroarty and Dobson 1974; Brown and Wilson 1975). Individual *Dermanyssus gallinae* were obtained from barn swallow (*Hirundo rustica*) nests. Mites were taken to the laboratory and carefully taken up with a spatula one by one while holding a sparrow with the other hand. Feathers were lifted and a total of 26 mites deposited on each bird (13 on the abdomen and 13 on the back). This is an upper limit of *Dermanyssus* intensity (number of parasites on a bird: Margolis et al. 1982) found in house sparrows (McGroarty and Dobson 1974). All birds were experimentally parasitized. Immediately after infestation, birds were held in the hand for 3 min to allow the mites to settle onto the bird. When the bird was to be released into the aviary, the holding bag was checked and mites which had dropped off the bird were reintroduced onto the host. The bird was finally released after 3 min. All birds were parasitized at the same time on the same day. Final intensities of experimental mites were measured using the dust-ruffling method (see above) 13 days after initial infestation.

Hormonal treatments and analyses

Birds were subdivided into two treatments and two controls in each cage at the end of stage I. Treatments were allocated randomly with respect to dominance status in the pre-implantation phase (stage I); (Likelihood ratio χ^2_9=9.45, P>0.39). Birds in the high-T treatment were castrated and implanted with a subcutaneous silastic tube containing crystalline T, sufficient to give T titres in the plasma at the upper end of the range recorded in free-living males in the breeding season. The low-T-treatment birds were also castrated and implanted to show plasma T titres equivalent to the lower end of the range observed in free-living males during the breeding season (Evans et al. 2000). Castrated controls were operated on and implanted with an empty silastic tube. Finally, intact males were sham operated and provided with an empty implant.

Blood samples for T and B assays were taken during stage II, before the initial deparasitization, and also at the end of stage IV, just before the final collection of experimental mites from the birds. We collected a variable amount of blood [up to about 200 µl per (bird×sample)], after puncturing the vena ulnaris, into heparinized capillary tubes. Samples were then centrifuged for 10 min at 8500 rpm and the plasma separated and stored at –20°C until assayed. Only one blood sample was taken for T analyses in each case, but samples for B analyses were taken at 0, 10 and 30 min after capture using the capture-handling-restraint technique (Wingfield et al. 1992). In this way we could compare both basal and maximum levels of B obtained during the period of sampling. Since B levels can fluctuate daily in the plasma (Webb and Mashaly 1985; Dallman 1991; but see Marra et al. 1995), blood samples were mainly taken in the afternoon (83% of samples).

T concentrations were measured in plasma by direct radioimmunoassay (antiserum code 8680-6004; Biogenesis, Poole, UK] and [^{125}I]-T label [Immunodiagnostic Systems, Boldon, UK]). Sensitivity was 0.1 nmol l^{-1}, and the interassay coefficient of variation was 9.8%. B concentrations were measured by radioimmunoassay after extraction of plasma in diethyl ether, using antiserum code B21-42 (Endocrine Sciences, Tarzana, Calif.) and [1,2,6,7-^{3}H]-B label (Amersham, UK). Sensitivity was 4 nmol l^{-1} and the interassay coefficient of variation was 11.9%.

Statistical analyses

Non-parametric and parametric statistics were used throughout, the latter being used after logarithmic or square root transformation of data. Since the distribution of ectoparasites usually follows a negative binomial distribution (e.g. Poiani 1992), we used the following natural logarithmic transformation whenever parametric statistics were used to analyse ectoparasite intensities: $y=\ln(x+0.5k)$, where k is the exponent of the negative binomial distribution. This is a simpler transformation than the usual inverse hyperbolic sine transformation (Anscombe 1948), and it is valid when the mean of the distribution is large (i.e. >20) and $k\geq1$. The exponent k was estimated using the formula: $k=m^2(s^2-m)^{-1}$, where m is the mean and s^2 is the variance of the distribution (Bliss and Fisher 1953).

Results

There was no cage effect for any of the variables studied: ectoparasite loads (Kruskal-Wallis ANOVAs, P>0.05); time spent grooming (Kruskal-Wallis ANOVAs, P>0.05); circulating levels of steroid hormones (one-way ANOVAs, P>0.05). Therefore individual birds were treated as independent data points in the statistical analyses (see also Veiga and Puerta 1996).

How did grooming affect ectoparasite loads?

Sparrows performed only self-grooming, never allogrooming. Time spent grooming was greater in the

Table 1 Total time spent grooming (mean±SD s h^{-1} observation, *n* in parentheses) at different stages of the experiment for the different experimental groups. Values for morning and afternoon observations are given separately. For post-implantation, only the second hour of observation is shown (*T* testosterone)

		High T	Low T	Intact	Castrated
Post-implantation (stage II)	a.m.	56.3±35.5 (3)	25.0±35.5 (3)	14.6±10.5 (3)	51.5±67.1 (2)
	p.m.	62.2±39.8 (5)	44.4±58.8 (5)	45.5±58.6 (2)	10.0±11.7 (5)
Post-deparasitization (stage III)	a.m.	14.3±21.3 (3)	37.3±61.2 (3)	41.5±50.2 (2)	33.3±5.5 (3)
	p.m.	27.6±27.2 (5)	17.8±21.7 (5)	7.0±9.8 (2)	4.7±8.1 (4)
Post-experimental infestation (stage IV)	a.m.	39.7±29.3 (8)	83.2±94.8 (7)	31.7±33.3 (4)	74.6±84.8 (6)
	p.m.	24.3±14.1 (8)	85.4±120.1 (7)	14.5±15.5 (4)	27.3±27.9 (6)

Table 2 List of ectoparasites found on house sparrows

Taxon	Total number of individuals	
	Initial deparasitization (end stage II)	Final deparasitization (end stage IV)
Siphonaptera		
Ceratophyllidae		
Ceratophyllus gallinae	1	0
Phthiraptera		
Philopteridae		
Philopterus fringillidae	153	44
Brueelia sp.	190	8
Acarina		
Dermanyssidae		
Dermanyssus gallinae	2	0
Analgidae	24	0
Proctophyllodidae	544	96

Table 3 Ectoparasite loads (mean±SD, *n* in parentheses) at different stages of the experiment for the different experimental groups

		High T	Low T	Intact	Castrated
Initial deparasitization (end stage II)		78.0±142.6 (8)	17.6±9.9 (8)	19.8±12.3 (5)	7.1±4.6 (7)
Final deparasitization (end stage IV)	Natural ectoparasites	7.7±8.5 (8)	7.1±7.4 (7)	4.7±4.5 (4)	3.6±2.0 (5)
	Experimental mites	1.0±1.4 (8)	0.28[a]±0.4 (7)	0.25±0.5 (4)	0.20±0.4 (5)
	Total ectoparasites	8.7±8.5 (8)	7.4±7.5 (7)	5.0±4.2 (4)	3.8±2.0 (5)

[a] Second decimal values are given here in order to show numerical differences

morning than in the afternoon (Table 1), at least for stage IV data (the only stage where all cages were observed both in the morning and the afternoon) (Wilcoxon signed-ranks test, $P<0.01$). Grooming data were therefore either analysed separately for morning and afternoon, or they were balanced in the analyses.

All birds were observed for 2 h during stage II post-implantation. Grooming increased during the second hour [13.64±18.90 s (mean±SD), $n=28$ vs 37.46±41.09 s, $n=28$; Wilcoxon signed-ranks test, $P<0.0005$]. We therefore used only second-hour data for stage II, which provide information just prior to deparasitization.

Although time spent grooming was shorter after the birds had been deparasitized (stage II vs stage III; Table 1), the difference was not statistically significant (Wilcoxon signed-ranks test, $P>0.10$). Grooming did increase, however, soon after experimental infestation with *D. gallinae* mites (Table 1; Wilcoxon signed-rank test, $P<0.005$).

The number and kind of ectoparasites found on the sparrows are shown in Table 2. The dust-ruffling method had a maximum sampling efficiency [(ectoparasites sampled/ectoparasites present)×100] of 87.7% if all parasites were considered and a maximum efficiency of 74.1% if only feather lice were considered. These estimates can be considered as upper limits to the extraction efficiency of the dust-ruffling method.

There was a clear drop in ectoparasite counts after the birds had already been deparasitized (stage II vs stage IV; Wilcoxon signed-ranks test $P<0.0005$; Table 3). The correlation between time spent grooming and ectoparasite

Table 4 Mean (±SD, *n* in parentheses) testosterone (T) and corticosterone (B) values (nmol l^{-1}) for male house sparrows at stage II (pre-initial deparasitization) and stage IV (after experimental infestation)

Hormone	High T	Low T	Intact	Castrated
T (stage II)	14.0±5.1 (8)	2.6±1.7 (8)	0.6±0.0 (5)	0.3±0.1 (7)
T (stage IV)	10.5±6.3 (8)	1.9±1.0 (7)	0.5±0.1 (4)	0.1±0.1 (5)
B_{basal} (stage II)	12.8±2.8 (8)	9.4±4.0 (8)	9.5±2.8 (5)	7.6±3.9 (7)
B_{max} (stage II)	25.3±5.0 (8)	18.4±6.0 (8)	16.8±3.4 (5)	16.5±4.5 (7)
B_{basal} (stage IV)	13.9±4.0 (8)	13.2±6.3 (7)	14.1±5.7 (8)	8.6±2.0 (5)
B_{max} (stage IV)	21.3±8.5 (8)	22.4±6.8 (7)	27.7±20.4 (4)	17.7±4.1 (5)

Table 5 Linear regressions between $\log_{10}$[T] and $\log_{10}$[B] (basal and max) at both stage II and IV of the experiment

	Intercept	Slope	*r*	*n*	*P*
Stage II					
log T vs log B_{basal}	–0.908	1.219	0.397	29	0.037
log T vs log B_{max}	–3.023	2.577	0.546	29	0.003
Stage IV					
log T vs log B_{basal}	–1.432	1.534	0.449	24	0.032
log T vs log B_{max}	–2.052	1.765	0.386	23	0.077

loads in stage II was not significant (Spearman's rank correlation: $r_{(morning)}$=0.345, n=11, P>0.10; $r_{(afternoon)}$=0.097, n=17, P>0.25). However, there was a negative correlation between grooming and ectoparasite loads post-infestation (stage IV) (Spearman's rank correlation: $r_{(morning)}$=–0.471, n=24, P<0.025; $r_{(afternoon)}$=–0.118, n=24, P>0.10), although the test was significant for morning grooming only. In addition, the observed increase in time spent grooming post-experimental infestation (see above) was associated with a dramatic drop in experimental mites at the end of a 13-day period: 26 mites per bird initial versus 0.5 mites per bird final.

Were plasma levels of T and B correlated with ectoparasite loads after controlling for the effect of grooming?

Grooming during stage II did not differ among T treatments and controls (Table 1; Kruskal-Wallis ANOVA: $H_{(morning)}$=2.23, P>0.50; $H_{(afternoon)}$=5.71, P>0.10). The difference was also not significant during stage III post-initial deparasitization (Kruskal-Wallis ANOVA: $H_{(morning)}$=2.30, P>0.10 and $H_{(afternoon)}$=3.21, P>0.25). The same result was obtained for grooming post-experimental infestation (stage IV) (Kruskal-Wallis ANOVA: $H_{(morning)}$=1.49, P>0.50 and $H_{(afternoon)}$=2.87, P>0.25).

Total ectoparasite loads differed among treatments at the end of stage II (Kruskal-Wallis ANOVA: H=8.38, P<0.05; see Table 3). High-T sparrows had the largest loads of ectoparasites, followed by intacts, low T and castrated. The only statistically significant difference, however, was between high-T and castrated groups (Dunn's test, P<0.05). Treatments did not differ significantly in total ectoparasite loads after experimental infestation (end of stage IV) (Kruskal-Wallis ANOVA: H=1.16, P>0.75), although high-T sparrows had numerically more ectoparasites than low-T birds, followed by intacts and castrated. Experimental mites were numerically more numerous on high-T individuals, followed by low T, intacts and castrated, although the differences were not significant (Kruskal-Wallis ANOVA: H=2.27, P>0.50). However, the distribution of experimental mites did not differ from that found among total ectoparasites in stage II [Kolmogorov-Smirnov two-sample test: χ^2_2=0.098, P>0.95, with an upper boundary to the power of the test of 0.48 (Yu 1971)]. This suggests that the above difference in ectoparasite loads detected among treatments at the end of stage II is unlikely to be just a result of initial sampling bias of birds.

Table 4 summarizes plasma concentrations of T and B found in birds in the different experimental groups at both stage II, before they were deparasitized, and at stage IV, after experimental infestation with mites. T and B are correlated (Table 5). To determine the specific effect of each hormone, we calculated log-residuals (=log observed–log expected from the regression) for T based on each of four regressions with B. Basal and maximum B were treated separately at each of the two stages. Log_{10}T-residuals (T-residuals hereafter) were not correlated with $\log_{10}$B (B hereafter) (P>0.10).

Treatments did not differ in their basal level of B (ANOVA: $F_{3,24}$=2.395, P=0.094) in the period prior to the initial deparasitization (stage II), although the difference approached statistical significance. High-T birds showed higher basal concentrations of B than the rest, whereas castrated individuals had the lowest. A significant difference was found for B_{max} (ANOVA: $F_{3,24}$=3.897, P=0.021), with high-T birds having the highest levels (Fisher's PLSD test, P<0.05). T-residuals also differed among treatments in the same stage ($F_{3,34}$=19.079, P=0.0001 with respect to B_{basal} and $F_{3,24}$=12. 404, p=0.0001 with respect to B_{max}). The relationship among treatments was high T>low T>intacts>castrated in all cases (Fisher's PLSD test, P<0.05). The situation changed somewhat at the end of stage IV, after initial deparasitization and experimental infestation. Although T-residuals followed the same pattern as in stage II ($F_{3,20}$=11.764, P=0.0001 with respect to B_{basal} and $F_{3,20}$=24.757, p=0.0001 with respect to B_{max}; Fisher's PLSD test, P<0.05), B did not differ significantly among treatments (B_{basal}: $F_{3,20}$=1.613, P=0.218; B_{max}: $F_{3,20}$=0.523, P=0.671). Hormone concentrations did not differ significantly between stages II and IV (paired t-tests; P>0.30), except for B_{basal} which significantly increased post-experimental infestation (stage IV) (paired t-test: t_{23}=–1.866, p=0.037).

Table 6 Spearman's rank correlations between both plasma corticosterone (B_{basal} and B_{max}) and log_{10} T-residuals (with respect to B_{basal} and B_{max} regressions) and dominance status at both stage II (post-implantation) and stage IV (post-infestation)

	Stage II			Stage IV		
	rho	*n*	*P*	rho	*n*	*P*
B_{basal}	–0.408	28	<0.025	–0.210	24	>0.10
B_{max}	–0.381	28	<0.025	–0.370	24	<0.05
log T-residuals$_r$ (B_{basal})	–0.226	28	>0.10	–0.382	24	<0.05
log T-residuals (B_{max})	–0.280	28	>0.05	–0.451	24	<0.025

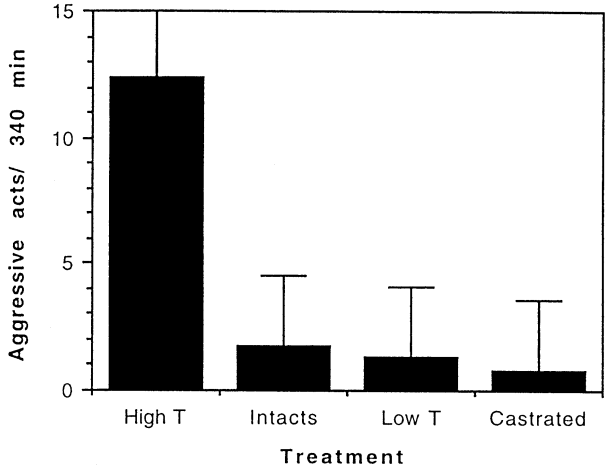

Fig. 1 Accumulated aggression rates (mean+SE) shown by male house sparrows in captivity throughout the experiment (only birds surviving to the end of the experiment are considered)

In some cases, B and T concentrations were positively correlated with ectoparasitic loads, after taking into account the effect of grooming in a multiple-regression analysis. For example, ectoparasite (total) intensity (i.e. number of ectoparasites found in each bird) increased with increasing values of B_{basal} after controlling for grooming (r_p=0.384, $F_{1,22}$=3.802, P<0.05) during stage IV, afternoon observations. There was also a marginally non-significant increase in T-residuals (with respect to B_{basal}) with ectoparasite loads during stage II (r_p=0.595, $F_{1,9}$=4.945, 0.10>P>0.05) after controlling for time spent grooming (morning observations).

Total ectoparasites at stage IV, post-infestation, were negatively correlated with grooming after controlling for the effect of B_{basal} (r_p=–0.556, $F_{1,22}$=9.843, P<0.005 for morning observations and r_p=–0.326, $F_{1,22}$=2.609. 0.10>P>0.05 for afternoon observations). The numerical relationship is for both natural ectoparasites (r_p=–0.377, $F_{1,22}$=3.64, 0.10>P>0.05) and experimental mites (r_p=–0.181, $F_{1,22}$=0.746, P>0.25) to decrease independently with time spent grooming.

Rate of aggression was positively correlated with time spent grooming during stage II (Spearman's rank correlation: r=0.614, n=24, P<0.001). Aggression rate was not correlated with ectoparasite loads (r=0.063, n=28, P>0.25) in the same period. There was no significant correlation between rate of aggression and either grooming (r=–0.114, n=24, P>0.25) or total ectoparasite loads (r=–0.057, n=24, P>0.25) during stage IV.

The higher the dominance status of males, the higher the value of B and T-residuals (Table 6).

Rates of aggression decreased with time: stage II=2.75±4.99 (aggressive acts per 2 h, mean±SD, n=28) versus stage IV=0.96±1.48 (n=25; Wilcoxon signed-ranks test: Z=–1.897, p=0.029).

If only birds surviving up to the end of the experiment are considered, aggression rates differed among treatments throughout the whole experiment (Fig. 1; Kruskal-Wallis ANOVA: H=8.14, P<0.05). A Dunn's test for multiple comparisons indicates that high-T birds are the most aggressive (P<0.01).

In general, birds implanted with high T increased their social dominance rank compared with the pre-implantation status (stage I vs stage II). The same was true for low-T birds. The situation was less clear cut for intacts and castrated birds, and there was even one case of a castrated bird jumping from position three in the hierarchy to position one after castration! A total of five birds died of natural causes during this study; none of them was a high-T bird (two intacts, one low T and two castrated).

Discussion

Unfortunately, decreased sample sizes following the death of some birds during the experiment and large variances in the data might have contributed to some results being statistically non-significant. Although we will discuss some of these results, especially when probability approaches the critical level, we are aware that they still await confirmation or refutation by future tests.

How did dominance status affect ectoparasite loads?

Dominance is defined here on the basis of direction and frequency of aggression among individuals (see Methods). We observed a total of 136 instances of aggressive acts, including 6 (4.4%) reversals (i.e. subordinate to dominant aggression).

How did grooming affect ectoparasite loads?

We found a direct relationship between the amount of time spent grooming and changes in ectoparasitic loads in wild-caught male house sparrows held in all-male groups in captivity. Grooming decreased after the birds had been deparasitized and increased after experimental

infestation with haematophagous *D. gallinae* mites (see also Mooring et al. 1996b for similar results in impala *A. melampus*). The more time birds spent grooming, the smaller the measured ectoparasite loads (stage IV, post-infestation). The above results suggest that grooming may be a behavioural response to ectoparasitism in house sparrows and that such behaviour is potentially capable of keeping ectoparasite infestations at bay (Clayton 1991b; Cotgreave and Clayton 1994; Mooring 1995). It is also possible that grooming might have been a response to succesful feeding bouts by ectoparasites (especially experimental mites), thus leading to earlier parasite departure from the host associated with longer time spent grooming.

Were plasma levels of T and B correlated with ectoparasite loads after controlling for the effect of grooming?

In general, both plasma B and B-independent plasma T (T-residuals) were higher in the high-T treatment than in low-T, intact and castrated sparrows. Castrated individuals had the lowest levels of both B and T-residuals. That is, in this sample of house sparrows, T and B increased independently (see also Silverin 1998). Since both T (McDonald et al. 1981; Grossman 1985; Barnard et al. 1993; Weatherhead et al. 1993; Zuk et al. 1995; Salvador et al. 1996; Folstad and Skarstein 1997) and B (Nelson 1962; Siegel 1980; McDonald et al. 1981; Harvey et al. 1984; Sapolsky 1991, 1992; Barnard et al. 1993; Hadley 1996; Roitt et al. 1996) have immunosuppressive effects, high-T individuals are expected to be at an immunocompetence disadvantage compared to individuals in the other treatments. In fact, high-T individuals harboured more ectoparasites (both natural and experimental) than low-T, intact and castrated birds, whereas castrated individuals harboured the least number of ectoparasites. Ectoparasite intensities increased with both B_{basal} and T-residuals after accounting for the effect of grooming in a multiple-regression analysis. This suggests that grooming may not completely compensate for the negative effect of steroid hormones on the activity of the immune system, resulting in higher susceptibility to ectoparasite infections in birds with higher circulating levels of steroids.

That ectoparasite loads were higher on high-T birds at the end of stage II might have just been a result of differential use of nest boxes by high-T individuals during stage I and part of stage II (nest boxes were sealed in the last third of stage II), rather than a result of differential immunocompetence. Although both effects do not exclude each other, boxes were clean when first introduced into the cages and they were sealed when ectoparasite loads were still relatively low as judged by the difference in time spent grooming between the first and the second hour of observation during stage II (boxes were sealed before those observations took place). But above all, the mite infestation experiment shows the same pattern for experimental mites as for natural ectoparasites pre- and post-infestation, and the infestation experiment was carried out after we had sealed the nest boxes. This suggests that the previous short-term effect of nest boxes was not a determinant of ectoparasite distribution in this experiment.

How did dominance status affect ectoparasite loads?

In general, aggression rate (the variable defining dominance status) was not significantly correlated with either time spent grooming or ectoparasite loads (Mooring et al. 1996a), except at stage II when aggression rate was associated with a longer time spent grooming. However, it is unclear whether dominant males were engaged in relatively ineffective displacement grooming.

The higher the dominance status, the higher the plasma levels of T and B (see also Hegner and Wingfield 1987). In addition, T implants were associated with increased social dominance, compared with the pre-implantation situation. That is, high-T implants increased social dominance and, at least on some occasions, also increased susceptibility to ectoparasites.

How does this work relate to previous works on house sparrows?

Evans et al. (2000) found that high-T house sparrows were less immunocompetent (i.e. they were less able to mount an immune response to sheep erythrocytes injected intraperitoneally) than low-T birds and intacts, and suggested that the apparent relationship between T and immunosuppression was mediated by B, whereas the direct effect of T was to increase immunocompetence, possibly through the effect T has on dominance (see above) and access to food. Evans et al.'s (2000) work included data for both the 1995 and 1996 breeding seasons. The data for the 1996 breeding season alone (this work) show a different pattern, with both B and T depressing immunocompetence. The difference may be explained by a peculiarity in the experimental design used in this work. To control for nest box effect on ectoparasite loads, we sealed the nest boxes during stage II. This might have prevented dominant (mainly high-T) birds from controlling the nest boxes and having preferential access to relatively better thermoregulatory conditions (e.g. adrenocorticotropin hormone causes dose-related hypothermia: Hadley 1996). In this way, high-T sparrows were as exposed as the birds in the other treatments to the stressful conditions of the aviary. Evans et al. (2000) also showed that high-T birds moulted into larger bibs than sparrows in the other treatment groups and they also had a less competent immune system. This indicates that T controls changes in the size of the bib and it also promotes immunosuppression (alone and in association with B, see above) and increased ectoparasite loads as predicted by the ICHH.

Hillgarth and Wingfield (1997b) criticized the ICHH on the grounds that although low circulating T levels

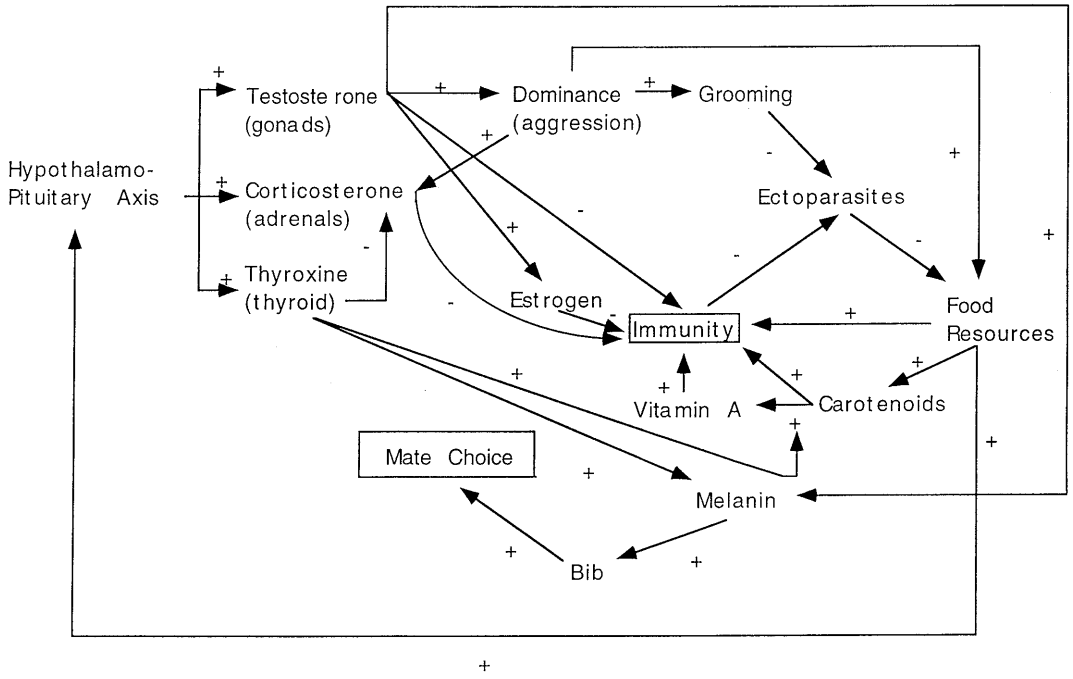

Fig. 2 Integrated model for immunocompetence effects (handicap and badge of status) in house sparrows showing the testosterone-corticosterone and thyroxine pathways. The *sign* by the side of an *arrow* indicates the kind of effect (permissive or inhibitory) the object at the basis of the arrow has on the object at its tip.Shown are only one-way, direct interactions, but many regulatory feedback mechanisms occur between the hypothalamopituitary axis and target tissues such as gonads, adrenals, thyroid and immune system (Marsh and Scanes 1994)

during moulting may affect the expression of secondary sexual traits, those same low T levels are unlikely to also be significantly immunosuppressive. Instead, they suggest that plumage coloration during the breeding season is associated with high circulating levels of T as a result of aggression in dominance interactions and that T levels associated with aggression are indeed capable of immunosuppressing the birds. Below we propose a new model to overcome such criticisms.

The integrated immunocompetence model of sexual selection

All models of sexual selection imply that secondary sexual traits have evolved under the selective pressure of increasing fitness through increased reproduction. Those same models, however, can be distinguished on the basis of their assumptions with regard to survival. Handicap models (Zahavi 1975; Grafen 1990) assume that an increased value of the secondary sexual trait (and hence increased attractiveness to the sexual partner) is associated with a decreased probability of survival of the trait's bearer. Some other secondary sexual traits, however, may be associated with increased probability of survival. Such survival-enhancing secondary sexual traits do not fit within the definition of a handicap (sensu Zahavi 1975), and we will refer to them as "badges of status" (Rohwer 1975; Dawkins and Krebs 1978).

Any specific hormone may be linked to behaviour, expression of secondary sexual traits, parasitism and immunity following both "handicap" and "badge of status" mechanisms. We call this the "integrated immunocompetence model" (IICM). The IICM makes the following major predictions:

(a) The same hormone can be involved in both survival-enhancing and survival-depressing mechanisms either simultaneously (e.g. by depressing the production of one kind of leukocyte, but enhancing the production of others) or sequentially (e.g. by being immunoenhancing at low concentrations, but immunosuppressing at high concentrations).
(b) Different hormones affecting expression of the same secondary sexual trait(s) may have either immunosuppressive or immunoenhancing effects.
(c) No matter what survival effects the hormone may have through its actions on the immune system, it should always have the effect of enhancing conspicuousness of the secondary sexual trait(s) used in mate attraction.

The ICHH, being a handicap model, does not make the above predictions.

Figure 2 summarizes the main aspects of the IICM (see also Marsh and Scanes 1994) with specific applications to the house sparrow. It is well known that the hypothalamopituitary axis controls the release of several

hormones into the blood stream (Hadley 1996). Three very important hormones under hypothalamopituitary control are T (mainly produced by the gonads), B (adrenals) and thyroxine (thyroid gland). Both T and thyroxine may affect the expression of a secondary sexual trait such as the bib in house sparrows. House sparrow bib coloration is melanin dependent (Ralph 1969) and steroid hormones are melanogenic in birds (and mammals) (Hadley 1996; Evans et al. 2000). We also know that the bib is a secondary sexual trait which can be used by house sparrow males in intra-sexual competition for mates (Møller 1987a). T-dependent secondary sexual traits are also known in other species (Höhn and Cheng 1967; Archawaranon et al. 1991; Saino and Møller 1994; Zuk et al. 1995; Salvador et al. 1996). It is important to emphasize that what matters in this sexual selection context is not only whether T determines presence or absence of the trait (Keck 1934), but that differences in plasma levels of T determine differences in the size of the bib.

Thyroxine, on the other hand, can also affect deposition of melanin in feathers (Frieden 1951 cited in Karkun and Landgrebe 1963; Ligon et al. 1990; see also Dawson 1998 for thyroid effects on melanization in house sparrows). That is, there are at least two hypothalamopituitary-dependent pathways controlling change in bib size through melanin, one involving the gonads and the other involving the thyroid. Indeed, there may also be a third pathway which has been proven for mammals but not yet for birds, and which involves the melanogenic effects of the pituitary adrenocorticotropin hormone (ACTH) (Hadley 1996). ACTH is also well known to trigger secretion of corticosteroids from the adrenals (Hadley 1996). Are those pathways also affecting the level of immunocompetence?

T can affect immunocompetence in several ways. It may depress immunocompetence directly (Grossman 1985; Zuk et al. 1995; Salvador et al. 1996; Evans et al. 2000) through reducing the circulating levels of IgY (humoral response) (Barnard et al. 1993), caused by reduced production of B-lymphocytes in the bursa of Fabricius (Zuk et al. 1995; Salvador et al. 1996). T can also reduce the cellular immune response through depressing the production of T lymphocytes in the thymus (Grossman 1985). There are also some indirect ways in which T can reduce the activity of the immune system. Oestrogen produced by the aromatization of T can inhibit the development of helper T cells (Grossman 1985). High plasma levels of T are also associated with elevated aggression rates leading to stress (Edwards and Rowe 1975; Mendoza et al. 1979; Searcy and Wingfield 1980; Wingfield 1984a, 1984b; Wingfield et al. 1987; Jones and Mench 1991; Gwinner and Gwinner 1994; Stoddart et al. 1994; Salvador et al. 1996; this work). Elevated stress triggers the release of corticosteroids in the plasma by the adrenals (Mendoza et al. 1979; Goo and Sassenrath 1980; Koob 1991; Miczek et al. 1991; Rivier 1991; Creel et al. 1996; this work). Furthermore, high circulating T can be associated with a rapid decrease in the levels of B-binding globulins in the plasma, thus increasing the concentration of free (active) plasma B (McDonald et al. 1981). Corticosteroids have well-established immunosuppressive effects (Nelson 1962; Siegel 1980; McDonald et al. 1981; Harvey et al. 1984; Sapolsky 1991, 1992; Barnard et al. 1993; Hadley 1996; Roitt et al. 1996; Evans et al. 2000), and such effects can lead to elevated parasite loads (Applegate and Beaudoin 1971; Esch et al. 1975; Halvorsen 1986; Barnard et al. 1993; this work). Moreover, by suppressing the immune system, both T and B can diminish the hypersensitivity reaction which may trigger grooming in ectoparasitized individuals (Wikel 1984; Mooring et al. 1996b; Hart 1997).

It should be noted, however, that the relationship among corticosteroids, immunosuppression and parasitism is not a straightforward one. Release of corticosteroids into the blood stream as a result of stress is an adaptive physiological response. The “overshooting prevention hypothesis” (Munck et al. 1984) states that release of corticosteroids by the adrenals during stress or infection is a mechanism to dampen the effects of the immune system in order to prevent autoimmune damage to the organism (see also Råberg et al. 1998 for a review). In fact, interleukin-1 (a cytokine involved in the immune response) directly stimulates the release of corticotropin-releasing factor (CRF) from the hypothalamus (Tsagarakis et al. 1989; Roitt et al. 1996), CRF can reduce the ulceration effect (autoimmune pathology) of stress (Tachè 1991) and lymphocytes can produce ACTH themselves in response to CRF (Roitt et al. 1996). In addition, B has physiologically adaptive effects on the maintenance of body temperature and circulating levels of glucose during stress (Dallman 1991). It is only when stress becomes chronic, as may have happened in the sustained situation of social conflict created in our experiment with house sparrows, that release of B and its immunosuppressive effects may become damaging (see also Zuk 1996; Creel et al. 1997). Dominant male house sparrows are also expected to be under sustained stress during the breeding season in the wild (Creel et al. 1996), as a result of resource-defence behaviours against male conspecifics (Møller 1987a, 1987b, 1987c, 1988; Veiga 1993, 1996) and defence against predators (Reyer et al. 1998). Chronic secretion of B is associated with several pathologies such as diabetes mellitus, ulcers, heart diseases, gastrointestinal disorders and hypothermia (Burks 1991; Grossman 1991; Taborsky and Porte 1991; Tachè 1991; Weiner 1991; Hadley 1996). What “chronic” means, however, will depend on the species, or individuals within species, since different organisms show different stress responses depending on their experience with stressors during ontogeny and selective pressures during phylogeny (Ader 1975; Wingfield et al. 1992; Liu et al. 1997; Romero et al. 1998).

In the light of the above evidence, it is puzzling that none of the dominant, high-T (implanted) house sparrows studied in this work died, whereas all other groups (low-T, castrated and intact) had some dead individuals.

Clearly, being a dominant individual with high levels of T and B may also imply some benefits, possibly in terms of immunocompetence (see also Evans et al. 2000).

Although high circulating levels of corticosteroids tend to reduce the production of lymphocytes, they also tend to increase the production of heterophils which are associated with immune defence against bacteria (Siegel 1980). Stress-induced elevation of circulating B can be associated with increased cell-mediated immunity (Dhabhar and McEwen 1996). More aggressive house sparrows groomed more in our work and more grooming kept ectoparasite loads relatively low, at least after experimental infestation with *D. gallinae* mites (see also Brooke 1985; Marshall 1987; Clayton 1991b; Cotgreave and Clayton 1994; Mooring 1995; Mooring and Hart 1995, 1997; Mooring et al. 1996a). More dominant (with usually high circulating levels of T and B) house sparrows were also likely to control limiting food resources such as arthropods which were not supplied by us, but which did enter the cages occasionally and over which the sparrows fought fiercely. Improved body condition due to preferential access to some critical food items may facilitate the activity of the immune system (Roitt et al. 1996; see also Saino et al. 1997b and references therein) and also affect the expression of the house sparrow's bib, since melanin is synthesized from tyrosine, an amino acid ingested with food (Murphy 1994; Veiga and Puerta 1996). In particular, a higher intake of carotenoids can have an important effect on immunocompetence (Lozano 1994; Bortolotti et al. 1996). The most abundant carotenoid is β-carotene which has pro-vitamin A activity (Bendich 1989). Vitamin A (also known as retinol) can be oxidized into retinoic acid by many cell types and retinoic acid can retard death of activated cells of the immune system (Martin 1995). β-Carotene also has direct positive effects on the immune system independent of its pro-vitamin A role (see reviews in Bendich 1989; Chew 1993). Thus, the testosterone-corticosterone-dependent pathway may define a mechanism for both a handicap principle for sexual selection (Zahavi 1975) and a more direct badge-of-status mechanism mediated by dominance (Møller 1988; Owens and Hartley 1991) in house sparrows.

The thyroxine-dependent pathway (Fig. 2) involves the concomitant melanogenic and immunoenhancement effect of thyroxine. Thyroxine is secreted by the thyroid gland and is required for hepatic conversion of carotenes to vitamin A (Hadley 1996). We have already mentioned that vitamin A has positive effects on the immune system (Martin 1995). Thus the thyroxine pathway may not underlie a classical handicap mechanism (Zahavi 1975); rather, thyroxine-dependent melanization is a direct signal for the bird's capacity to access food (e.g. carotenes), as a result of their higher dominance status. The thyroid gland can also enhance immunocompetence through the inhibitory effect 3,5,3'-triiodothyronine (T_3) exerts on B secretion by the adrenals (Carsia et al. 1997). It is unknown whether T_3 is also melanogenic in birds.

An additional pathway is also possible, which involves the melanogenic effect of ACTH, which is secreted by the pituitary and which also stimulates secretion of B from the adrenals. It is also interesting to note that avian lymphocytes can produce pro-opiomelanocortin (POMC) (Marsh and Scanes 1994), suggesting mutual regulation of the POMC-immune system. POMC is mainly produced by the pituitary and it acts as pro-hormone for ACTH, β-endorphin and melanocyte-stimulating hormone (MSH) (Hadley 1996). That MSH controls deposition of melanin in feathers as it does in mammalian skin (Hadley 1996) has yet to be demonstrated.

In conclusion, our work and that of Evans et al. (2000) partly support the ICHH in house sparrows. However, the immunocompetence handicap mechanism does not seem to be the only one operating in the context of sexual selection in this species, since hormones such as T can also have fitness-enhancing effects through increased dominance, thus affording the birds better access to limiting resources such as food and shelter (e.g. see results of bird survivorship during our experiment). The picture that emerges from our works is much more complex, pointing to the existence of both survival-decreasing (i.e. handicaps) and survival-enhancing (i.e. badges of status) sexual selection mechanisms, which can operate at the same time (see also Nolan et al. 1998), in the same organism, sometimes involving the same hormones (e.g. T). This has prompted us to propose a new "integrated immunocompetence model", which considers the concomitant effect of handicaps and badges of status in sexual selection. Multiple physiological and behavioural pathways ensuring the honesty of one specific signal (IICM) may have the same selective advantage as multiple signals of different origin (Møller and Pomiankowski 1993; Johnstone 1995; Marchetti 1998): they all provide reliable tests for the quality of the signaller as a potential sexual partner. Further study will be necessary, however, to establish the relative importance of both handicap and direct-benefit mechanisms in the maintenance of sex-specific plumage in the house sparrow and other species, under variable environmental conditions. Our suggestion is that, whatever their relative importance, both mechanisms may act at the same time, thus reinforcing the value of the trait as a honest signal.

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