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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.* *[Behav Ecol 13:401–407 (2002)]*

The evolution of conspicuous signals is a pathway for resolving conflicts of interest in an economical manner. Instead of solving a conflict by costly fighting, contestants may assess each other's quality by visual signals that indicate the bearer's quality honestly. Honesty is maintained by the cost of producing or carrying the signal and by the fact that signals are more costly to poorer phenotypes (e.g., Zahavi, 1975, 1977; Grafen, 1990). Parasites are expected to influence signal expression by living off their host's resources and by influencing the trade-offs for resource allocation among various fitness components within hosts. Milinski and Bakker (1990) have shown both that an endoparasite *Ichthyophthirius multifiliis* reduces the intensity of a carotenoid-based red signal in male sticklebacks *Gasterosteus aculeatus* and that females select mates on the basis of this trait. Similarly, male house finches, *Carpodacus mexicanus,* experimentally infected with coccidiosis grow a less red plumage and are less often selected by females (Hill, 1990; Hill and Brawner, 1998). In contrast to carotenoid-based signals, there is no experimental evidence yet for parasites to affect melanin-based signals. Møller et al. (1996) suggested that ectoparasite load reduces the size of the melanin-based breast badge in house sparrows, whereas in house finches Hill and Brawner (1998) did not find a correlation between experimental coccidial infection and melanin-based plumage colors.

Great tits (*Parus major*) show both a carotenoid-based yellow breast plumage and a melanin-based black breast stripe. Melanins are synthesized by the birds before deposition in the melanophores of the feathers (Brush, 1978). This process depends on body condition during molt (Veiga and Puerta, 1996). Correlational evidence suggests that female great tits assess future mates by the size of the breast stripe (Norris, 1990). In intrasexual interactions birds with experimentally enlarged breast stripes are more dominant and gain more conflicts (Järvi et al., 1987). Carotenoids, in contrast to melanins, cannot be synthesized by birds and thus have to be ingested with food. It has been suggested that restricted availability of carotenoid-containing food (Hill, 1992; Hill and Montgomerie, 1994; Slagsvold and Lifjeld, 1985), low body condition of the individual bird (Hill and Montgomerie, 1994), and detrimental effects of the carotenoids on the organism (Olson and Owens, 1998) may limit the expression of the carotenoid-based plumage coloration. In great tits, correlational evidence suggests that brighter males signal low parasite level and thus better quality (Dufva and Allander, 1995; Hõrak et al., 2000).

In summary, experimental evidence that parasites affect melanin- and carotenoid-based signals is scant. Here we investigate, by experimental infestation, the influence of a hematophageous ectoparasite (*Ceratophyllus gallinae*) that affects reproductive success and reproductive effort (e.g., Christe et al., 1996; Richner et al., 1993) on the expression of a melanin- and a carotenoid-based plumage trait in great tits (*P. major*). We assessed the effect of flea infestation during reproduction on the plumage coloration of the subsequent year and predicted a change in signal size or intensity as a function of the applied ectoparasite load. Whether ectoparasites change their hosts' plumage coloration directly or cause a change indirectly by imposing higher reproductive effort requires further work beyond the present study.

MATERIALS AND METHODS

Experimental setup

The experiment was performed on a great tit population breeding in nest-boxes in the Forst, a forest near Bern, Switzerland (46°54′ N, 7°17′ E to 46°57′ N, 7°21′ E). On a first set of birds, the parasite load was manipulated in 1997, and the change in plumage color from 1997 to 1998 was assessed (i.e., after the annual molt). On a second set the same procedure was used from 1998 to 1999. We eliminated nest-based

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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: $t = 0.048$, $p = .962$ $N = 74$). 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 adults 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 ± 3; 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 photograph 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 is 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, $F_{1,12} = 68.84$, $p < .001$; breast stripe blackness: $r = .9925$, $F_{1,12} = 242.74$, $p < .001$). Similarly, repeatabilities of the carotenoid-based measurements were highly significant (H: $r = .80$, $F_{1,12} = 9.11$, $p = .01$; S: $r = .842$, $F_{1,12} = 11.618$, $p < .01$; B: $r = .824$, $F_{1,12} = 10.374$, $p < .01$), 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: $\chi^2 = 0.026$, $N = 74$, $p > .5$). 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 ($\Delta D = 0.02$, $N = 116$, $p = .88$) nor the set of birds manipulated for parasite exposure in both 1997 or 1998 (hereafter referred to "year of experimental infestation") influenced the recapture rate significantly ($\Delta D = 2.33$, $N = 116$, $p = .13$). 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%; $\Delta D = 4.42$, $N = 116$, $p = .04$). 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

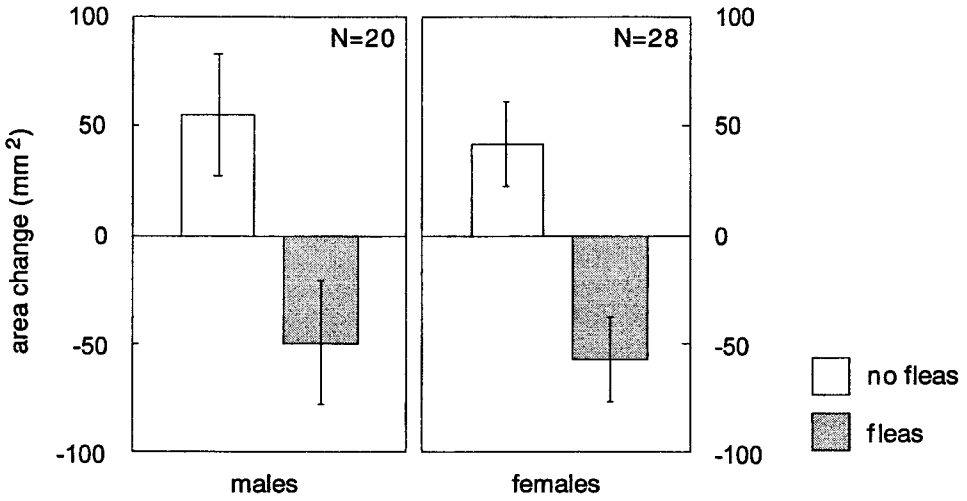

Figure 1
Breast stripe area change in relation to sex and ectoparasite treatment. For statistics, see Table 1.

from (or included last into) the model (Crawley, 1993). The change in deviance is asymptotically distributed as χ^2 with corresponding degrees of freedom (Crawley, 1993). The scale was estimated at a value of 1.38. 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: $t_{1,65} = 0.31$, $p = .76$; male saturation: $t_{1,65} = -1.34$, $p = .19$; male brightness: $t_{1,65} = -0.41$, $p = .68$; breast stripe area: $t_{1,65} = 0.43$, $p = .67$; male breast stripe blackness: $t_{1,65} = -0.50$, $p = .62$; female hue: $t_{1,63} = -2.45$, $p = .02$; female saturation: $t_{1,63} = -1.62$, $p = .11$; female brightness: $t_{1,63} = -0.25$, $p = .81$; female breast stripe area: $t_{1,63} = 0.48$, $p = .64$; female breast stripe blackness: $t_{1,63} = -1.56$; $p = .13$). Because there were no differences 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 appear 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 explained 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 subsequent year as repeated measures, parasites, year of experimental infestation, and age as factors and body condition as a covariate (see Table 2), was conducted using the JMP statistical 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 influence 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 Appendix 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 experimental 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 ($F_{1,58} = 7.93$, $p = .007$). Therefore, in addition to the univariate repeated-measures ANOVAs presented in Table 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 infestation, 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 seasons. To avoid pseudoreplication we included the 11 individuals only in the data set of the first year of experimental infestation. For the analyses we used the JMP statistical package (Sall and Lehman, 1996). Normality was evaluated by a Lilliefors test (Wilkinson, 1989). Significance levels are two-tailed with a .05 significance level. Bonferroni corrections were applied to adjust the p values for the increased probability of obtaining statistical significance from multiple testing (Rice, 1989). Means ± 1 SE are given.

RESULTS

Melanin-based plumage coloration

Male breast stripes covered an average area of 682 ± 21.5 mm^2 in the first year and 676 ± 25.6 mm^2 in the subsequent year. In females the breast stripes covered an average area of 351 ± 15.2 mm^2 in the first year and 346 ± 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 × parasites).

Table 1
Area and blackness change of the breast stripe

		Males		Females	
	Factor	$F_{1,15}$	p	$F_{1,23}$	p
Area	Area change-dependent (within subjects)				
	Area change	1.602	.225	0.297	.591
	Area change × parasites	7.034	.018*	7.230	.013*
	Area change × year of experimental infestation	0.462	.507	1.689	.206
Blackness	Blackness change-dependent (within subjects)				
	Blackness change	0.458	.509	0.986	.331
	Blackness change × parasites	0.011	.917	4.565	.044
	Blackness change × year of experimental infestation	0.191	.669	0.650	.428

Repeated-measures ANOVA with area or blackness in the first year and area or blackness in the subsequent year as repeated measures, parasites, year of experimental infestation and age as factors, and body condition as a covariate. 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

Breast stripes of parasitized males decreased by 49 ± 28.0 mm² (7.2%), and of parasitized females by 57 ± 19.2 mm² (16.2%), while parasite-free males increased their breast stripes by 55 ± 27.9 mm² (8.1%), and parasite-free females by 42 ± 18.8 mm² (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 × 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 × 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 × 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

Table 2
Parasite influence on carotenoid-based male and female plumage coloration

		Males		Females	
	Factor	$F_{1,15}$	p	$F_{1,22}$	p
PC1	Color change-dependent (within subjects)				
	Color change	0.000	.984	0.148	.704
	Color change × parasites	1.143	.302	0.599	.447
	Color change × year of experimental infestation	0.868	.366	14.691	.001*
Hue	Color change-dependent (within subjects)				
	Color change	10.574	.005*	10.408	.004*
	Color change × parasites	2.406	.142	0.427	.520
	Color change × year of experimental infestation	0.042	.841	7.542	.012
Saturation	Color change-dependent (within subjects)				
	Color change	1.844	.195	6.458	.012
	Color change × parasites	0.427	.523	0.192	.666
	Color change × year of experimental infestation	0.187	.672	1.133	.299
Brightness	Color change-dependent (within subjects)				
	Color change	0.053	.821	0.342	.564
	Color change × parasites	2.760	.117	1.331	.261
	Color change × year of experimental infestation	4.721	.046	22.093	.001*

Repeated-measures ANOVA with breast coloration in the first and subsequent year as repeated measures, parasites, year of experimental infestation and age as factors, and body condition as a covariate. 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.

Appendix Table A
Multivariate analysis of area and blackness change of the breast stripe

		Males		Females	
	Factor	$F_{1,15}$	p	$F_{1,23}$	p
Area	Area change-dependent (within subjects)				
	Area change	0.486	.496	0.387	.540
	Area change × parasites	6.315	.024*	10.212	.004*
	Area change × year of experimental infestation	1.033	.326	1.852	.187
Blackness	Blackness change-dependent (within subjects)				
	Blackness change	0.043	.839	1.506	.232
	Blackness change × parasites	0.974	.339	3.423	.077
	Blackness change × year of experimental infestation	0.191	.669	1.591	.220

Repeated-measures ANOVA with area or blackness in the first year and area or blackness in the subsequent year as repeated measures and parasites, year of experimental infestation and age as factors and body condition as a covariate. 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.

by the year of experimental infestation (Table 2; color change × year of experimental infestation). The multivariate approach (Appendix Table B) led to similar results, except in male brightness being significantly different between males of different ages (color change-independent between subjects factor [not presented in Appendix Table B]) and in the color change of female hue, being significantly different between years (Appendix Table B).

DISCUSSION

This study shows experimentally that ectoparasites can induce a change in the size of a melanin-based plumage trait that had been suggested to function as an honest signal during processes of inter-and intrasexual selection in male great tits. It demonstrates that melanin-based traits of adults are dependent on the parasite load during reproduction. The blackness of the breast stripe was not significantly modified. The multivariate approach led to the same results, suggesting that there is no underlying trade-off between signal size and signal intensity. It is interesting that the effect of parasites on signal size was also found in females. This result is not surprising considering the study of Wilson (1992), which suggested a correlation between the size of the female breast stripe and social dominance. Thus, in both male and female great tits, the size of the breast stripe is a potential signal to conspecifics for individual parasite load and/or resistance against this ectoparasite, as had been suggested in barn owls for another melanin-based trait (Roulin et al., 2001).

The mechanism leading to reduced breast stripe size cannot be revealed by our experiment and needs further investigation. Both a direct mechanism through blood sucking and/or disease transmission by the fleas and an indirect effect due to flea-induced higher reproductive investment (Christe et al., 1996; Richner and Tripet, 1999) are conceivable.

In the house sparrow (*Passer domesticus*), nutritional constraints affect body condition and influence the expression of badge size (Veiga and Puerta, 1996), whereas in our study body condition alone, as measured 13 days after hatching, did not explain a significant proportion of the total variance in breast stripe area. Although in house sparrows the body condition was directly manipulated via access to food, we did not manipulate body condition directly. It might be for this reason that body condition affected breast stripe area in house sparrows, but not in our study where body condition was merely a correlational variable. Moreover, it is conceivable that body condition later in the year, when melanins are deposited during molt, is only poorly correlated with the body condition measured during parasite exposure. Therefore only body condition measured and manipulated during molt could reveal the relationship with breast stripe area. In contrast to our study, a melanin-based trait in another species, the black tail coloration of house finches (*Carpodacus mexicanus*), was unaffected by coccidial infection (Hill and Brawner, 1998). It has been suggested that the honesty of melanin-based signals is maintained by the costs arising from social dominance interaction (Møller, 1987; Veiga, 1993) and not by the costs of the melanin synthesis per se (Gray, 1996). It has further been suggested that the black tail of house finches is not used in conspecific signaling (Hill and Brawner, 1998), implying that tail color has not been selected to indicate parasite exposure.

In contrast to previous studies (Hill and Brawner, 1998; Milinski and Bakker, 1990), we did not find an effect of the parasites on carotenoid-based coloration of the plumage. There are at least four not mutually exclusive explanations. First, parasites may have affected the coloration exclusively in the UV range of the visual spectra not measured here. Second, both of the above-mentioned studies used endoparasites. Coccidia infections, as used in the study on house finches (Hill and Brawner, 1998), inhibit the uptake of nutrients in the gut, including carotenoids (Augustine and Ruff, 1983; Ruff et al., 1974). *Ichthyopthirius multifilis,* as used in the stickleback experiments, feeds on cells and blood below the epidermis (Schäperclaus, 1990) and reduces the condition of the fish (Milinski and Bakker, 1990), whereas hen fleas do not directly inhibit the uptake of nutrients, nor do they feed on body cells. This might explain the absence of negative effects of hen fleas on the carotenoid-based plumage coloration. Third, the chemical pathways of the carotenoid deposition in house finches and sticklebacks are different from those in great tits. The red coloration of house finches and sticklebacks originates from carotenoids being modified before deposition (Brush, 1990; Wedekind et al., 1998), whereas the yellow carotenoid-based coloration of the great tits originates from carotenoids being deposited unmodified in the feathers (Partalli et al., 1987). If carotenoid modification is costly, it is conceivable that the altered energy balance due to parasites reduces the quantity of energy available for the chemical processes before carotenoid deposition. Therefore, differences in red but not yellow coloration are predicted. Finally, in contrast to house finches, this carotenoid-based trait may not be used in

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

		Males		Females	
	Factor	$F_{1,15}$	p	$F_{1,22}$	p
Hue	Color change-dependent (within subjects)				
	Color change	7.748	.014	9.139	.006*
	Color change × parasites	2.013	.176	0.799	.381
	Color change × year of experimental infestation	0.003	.957	15.035	.001*
Saturation	Color change-dependent (within subjects)				
	Color change	0.628	.440	3.794	.064
	Color change × parasites	1.274	.277	0.017	.899
	Color change × year of experimental infestation	0.474	.502	1.619	.217
Brightness	Color change-dependent (within subjects)				
	Color change	0.730	.406	0.077	.785
	Color change × parasites	3.388	.086	1.013	.325
	Color change × year of experimental infestation	3.328	.088	20.852	.001*

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.

conspecific 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 another parasite species than hen fleas, as suggested by two recent studies (Dufva and Allander, 1995; Hõrak et al., 2000). Additionally it would be important to investigate whether a signal (melanin- or carotenoid-based) reflects 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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