Differential effects of a parasite on ornamental structures based on melanins and carotenoids

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Models of sexual selection predict that ornamental coloration should be affected by parasites in order to serve as honest signals. Animals are commonly infested by a range of parasite species and often simultaneously display several ornaments. Thus the specific effect of a given parasite on ornaments is important for the understanding of the signal. Here we investigate experimentally the effect of an ectoparasite on carotenoid- and melanin-based traits in breeding great tits Parus major. In the experiment, nests were either infested with hen fleas, Ceratophyllus gallinae, or kept free of parasites. The color of the two traits and the size of the melanin-based breast stripe were assessed both in the year of experimental parasite infestation and during the following breeding season, after the annual molt. The size of the breast stripe of infested males and females significantly decreased, but increased significantly in uninfested males and females. The blackness of the breast stripe and the carotenoid-based plumage coloration was unaffected. Our experiment demonstrates that the size of the melanin-based breast stripe of adults depends on parasite infestation, suggesting that the trait can serve as an honest signal of previous parasite exposure. Key words: carotenoids, Ceratophyllus gallinae, great tits, hen fleas, honest signaling, melanins, ornaments, parasites, Parus major.

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ectoparasites by subjecting all nests to a heat treatment inside a microwave oven on the day the birds laid their second egg, following Heeb et al. (1996). Nests were then alternately assigned to a group left free of parasites or to a group infested with 40 adult hen fleas Ceratophyllus gallinae obtained from naturally infested nests of the same forest. There was no significant difference in laying date between nests allocated to the two experimental treatments (Student’s t test: t = 0.048, p = 0.962, N = 74). In 1997, 14 nests were experimentally infested while 15 nests were kept free of parasites, and in 1998, 22 nests were infested and 23 were kept parasite-free. Thirteen days after hatching, parents were caught in the nest-box, and a digital photograph of the breast plumage was taken. The year following parasite manipulation, all breeding birds were captured again and photographed. For each adult we measured body mass to the nearest 0.01 g using an electronic scale (Sartorius) and measured the length of the metatarsus to the nearest 0.1 mm using slide calipers (Svensson, 1992). Age was determined by the presence or absence of molt limits (Jenni and Winkler, 1994). Breeding birds were classified as either first-year birds (molt limits present) or birds older than one year (molt limits absent). Fifteen days after hatching each nestling was weighed and the length of the metatarsus measured (Svensson, 1992).

Quantifying the carotenoid-based yellow plumage coloration

Great tits were placed in a box covered with a photographic filter lens (Hoya UV-filter, 59 × 74 mm), including a thin cardboard at one end to protect the bird’s eyes from the flash. This box was placed in a standard position inside a larger opaque camera box. Two flashes (Nikon SB26) were mounted at an angle of 13° to the optical axis, 10 cm below and 20 cm beside the front lens of the camera (Nikon E2 with a 105 mm f/2.8 Nikkor objective) measuring the red, green, and blue values (RGB) of the color. The distance between the feathers and the front lens was fixed to 50 cm. The settings of the camera and flashes were always identical, and thus all photographs received a standard light exposure. Standard white chips (Kodak Color Control Patches with R = 255, G = 255, B = 255) were fixed to each side of the filter for calibration of the equipment during color analysis.

The photographs were imported into the Adobe Photoshop program, and a virtual second layer, holding 10 square measurement areas of 400 pixels each, was placed over the photograph. Then the program calculated the mean RGB values of each square. The squares falling on the black breast stripe were not used for the quantification of the yellow coloration. There was no correlation between the color values and the number of squares analyzed in the 52 recaptured birds (R: r = -0.14, p = .27; G: r = -0.16, p = .21; B: r = .121, p = .34), demonstrating that the exclusion of squares did not bias the results. Both the photographing and the analyses were done blindly with respect to origin and condition of the birds. Mean RGB values per bird were converted to hue-saturation-brightness (HSB) values by the algorithm described in Foley and Van Dam (1984). The variation in light exposure, as assessed from the measurements of the white reference chips, was small (R: 254 ± 1, G: 254 ± 1, B: 243 ± 5; mean ± SD; N = 131), and therefore no correction of the color values measured on the plumage was required.

The measures of color used here do not correspond exactly to the colors perceived by birds. Also, birds possess biologically functional receptors for UV light (e.g., Cuthill et al., 2000), to which our equipment was insensitive. However, as also remarked by Bennett et al. (1994: 855), “for heuristic purposes, it may be useful to express color patterns in subjective terms that humans can readily understand.”

Quantifying the melanin-based black breast stripe

The black area of the breast stripe was measured with the NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/) after calibration of the digital photographs using a standard distance. Pixels with gray values between 150 and 254 (i.e., the ones appearing as black on the digital photograph) were included for measuring the area of the black breast stripe. In males the breast stripe joins the base of the legs (Gosler, 1993), and therefore the area between the base of the neck and the base of the legs was measured. Compared to males, in females the breast stripe is much reduced and usually does not reach the base of the legs (Gosler, 1993). To avoid measuring plumage parts not belonging to the breast stripe, the area between the base of the neck and the end of the breast stripe was measured. The five females in the sample with a breast stripe reaching the base of the legs were analyzed like the males. Skeletal size and body condition, defined as the residuals of the regression of body mass on tarsus, of adult great tits remained constant from one year to the other (paired t test: male tarsus length t = -0.038, n = 22, p = .97; male body condition t < -0.001, n = 22; p = 1; female tarsus length t = -1.32, n = 30, p = .2; female body condition t < -0.001, n = 30, p = 1). Therefore, differences in male breast stripe area are not due to different skeletal size or different chest-diameters between years and thus must be the result of differences in mean width. In females, area differences might be due to changes in mean width and length of the breast stripe. The blackness of the breast stripe was calculated by averaging the gray values of the pixels belonging to the breast stripe. Repeatability (Lessells and Boag, 1987) among 14 adult birds (seven females and seven males) measured twice during the same capture occasion in winter 1997 was highly significant (breast stripe area: r [repeatability] = 0.923, F112 = 68.84, p < .001; breast stripe blackness: r = .9925, F112 = 242.74, p < .001). Similarly, repeatabilities of the carotenoid-based measurements were highly significant (H: r = .80, F112 = 9.11, p = .01; S: r = .842, F112 = 11.618, p < .01; B: r = .824, F112 = 10.374, p < .01), indicating that the measuring of coloration and area by our photographic system is well standardized.

Mortality and recaptures

Seven broods in 1997 (four infested, three uninfested) and nine broods in 1998 (four infested, five uninfested) failed before the nestlings fledged. Brood failures were not influenced by the treatment (chi-square test: χ2 = 0.026, N = 74, p > .5). Of the 22 successful nests in 1997, 13 males (eight infested, five uninfested) and 17 females (nine infested, eight uninfested) were recaptured during the subsequent breeding season. Of the 36 successful nests in 1998, 15 males (five infested, 10 uninfested) and 18 females (seven infested, 11 uninfested) were recaptured. Neither the treatment (ΔD = 0.02, N = 116, p = .88) nor the set of birds manipulated for parasite exposure in both 1997 or 1998 (hereafter referred to as “year of experimental infestation”) influenced the recapture rate significantly (ΔD = 2.33, N = 116, p = .13). Therefore differences between treatment groups are not due to a treatment-dependent recapture bias. Females were recaptured more often (57.6%) than males (38.6%; ΔD = 4.42, N = 116, p = .04). The proportion of recaptured adults was analyzed using logistic regression analysis with binomial errors and a logit link, taking recapture as the dependent binomial variable. The statistical significance of a recapture bias in relation to the independent variables was assessed from the change in deviance (denoted as ΔD) when a variable was excluded first.
from (or included last into) the model (Crawley, 1993). The
change in deviance is asymptotically distributed as $\chi^2$ with
corresponding degrees of freedom (Crawley, 1993). The scale was
estimated at a value of 1.58. Statistical analysis of the recapture
rate was carried out using the statistical package GLM Stat
(Beath, 1997).

With respect to initial plumage characteristics, recaptured
males and females were not significantly different from birds
not recaptured (Student’s $t$ tests: male hue: $t_{65} = 0.31$, $p =
.76$; male saturation: $t_{65} = -1.34$, $p = .19$; male brightness:
$t_{65} = -0.41$, $p = .68$; breast stripe area: $t_{65} = 0.43$, $p = .67$;
male breast stripe blackness: $t_{65} = -0.50$, $p = .62$; female
hue: $t_{65} = -2.45$, $p = .02$; female saturation: $t_{65} = -1.62$,
$p = .11$; female brightness: $t_{65} = -0.25$, $p = .81$; female
breast stripe area: $t_{65} = 0.48$, $p = .64$; female breast stripe
blackness: $t_{65} = -1.56$, $p = .13$). Because there were no dif-
fences in nest failures and recapture rates between treatment
groups and recaptured birds did originally not differ
significantly in the measured plumage traits from birds not
recaptured the subsequent year, birds used in our analysis ap-
ppear to represent a random sample of the original sample.

**Statistical analysis**

The overall plumage coloration was evaluated using the first
principle component (PC1) from a Principal Components
Analysis including hue, saturation, and brightness. PC1 ex-
plained 55.9% of the total variance in male and 50.7% in
female plumage coloration, respectively. A repeated-measures
ANOVA with breast coloration in the first and in the subse-
quent year as repeated measures, parasites, year of experi-
mental infestation, and age as factors and body condition
as a covariate (see Table 2), was conducted using the JMP statis-
tical package (Sall and Lehman, 1996). In addition to these
univariate analyses, we used multivariate analyses to test the
pure variation of a single color parameter without the influ-
ence of the intercorrelated color parameters. Therefore each
color parameter was corrected for both intercorrelated color
parameters (e.g., hue for saturation and brightness) by taking
the residuals of a model with one color parameter as the
dependent variable and the two others as covariates, before
analyzing the data with a repeated-measures ANOVA (see Ap-
pendix Table B). Because all between-subject factors and the
within-subject interactions of age and body condition were not
significant ($p > .1$) in both analyses, only the within-subject
analyses with the factors parasites and year of experimental
infestation are presented in Table 2 and Appendix Table B,
with the exception of the significant effect of year of experi-
mental infestation in hue, as mentioned in the results section.

Blackness of the breast stripe was positively correlated with
the breast stripe area in both males ($F_{1,64} = 6.62$, $p = .012$)
and females ($F_{1,56} = 7.93$, $p = .007$). Therefore, in addition
to the univariate repeated-measures ANOVAs presented in Ta-
ble 1, a multivariate analysis was conducted. Each parameter
was first corrected for the intercorrelated parameter (e.g.,
breast stripe area for blackness) by taking the residuals of a
correlation with one parameter as the dependent variable and
the other one as the independent variable. Residuals were
then used for the repeated-measures ANOVA (see Appendix
Table A). Experimental treatment, year of experimental in-
festation, and age were included as factors and body condition
as a covariate into the model. Because the factors age and
year and the covariate body condition and their interactions
were not significant ($p > .1$), they are not shown in Table 1
and Appendix Table A. Parasite load of six males and five
females was manipulated in both years 1997 and 1998, and
these birds were captured in each of the three breeding sea-
sons. To avoid pseudoreplication we included the 11 individ-
uals only in the data set of the first year of experimental in-
festation. For the analyses we used the JMP statistical package
(Sall and Lehman, 1996). Normality was evaluated by a Lillie-
fors test (Wilkinson, 1989). Significance levels are two-tailed
with a .05 significance level. Bonferroni corrections were ap-
p lied to adjust the $p$ values for the increased probability of
obtaining statistical significance from multiple testing (Rice,
1989). Means $\pm$ 1 SE are given.

**RESULTS**

**Melanin-based plumage coloration**

Male breast stripes covered an average area of $682 \pm 91.5$ mm$^2$
in the first year and $676 \pm 25.6$ mm$^2$ in the subsequent year.
In females the breast stripes covered an average area of $351
\pm 15.2$ mm$^2$ in the first year and $346 \pm 14.4$ mm$^2$ in
the subsequent year. The sexual size difference of the breast stripe
was highly significant ($t = -13.18$, $n = 52$, $p < .0001$).

Parasite exposure during breeding significantly affected the
area of the breast stripe the subsequent year in both males
and females (Figure 1, Table 1; area change $\times$ parasites).

**Figure 1**

Breast stripe area change in relation to sex and ectoparasite treatment. For statistics, see Table 1.
Breast stripes of parasitized males decreased by $49 \pm 28.0$ mm$^2$ (7.2%), and of parasitized females by $57 \pm 19.2$ mm$^2$ (16.2%), while parasite-free males increased their breast stripes by $55 \pm 27.3$ mm$^2$ (8.1%), and parasite-free females by $42 \pm 18.8$ mm$^2$ (12.0%). Neither the year of experimental infestation nor the age of the birds explained a significant part of the variation of the area change in males and females. Similarly, breast stripe area was not correlated with body condition. Parasite exposure had no significant effect on the blackness of the breast stripe in both males and females (Table 1; blackness change $\times$ parasites). The multivariate approach (Appendix Table A) led to the same results, suggesting the lack of an underlying trade-off between breast stripe area and blackness.

### Carotenoid-based plumage coloration

Carotenoid-based plumage color was not affected by parasites in either males or females (Table 2; color change $\times$ parasites, $p > .05$). The year of experimental infestation explained a significant proportion of the variation in hue (color change-independent variation [between subjects, not shown in Table 2]: males: $F_{1,15} = 11.085, p = .005$; females: $F_{1,22} = 13.146, p = .002$) but not in overall plumage coloration, saturation, and brightness ($p > .1$). In males, the color change (Table 2; color change $\times$ year of experimental infestation) was not significantly different between the two data sets in any of the four color variables, whereas in females the change in overall plumage coloration and brightness was significantly affected.
Discussions and conclusions

The results show that ectoparasites can affect the signalization of melanin-based traits in great tits. The reduction in size of the breast stripe in males is consistent with the hypothesis that melamins are deposited during molt. However, in females, the reduction in size is not as pronounced. This suggests that the melanin-based trait may have a different function in males and females.

The study also shows that the effect of ectoparasites on signalization is mediated by the costs of the parasites. This is supported by the finding that the reduction in signal size is more pronounced in males than in females. The costs of the parasites may include the energy required for the development and maintenance of the parasite, which is likely to be higher in males than in females.

The study provides further evidence for the hypothesis that the honesty of melanin-based signals is maintained by social dominance. This is supported by the finding that the reduction in signal size is more pronounced in males than in females. This suggests that the honesty of melanin-based signals is maintained by the costs of the parasites, which are likely to be higher in males than in females.

In conclusion, the study shows that ectoparasites can affect the signalization of melanin-based traits in great tits. The effect of ectoparasites on signalization is mediated by the costs of the parasites, which are likely to be higher in males than in females. This provides further evidence for the hypothesis that the honesty of melanin-based signals is maintained by social dominance.
conspicuous signaling in great tits, implying that it has not been selected to indicate parasite exposure. 

Slagsvold and Lifjeld (1985) found an effect of habitat type on yellow plumage coloration. The significant color change of individuals (Table 2; color change, \(p < .01\)) suggests that this effect may also arise due to varying carotenoid availability between years.

The present study demonstrates that ectoparasite exposure reduces the expression of a melanin-based signal, suggesting that the size of the breast stripe can be a signal of previous parasite exposure. Further work should focus on whether the carotenoid-based breast coloration of great tits is signaling the presence of a single parasite species only, or whether different parasite species affect the same signal similarly, revealing the specificity of such signals.

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REFERENCES


Appendix Table B

Multivariate analysis of parasite influence on carotenoid-based male and female plumage coloration

<table>
<thead>
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<th>Factor</th>
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<td></td>
<td>(F_{1,15})</td>
<td>(p)</td>
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<td>Hue</td>
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<td>Saturation</td>
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<td>Color change (\times) parasites</td>
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Repeated-measures ANOVA with breast coloration in the first year and breast coloration in the subsequent year as repeated measures and parasites, year of experimental infestation and age as factors and body condition as a covariate. Each color parameter was corrected for the two correlated color measures (H for S and B; S for H and B, B for H and S) before analyzing the data. As all between-subject factors and the within-subject interactions with age and body condition were not significant (\(p > .1\)), only the within-subject analysis with the factors parasites and year of experimental infestation is presented.

* \(p < .05\) after Bonferroni correction.


