Do male ornaments signal immunity in the common yellowthroat?

Julia C. Garvin,[a] Peter O. Dunn,[a] Linda A. Whittingham,[a] Douglas A. Steeber,[a] and Dennis Hasselquist[b]
[a]Department of Biological Sciences, PO Box 413, University of Wisconsin-Milwaukee, Milwaukee, WI 53201,USA and [b]Department of Animal Ecology, Ecology Building, Lund University, SE-223 62 Lund, Sweden

Male ornaments may advertise genetic benefits to females choosing mates. These benefits may come in the form of genes for resistance to parasites and disease. Thus, females that prefer more ornamented males as mates may receive genes for enhanced immune system function for their offspring. The common yellowthroat (*Geothlypis trichas*) is the only species to date in which a male plumage ornament (size of the black facial mask) is known to be related to extrapair mating, and extrapair young are more immunocompetent than their within-pair half-sibs (at least in colder years). To investigate whether male mask size signals superior immune function, we examined male ornaments (mask and bib size and color) in relation to measures of overall health (hematocrit) and immune system function (plasma immunoglobulin G [IgG] concentration and cutaneous immune activity). We also investigated the role that testosterone may play in mediating the relationship between ornaments and immunity. Male mask size was correlated positively with IgG level, suggesting that male mask size may indicate humoral immunity. However, mask size was correlated negatively with hematocrit and cutaneous immune activity (our measure of nestling immunocompetence). Bib size and color were not related to these indices of immune function or health. Plasma testosterone level was neither related to immune function or health nor to the expression of male ornaments. These results suggest that there might be a trade-off between immune system components, as well as between immunity and the production or advertisement of male ornaments. *Key words:* genetic benefits, immune system, mate choice, secondary sexual traits, sexual selection, testosterone. *[Behav Ecol]*

Male ornaments and courtship displays are thought to have evolved as honest indicators of quality to females seeking mates (Andersson 1994). In some species, females receive only sperm from males and no direct resources, so male ornaments may advertise indirect genetic benefits to females, including resistance to parasites and pathogens (immunocompetence). Several hypotheses attempt to explain how male ornaments may be costly and act as honest signals of immunocompetence to females searching for mates (Westneat and Birkhead 1998).

In general, ornaments may be honest indicators if males face a trade-off between producing or advertising an ornament and maintaining their immune system. This trade-off could occur for 2 reasons: 1) limited resources (e.g., energy or limiting nutrients) are needed for both (Wedekind and Folstad 1994; von Schantz et al. 1999; Blount et al. 2003) and (2) androgens, such as testosterone (T), may mediate this trade-off because they play a role in ornament development and breeding behavior, but they also inhibit the immune system (e.g., immunocompetence handicap hypothesis, Folstad and Karter 1992). There are numerous correlative studies that have examined T and immunosuppression in males, but the relationship remains unclear (reviewed in Roberts et al. 2004). In contrast, experimental studies indicate that there is often a negative trade-off between ornament production and immune system function (Verhulst et al. 1999; Faivre, Grégoire, et al. 2003).

There are only a handful of avian species in which a sexually selected male ornament is associated with both female choice of social mates and male immunocompetence (Faivre, Préault,

et al. 2003; Saino et al. 2003; Mougeot and Redpath 2004; Ekblom et al. 2005). In these cases, females could potentially use the expression of sexual ornaments to choose social mates with stronger immune responses. However, to date, there are no studies that have examined whether these signals might also function in extrapair mate choice, where it is more likely that the benefits are solely genetic.

In this study, we examined the relationships between male ornaments and adult immunocompetence in the common yellowthroat (*Geothlypis trichas*), a socially monogamous wood warbler in which both social and extrapair mating success is related to the size of the male's black facial mask (46% of broods contain extrapair young, Thusius et al. 2001). We have evidence from mate choice experiments and radio tracking that females prefer males with larger masks (Tarof et al. 2005; Pedersen et al. 2006). Furthermore, common yellowthroats are one of a few species studied to date in which extrapair young are more immunocompetent than within-pair young, at least in a year with colder temperatures (Garvin et al. 2006). Thus, a remaining question is whether the ornaments of male common yellowthroats signal their immunocompetence and general health to females.

Here, we examined this question using hematocrit as a measure of general health and immunoglobulin G (IgG) concentration and response to a subcutaneous injection of phytohemagglutinin (PHA) as 2 measures of immunocompetence. If male ornaments signal health and immune function, then we would expect higher IgG concentration and larger responses to PHA, as well as higher hematocrit levels, from more ornamented males. However, studies have shown that different branches of the immune system may be related independently to the same trait (Johnsen and Zuk 1999; Blount 2003; Faivre, Préault, et al. 2003), and even one type of test (e.g., PHA) can involve both innate and adaptive components of the immune system (Martin et al. 2006). Thus, care must be

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Address correspondence to P.O. Dunn. E-mail: pdunn@uwm.edu.

taken when interpreting the results of immune activity tests (Adamo 2004). We also measured endogenous levels of T to examine whether T is related to immunity and ornamentation as predicted by the immunocompetence handicap hypothesis (Folstad and Karter 1992). If T is immunosuppressive as predicted, we would expect individuals with higher levels of T to have decreased measures of immunity compared with individuals with lower T levels.

METHODS

Common yellowthroats breed from May to August in wetlands throughout most of North America. Males arrive first and establish territories where they display to attract females (Guzy and Ritchison 1999). Visual displays include the black facial mask that is pigmented with melanin and a bright yellow bib (throat, breast, and belly) that is pigmented with carotenoids, primarily lutein (McGraw et al. 2003). Females choose a mate shortly after arriving and build a well-concealed nest less than 1 m above the ground. Only females incubate, but both sexes feed the nestlings. Molt occurs after the breeding season and extends into the fall migration (August–September, Guzy and Ritchison 1999).

This study was conducted at the University of Wisconsin-Milwaukee (UWM) Field Station in Saukville, WI (43°23'N, 88°01'W) from May to August 2003–2005. Males were captured in mist nets throughout the breeding season and banded with unique combinations of 3 colored plastic bands and a United States fish and Wildlife Service band. Small (30–50 μl) blood samples were collected in capillary tubes from all individuals and centrifuged. Hematocrit, the proportion of red blood cells to total volume, was measured after centrifugation and used to indicate metabolic activity and current health of individuals (Pap and Márkus 2003). Low hematocrit levels may indicate anemia caused by blood parasites or various infections (Ots et al. 1998), but they may also increase in response to high workload (Pap 2002). Blood plasma was stored at –20 °C for later ELISA (enzyme-linked immunosorbent assay) analysis of immunoglobulin levels. We measured body mass; length of the wing, tail, and tarsus of adults; and photographed males to estimate the size of the plumage ornaments. Mask and bib size were measured using image analysis software (details in Thusius et al. 2001), and size estimates were highly repeatable between multiple pictures of both the mask and bib ($F_{126,127} = 80.7$, $R = 0.98$ and $F_{126,127} = 9.11$, $R = 0.80$ for mask and bib, respectively) as well as between persons ($F_{15,25} = 79.0$, $R = 0.98$ and $F_{16,17} = 44.8$, $R = 0.96$ for mask and bib, respectively). Bib color was estimated by measuring hue, saturation, and brightness (H, S, B) of the bib using Adobe Photoshop Elements 2.0 (Adobe Systems, San Jose, CA, see Tarof et al. 2005). We repeated our analyses using the scores from a principal components analysis of all ornaments (mask and bib size and bib H, S, B), and the results were similar (not shown).

Immunoassays

Cutaneous immune activity was measured as the local inflammatory response to a subcutaneous injection of PHA. The PHA skin test has been considered a condition-dependent indicator of an individual's cell-mediated immunity (Goto et al. 1978; Cheng and Lamont 1988), and it is correlated with survival in the wild (Navarro et al. 2003; reviewed in Norris and Evans 2000). However, recent papers have shown that the swelling response involves multiple immune cell types and measures aspects of both the innate and the adaptive (humoral and cell mediated) branches of the immune system, and swelling size should be interpreted as a measure of cuta-

neous immune activity, rather than cell-mediated immunity alone (Kennedy and Nager 2006; Martin et al. 2006). Captive adult males ($n = 27$) were tested with a subcutaneous injection (10 μl) of 0.05 mg PHA (Sigma-Aldrich, Inc., St Louis, MO) dissolved in saline into the right wing web with an equal volume of saline injected into the left wing web as a control (Lochmiller et al. 1993). Wing thickness was measured to the nearest 0.01 mm before injection and 24 (± 1) h later using a digital thickness gauge (Mitutoyo model 700-118). Several measurements were taken (all by J.C.G.) of each wing web until a consistent value was reached and recorded. Immune response was measured as the increase in wing thickness of the PHA-injected wing minus the change in the control wing. Due to the difficulty of recapturing adult males in the field at an exact time, we conducted these tests on wild males captured on their territories and brought into captivity for the immunoassay. After PHA injection, individual males were held for 24 h in separate cages of an outdoor aviary. Males were given food (mealworms and fly larvae) and water ad libitum and quickly adapted to the aviary, which was designed to mimic their natural habitat (see Tarof et al. 2005). All males except one gained weight while in captivity; the remaining male maintained his original weight. After the final measurement, males were returned to their territories. This test was conducted within a 3-week period (8–28 June 2004) during the incubation and early nestling periods. In our analyses of cutaneous immune response, we included the effects of air temperature (during the 24 h after injection) and body mass because these factors influence cutaneous immune response (Lifjeld et al. 2002; Garvin et al. 2006). Air temperature was measured at an automated weather station located on the study site.

Different components of the immune system are not necessarily correlated with each other (Johnsen and Zuk 1999; Blount 2003; Faivre, Préault, et al. 2003), so we also examined humoral immunity by assaying the circulating IgG concentration in each male's plasma ($n = 110$ over the 3 years). IgG proteins are the most important serum proteins involved in humoral responses as they constitute the main class of antibody molecules and are thought to indicate the health or nutritional state of free-living birds (Gustafsson et al. 1994; Goldsby et al. 2003; Morales et al. 2004). High IgG concentrations are often suggested to indicate stronger humoral immunity (Johnsen and Zuk 1999; Saino et al. 2001); however, it could also indicate immune response to current infections, and, thus, interpretations must be made with caution (Norris and Evans 2000). Indeed, both high and low IgG concentrations can be interpreted as symptomatic of disease and infection (see p. 1485 of Morales et al. 2004); therefore, using multiple assays in studies of immunocompetence is recommended (Norris and Evans 2000).

Plasma IgG concentration of individuals was measured using an indirect ELISA. Our protocol was adapted from Hasselquist et al. (1999) with variations described below. Polystyrene 96-well plates were coated overnight with a commercial goat antibird IgG antibody in 0.1 M borate-buffered saline (5 μg/ml; Bethyl Laboratories Inc., Montgomery, TX). Plates were washed with 0.01 M Tris-buffered saline (TBS, pH 7.5) and then blocked with 3% powdered milk in TBS. Plasma samples were diluted to 1:25 000, 1:50 000, 1:100 000, and 1:200 000 in 1% powdered milk in TBS. After 2 washes, the samples were added to the plates in duplicate. Plasma of a single individual diluted to 1:25 000 was used as a positive control. After incubation and 4 washes in TBS and 0.05% Tween 20, rabbit antipasserine plasma was added (diluted 1:2000, produced against red-winged blackbird *Agelaius phoeniceus* antibodies, Hasselquist et al. 1999). After incubation and wash, a commercial horseradish peroxidase–conjugated goat antirabbit

antibody (Southern Biotechnology Associates, Inc., Birmingham, AL) diluted to 1:20 000 in 1% powdered milk in TBS was added to the plates. After a last incubation and wash, the peroxidase substrate orthophenylenediamine-HCl (0.67 mg/ml, Acros Organics USA, Morris Plains, NJ) was added to the wells. Kinetic readings of the plates were taken every 30 sec for 10 min at 450 nm and analyzed using SoftMax PRO plate reader software (version 4.6, Molecular Devices, Sunnyvale, CA; 2003). The mean of each duplicate was used as the IgG titer and was given as the slope of the substrate conversion over time (Vmax) measured in the units $10^{-3} \times$ optical density per minute (mOD/min). Vmax ranged from 57.5 to 131.0 mOD/min. In our analyses, we included absorbance values for positive and negative controls on each plate to account for variation between plates. Individual antibody levels at the 4 different dilutions were all positively correlated with each other (all pairwise correlations $P < 0.0001$); for simplicity, we used results from the 1:25 000 dilution for analyses ($n = 110$) because it was the most consistent dilution. Repeatability between duplicate samples of the same individual on the same plate was high ($R = 0.948$, ANOVA $F_{105,104} = 37.5$, $P < 0.0001$ for all individuals).

Testosterone assay

We used a commercial competitive immunoassay (T Enzyme Immunoassay Kit; Assay Designs, Inc., Ann Arbor, MI) to measure the total circulating levels of T in male plasma samples ($n = 49$ over all 3 years; sufficient plasma for this analysis was not available from all males). Samples (5 µl plasma per well, diluted 1:20 in kit buffer) were run in duplicate and according to manufacturer specifications. Each ELISA plate contained a complete standard curve as well as positive and negative controls. Plasma T concentrations (ng/ml) were calculated from the standard curve (R^2 ranged from 0.997 to 1) using SoftMax PRO plate reader software. The intraassay coefficient of variation as calculated from the standards was less than 5% for all 4 plates. T concentrations of all samples were within the sensitivity range of the assay. Validation tests of this commercial immunoassay have demonstrated accurate measures of T in house finches (*Carpodacus mexicanus*, Deviche and Cortez 2005). T concentrations were natural log transformed to achieve a normal distribution.

Statistical analysis

We used multiple regression to test the prediction that male ornaments were related to immune system function and health. We conducted separate multiple regression models for each measure of immunity and health in relation to all 3 ornaments (mask and bib size and bib color) and potentially confounding variables such as date, body mass, and air temperature (for PHA response). Adding breeding stage (prelaying, incubation, nestling, fledgling, and postbreeding) to these analyses did not change the results qualitatively (not shown), but they reduced sample sizes considerably because many birds were sampled outside our main study area and had unknown nesting histories. To find the ornament most closely correlated with immunity or health and to choose a final model that was simple yet had high predictive power, we used backward stepwise regression with mixed selection (P to enter or leave = 0.15) in the statistical program JMP (version 5.0.1; SAS Institute, Cary, NC; 2003). We compared the final stepwise regression model with similar models containing other important predictors (ornaments) using Akaike's Information Criterion, which is a statistical model selection criterion that accounts for model complexity (tested in Helu et al. 2000). In all cases, the 2 methods produced concordant results. We analyzed the relationship between ornaments and T in the

same manner. The effect of T on immune parameters was analyzed using bivariate regression analyses. Sample sizes differ because not all measurements were available for all males. All tests were 2 tailed, and means are reported with their standard error (SE) unless stated otherwise.

RESULTS

Correlations between ornaments

We performed pairwise correlations between all ornaments to determine if they were independent of each other ($n = 114$ males). Bib size was correlated positively with mask size ($r = 0.53$, $P < 0.001$) and bib saturation ($r = 0.35$, $P < 0.001$), but bib size was correlated negatively with bib hue ($r = -0.23$, $P = 0.01$), and it was not correlated with bib brightness ($r = -0.10$, $P = 0.27$). Mask size was correlated positively with bib saturation ($r = 0.20$, $P = 0.03$), but there was no correlation with bib hue or brightness ($P > 0.41$).

Ornaments in relation to immunocompetence and hematocrit

Cutaneous immune response was related negatively to mask size ($P = 0.024$) and positively to body mass ($P = 0.011$) in the final multiple regression model, which also included mean air temperature during the day after injection (Table 1, Figure 1). This sample consisted of 27 adult males in 2004 that were held 24 h in the aviary to measure their response to injection of PHA. Bib size, bib color (H, S, and B), and date were included in the initial set of predictors but were excluded in the final model (Table 1). There was no relationship between cutaneous immune response and hematocrit ($n = 27$, $r = 0.121$, $P = 0.548$), but there tended to be a negative relationship between cutaneous immunity and IgG level ($n = 27$, $r = -0.32$, $P = 0.099$).

Among 110 males sampled in the wild over all 3 years, plasma IgG levels were higher in males with larger masks ($P = 0.019$; Figure 1) and later in the season (date, $P < 0.008$; Table 1). Bib size, bib color, body mass, and year were included in the initial set of predictors but were excluded in the final stepwise model (Table 1).

Hematocrit was correlated negatively with IgG levels ($n = 106$ over all 3 years, $r = -0.257$, $P = 0.008$), so as might be expected, we found the opposite pattern between hematocrit and ornaments as was found with IgG. Hematocrit was correlated negatively with both mask size ($P = 0.011$) and date ($P < 0.001$) in the final stepwise model which also included body mass (Table 1, Figure 1). Thus, higher hematocrit levels were found earlier in the season and in males with smaller masks. Bib size, bib color (H, S, and B), and year were included in the initial set of predictors but excluded in the final model (Table 1).

Ornaments and immunocompetence in relation to testosterone

Plasma T concentrations were highly variable across our sample population (untransformed mean ± standard error: 2037 ± 251.0 ng/ml, $n = 49$, range: 425–8625 ng/ml), which may be due to the wide range of sampling dates (16 May–22 July; mean = 14 June) and the overall seasonal decline in T concentration ($r = -0.30$, $P = 0.039$), as found in other studies (Peters et al. 2001; Sockman et al. 2004). T concentration (log transformed) was correlated positively with body mass ($P = 0.016$) but tended to be lower in males with larger bibs ($P = 0.052$) and bibs with greater hue ($P = 0.018$; Table 2). T levels were greatest in 2005 (4373 ± 407 ng/ml) when many samples were taken earlier in the season and lower in 2003 (2266 ± 596 ng/ml) and 2004 (1750 ± 268 ng/ml). Mask size was not a predictor of T level in the final model (Table 2).

Table 1

Multiple regression models of immune response and health of male common yellowthroats in relation to ornaments

Predictors	Cutaneous immunity		IgG		Hematocrit	
	Estimate	P	Estimate	P	Estimate	P
Mask size	**−0.008**	**0.024**	**0.001**	**0.011**	**−0.027**	**0.011**
Bib size						
Bib hue						
Bib saturation						
Bib brightness						
Mass	**0.561**	**0.011**			1.43	0.103
Date			**0.002**	**0.003**	−0.090	**<0.001**
Year				**0.012**		
Air temperature	−0.045	0.179				
n	27		110		110	
Adjusted R^2	0.238		0.55		0.177	

The first 8 predictors were included in all initial models, whereas air temperature was only included in the model for cutaneous immunity (measured in 2004 only). The final stepwise model for immunoglobulin G (IgG) also included absorbance values for positive and negative controls on each plate to account for variation between plates. Variables included in the final stepwise model are indicated by their estimates and P values. Significant P values are in bold.

There was no evidence of immunosuppression by T. Neither the cutaneous immune response ($n = 19$; slope $= 0.46 \pm 0.29$, $r^2 = 0.13$, $P = 0.129$) nor IgG levels were related to T concentration ($n = 49$, slope $= 0.11 \pm 0.04$, $r^2 = 0.002$, $P = 0.768$). T concentration was also not related to hematocrit ($n = 48$, slope $= 0.36 \pm 0.99$, $r^2 = 0.003$, $P = 0.716$).

DISCUSSION

We investigated whether male ornaments that are known to be preferred by females in social and extrapair mate choice (Thusius et al. 2001) signal immunocompetence and general health. We found that male common yellowthroats with larger masks had higher IgG levels but lower cutaneous immune responses (PHA induced) and hematocrit levels. These opposing relationships could indicate trade-offs between components of immunity or between immune system function and ornamentation. Although the other ornaments (bib size and color) were not related to our measures of immunity or health, bib size was correlated positively with mask size, and larger bibs had more saturated yellow than smaller bibs, suggesting that the production of different plumage traits may not be independent. T levels were not correlated with ornamentation or measurements of immunity and health, which suggests that in this species T may not function as predicted by the immunocompetence handicap hypothesis, at least early in the breeding season. Below, we discuss how these results relate to sexual selection and honest advertisement in common yellowthroats and their implications for future studies of ecological immunology and behavioral ecology.

Signaling function of ornaments

Males with larger masks had higher IgG levels and, thus, stronger humoral immune function than males with smaller masks. Neither bib size nor color was related to humoral immunity. Males with larger masks are more likely to sire extrapair young (Thusius et al. 2001; Garvin et al. 2006), so females choosing males with larger ornaments may gain offspring with greater humoral immunity, if it is heritable (Råberg et al. 2003; Kilpimaa et al. 2005). The humoral component of the immune system is considered to be the most important defense against extracellular bacteria and parasites (Goldsby et al.

2003), and male common yellowthroats with larger masks are more likely to have blood parasites (hematozoa) than males with smaller masks (Dunn P, unpublished data). Thus, the higher humoral immune response of larger masked males may reflect the male's immunocompetence, but it may also reflect the immune response to current infections (Norris and Evans 2000). In our population, males with larger masks are dominant and preferred by females (Tarof et al. 2005), so it seems more likely that males with larger masks are more immunocompetent and can overcome the handicap of higher blood parasite loads.

Although mask size was related positively to a measure of humoral immunity, it was related negatively to cutaneous immune activity and hematocrit. Bib size and bib color were not related to either of these variables. These results are paradoxical, as extrapair males have larger masks and young sired by extrapair males have stronger cutaneous immune responses than their within-pair half-sibs, at least in colder years (Garvin et al. 2006). Thus, we would have predicted a positive relationship between mask size of adult males and cutaneous immune response. There are at least 2 nonexclusive explanations for these apparently incongruent findings. First, resources required for the synthesis or advertisement of ornaments, in this case the black facial mask, and those required for overall condition (as reflected by cutaneous immune response) and health maintenance may be limited, and, as a consequence, males may face a trade-off between allocating resources to ornaments at the expense of cutaneous immune activity or hematocrit (Wedekind and Folstad 1994; Westneat and Birkhead 1998). In contrast, the resources required for humoral immunity may not trade off with the costs of ornamentation. The immunocompetence handicap hypothesis specifically predicts that only those individuals with greater immune and health capacity can afford the cost of producing an elaborate ornament (Folstad and Karter 1992). However, as Getty (2002) cautions, these costs may be relative to the condition of the individual, so individuals in good condition suffer relatively less from immunosuppression than individuals in poorer condition, even if they have lower absolute measures of immunity or general health at a particular time.

Additionally, the relationship between ornaments and measures of immunity may be sensitive to the reproductive status of the male, as in red jungle fowl (*Gallus gallus*, Zuk
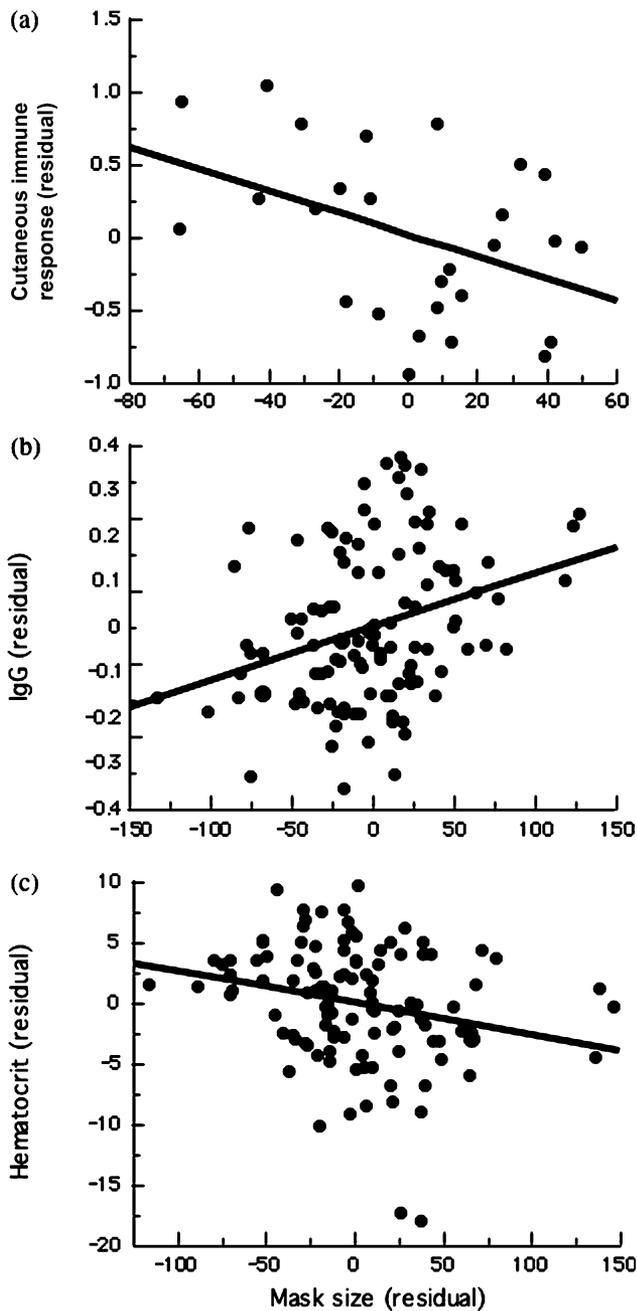
Figure 1

Measures of immunity and health in relation to male mask size.
a) Cutaneous immune response, b) humoral immunity measured as
plasma immunoglobulin G (IgG) concentration, and c) hematocrit
level. The residuals plotted here are from the multiple regression
analyses in Table 1.

Table 2

**Stepwise regression model of natural log–transformed plasma
testosterone levels in relation to male ornaments**

| | Testosterone | |
Predictors	Estimate	P
Mask size		
Bib size	−0.001	0.052
Bib hue	−0.040	**0.018**
Bib saturation		
Bib brightness		
Mass	**0.408**	**0.016**
Date	−0.008	0.144
Year		**0.005**
n	49	
Adjusted R^2	0.516	

All predictors on the left were included in the initial model, whereas
predictors included in the final model are indicated by their estimates
and P values. Significant P values are in bold.

nity (Greenman et al. 2005), and individuals expending rela-
tively more reproductive effort or increased activity (Ewenson
et al. 2003) may be expected to have lower cutaneous immune
responses. Finally, parasite prevalence is also related to sup-
pressed cutaneous immune activity (Johnsen and Zuk 1999;
Christe et al. 2000), and larger masked male yellowthroats are
more likely to have blood parasites, which might lead to lower
cutaneous immunity. Together, these studies suggest that
negative relationships between ornaments and cutaneous im-
mune activity during the breeding season may be an adaptive
response to divert resources to reproduction and suppress
some costly immune responses.

Trade-offs between different aspects of health and immunity

A second, and perhaps simpler, explanation for the different
relationships between mask size and immunocompetence is
that it may reflect a trade-off in the allocation of resources
between different immune and health functions, rather than
a direct trade-off between immunocompetence and the orna-
ment. For example, we found that IgG and hematocrit levels
were inversely related to each other; a similar relationship
has also been found in pied flycatchers (*Ficedula hypoleuca*,
Kilpimaa et al. 2004). However, in a multiple regression of
mask size on PHA response, IgG, hematocrit, body mass,
and air temperature, we still found negative relationships be-
tween mask size and both hematocrit ($F_{1,21} = 5.4$, $P = 0.03$)
and cutaneous immunity ($F_{1,21} = 6.4$, $P = 0.02$), suggesting
that our results were not simply due to a trade-off between IgG
and hematocrit or cutaneous immune response. These rela-
tionships could also be the result of seasonal variation, as date
had a significant effect on both IgG and hematocrit levels.
Note, however, that IgG increased and hematocrit decreased
with advancing date.

There tended to be a trade-off between cutaneous and hu-
moral immunity ($P = 0.099$) which Norris and Evans (2000)
suggested might occur when resources are limited. This trade-
off may be caused by nutritional and energetic cross-regulation
of differentiated T-helper lymphocytes, such that when the hu-
moral component of immunity is activated, the cell-mediated
component is downregulated (Long and Nanthakumar 2004).
Consistent with this idea, Northern bobwhite chicks (*Colinus
virginianus*) raised on a poor diet had suppressed cutaneous
but not humoral immunity (Lochmiller et al. 1993). More re-
cently, it has been shown that male ornamentation can also be
related to these different components of the immune system

and Johnsen 1998). In house sparrows (*Passer domesticus*),
males with larger black bibs (a condition-dependent
melanin-based trait, Veiga and Puerta 1996, but see Gonzalez,
Sorci, Møller, et al. 1999) have greater cutaneous immune
activity in the nonbreeding season but lower cutaneous im-
mune activity during the breeding season (Gonzalez, Sorci,
and de Lope 1999). Thus, it is possible that male common
yellowthroats with larger masks have lower cutaneous immune
responses in the breeding season because they invest relatively
more in display, territory maintenance, pursuing extrapair
fertilizations, or mate guarding (Pedersen et al. 2006).
Indeed, induction of breeding can suppress cutaneous immu-

independently. In European blackbirds (*Turdus merula*), bill color is a sexually selected trait, and brighter bills are correlated positively with cutaneous immunity but negatively with humoral immunity (Faivre, Préault, et al. 2003). Thus, as we found in common yellowthroats, ornaments may vary in their relationships with different measures of immunity because of trade-offs within the immune system. Experimental studies will be needed to tease apart these relationships and potential trade-offs. Together, these results emphasize the importance of using more than one measurement of immune system function or health when investigating the signaling function of male ornaments (Norris and Evans 2000).

A role for testosterone?

Our study does not provide support for the hypothesis that T levels mediate the relationship between ornamentation and immunity (Folstad and Karter 1992). We found no relationship between T levels and any of our measures of immunity or health. This suggests that naturally occurring levels of T do not suppress immune system function in breeding male common yellowthroats. In contrast, T levels were related negatively to IgG levels in dark-eyed juncos, *Junco hyemalis* (Greives et al. 2006). We also found no correlation between T levels and the expression of male ornaments in common yellowthroats. This is surprising because males with larger masks are dominant over smaller masked males in aviary trials (Tarof et al. 2005) and T is often associated with social dominance (Duckworth et al. 2004). However, it may be that T levels are more important to ornament size during molt than during the breeding season.

Conclusions

Our study provides evidence that a male plumage ornament used in social and extrapair mate choice is a potential signal of enhanced humoral immunity (plasma IgG concentration). Thus, females could potentially gain offspring with greater humoral immunity by choosing mates with larger masks. In a study of nestlings, we found that, in a colder year, extrapair young had a stronger cutaneous immune response than within-pair young (Garvin et al. 2006). Although the relationship between mask size and cutaneous immune response was negative in adult males, contrary to what might be expected, the regulation of immunity in adults and nestlings is likely to be different due to the numerous differences between these life stages (e.g., activity level, infection rate, parasite exposure and prevalence, and stress) that can affect immune responses. Future investigations will need to assess these factors, as well as the relationship between male mask size and offspring humoral immunity.

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