

## THE EVOLUTION OF IMMUNE DEFENSE AND SONG COMPLEXITY IN BIRDS

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**Abstract.**—There are three main hypotheses that explain how the evolution of parasite virulence could be linked to the evolution of secondary sexual traits, such as bird song. First, as Hamilton and Zuk proposed a role for parasites in sexual selection, female preference for healthy males in heavily parasitized species may result in extravagant trait expression. Second, a reverse causal mechanism may act, if sexual selection affects the coevolutionary dynamics of host-parasite interactions per se by selecting for increased virulence. Third, the immuno-suppressive effects of ornamentation by testosterone or limited resources may lead to increased susceptibility to parasites in species with elaborate songs. Assuming a coevolutionary relationship between parasite virulence and host investment in immune defense we used measures of immune function and song complexity to test these hypotheses in a comparative study of passerine birds. Under the first two hypotheses we predicted avian song complexity to be positively related to immune defense among species, whereas this relationship was expected to be negative if immuno-suppression was at work. We found that adult T-cell mediated immune response and the relative size of the bursa of Fabricius were independently positively correlated with a measure of song complexity, even when potentially confounding variables were held constant. Nestling T-cell response was not related to song complexity, probably reflecting age-dependent selective pressures on host immune defense. Our results are consistent with the hypotheses that predict a positive relationship between song complexity and immune function, thus indicating a role for parasites in sexual selection. Different components of the immune system may have been independently involved in this process.

**Key words.**—Bird song, bursa of Fabricius, phytohemagglutinin, repertoire size, T-cell mediated immune response.

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Parasites impose important selective pressures on their hosts by affecting the evolution of their sexual characters (Hamilton and Zuk 1982). Since avian song is one of the most extensively studied secondary sexual traits (Searcy and Andersson 1986; Catchpole and Slater 1995), this trait is a good candidate for investigating the roles of parasites in signal expression both within and among species (Hamilton and Zuk 1982; Read and Weary 1990; Saino et al. 1997a; Buchanan et al. 1999; Møller et al. 2000; Duffy and Ball 2002; Garamszegi et al. 2003). However, tests are often criticized because of their narrow focus both in terms of secondary traits and of parasites considered (Clayton 1991; Weatherhead et al. 1993; Møller et al. 1999), and the relationship between parasitism and ornaments is less than clear. The effect of a given parasite on fitness and trait expression of a host is probably small, because hosts may be parasitized by many kinds of pathogens. The intensity of infection with different species of parasites generally is not strongly correlated, thus prevalence data of a specific pathogen does not reflect in general the impact of parasites on host fitness in the past (Weatherhead et al. 1993; Møller et al. 1999). Therefore, the association between sexual display and health status may be expected to be stronger if measures of general antiparasite defense such as immune responses are investigated, rather than infection with particular parasites (Møller et al. 1999).

The avian immune system is comprised of two major arms of defense, namely humoral and cell-mediated defense (Toiv-

anen and Toivanen 1987). The humoral immune response includes the activity of B-cells that differentiate in the bursa of Fabricius. The relative size of this immune defense organ in birds may reflect current health state and ability to respond to an infection (Rose 1981; Glick 1983; John 1994). Several parasite interactions can increase the size of this lymphoid tissue (Toivanen and Toivanen 1987; Glick 1994). For example, selection for increased and decreased immune response to an immune challenge by sheep red blood cells resulted in correlated response to selection for the size of the bursa of Fabricius (Parmentier et al. 1995). In addition, relative bursa size was related to hole-nesting habits, migration and coloniality in birds, which are all factors that can involve high risk of parasitism (Møller and Erritzøe 1996, 1998).

Cells differentiating into three populations of T-lymphocytes in the thymus are responsible for the cellular immune response in adult birds. The efficiency of the T-cell mediated components of the immune system may be assessed by phytohemagglutinin (PHA) challenge (Goto et al. 1978; McCorkle et al. 1980; Smits et al. 1999). Injection with PHA causes local activation and mitogenic proliferation of T-cells, followed by local recruitment of inflammatory cells and major histocompatibility complex molecules (Goto et al. 1978). The PHA test has become an established method, because it can be performed in a standard fashion across species and is highly repeatable (Tella et al. 2002). Further, measures of T-cell immune responses are not confounded by parasite infection levels, because studies that experimentally inoculated

nests with parasites did not find a change in PHA response compared with control nests (Brinkhof et al. 1999; Saino et al. 1998). Intraspecific correlates of T-cell response involve an array of factors such as diet, body condition, exposure of parasites, sex, molt, extrapair paternity and sexual ornaments (see review in Tella et al. 2002). Among species, PHA response was positively correlated with body size, host sociality, parasite-induced mortality, development rate, and longevity (Martin et al. 2001; Møller et al. 2001; Tella et al. 2002).

The aims of this paper were to study interspecifically the relationship between avian song complexity and immune response, as assessed by T-cell mediated immune response and relative bursa mass among passerine species. Previous comparative studies have provided equivocal results regarding the role of parasites in the expression of bird song. The original test by Hamilton and Zuk (1982) used blood parasite prevalence as a measure of parasite impact on hosts, but it was criticized by Read and Weary (1990), who failed to find an association between hematozoa prevalence and song elaboration after controlling for phylogenetic relationships. However, after recognizing the importance of the use of general antiparasite defenses, Møller et al. (2000) demonstrated that spleen size, reflecting the additive importance of efficient humoral and cell mediated immune responses, was positively correlated with song type repertoire size. Here, by using response to phytohemagglutinin challenge and the relative size of the bursa of Fabricius, we examine the relationship between bird song and parasitism while separating the effects of B- and T-cell mediated immune response. Throughout this paper we assume that larger relative bursa size and higher T-cell immune response represent superior ability to mount an immune response, and that increasing parasite virulence selects for greater investment in immune function.

There are at least three hypotheses that predict covariation between features of song and immune defense among species. First, sexual selection is expected to have favored trait expression and investment in immune response in species experiencing high parasite pressure, because of female mate preferences for parasite resistant males (Hamilton and Zuk 1982). Thus, secondary sexual characters such as songs will evolve to reveal the health status of males because only males in prime condition are able to produce the most exaggerated signals. Second, the causal association between parasite resistance and sexual displays may have acted in the opposite direction. Sexual selection may affect the coevolutionary dynamics of host-parasite interactions by selecting for increased virulence and hence high investment by hosts in immune function (van Baalen 1998). The arms race between parasites and their hosts may be particularly intense in sexually selected species, because 'good genes' sexual selection in hosts continuously selects for improved ability of parasites to exploit their hosts. Alternatively, if sexual selection results in an increased frequency of horizontal transmission of parasites because of repeated encounters between the most preferred males and females (Bull 1994; Frank 1996), this should result in an evolutionary increase in virulence being associated with sexual selection and thus song production. Sexual selection through sperm competition and the associated multiple matings by females gives rise to multiple contacts among hosts,

and any directly transmitted parasite may thus change its levels of virulence in response to such altered transmission patterns. The increased virulence in host species with frequent horizontal transmission of parasites should lead to selection for enhanced immune function independent of the benefits of female choice. Because greater song output and complexity have been associated interspecifically with factors that are presumed to produce more intense inter- or intrasexual selection (Read and Weary 1992), we predicted that measures of immune function are positively associated with song complexity under the two hypotheses above. Although each hypothesis relates to selection through parasitism, we are aware that by using comparative approaches it is impossible to distinguish between these two hypotheses. However, a positive relationship between song display and a measure of immune function would indicate a role of parasites in sexual selection. Third, the possession and the maintenance of ornaments having immuno-suppressive side effects (Sheldon and Verhulst 1996) may result in certain species investing heavily in song production by reducing the efficiency of immune defense. For example, Folstad and Karter (1992) have proposed a role for testosterone whereby parasitism can constrain signaling mechanisms. Their immunocompetence handicap hypothesis suggests that the immuno-suppressive effect of steroid production will enforce reliability of signals of parasite resistance among individuals. The interspecific consequence of this mechanism is that species investing more in the expression of bird song by simultaneously elevating testosterone levels should suffer from immuno-suppression, resulting in relatively poor immune defense. However, alternative mechanisms of immuno-suppression based on resource allocation may also be important (Wedekind and Folstad 1994; Sheldon and Verhulst 1996). Trait expression entails costs to individuals in terms of limiting resources, which may lead to fewer resources being devoted to other important functions, such as immune defense. The hypothesis of immuno-suppressive cost of ornamentation by testosterone or limited resource allocation predicts immune function will be negatively correlated with song complexity interspecifically.

We tested whether passerine bird species that have evolved complex songs had higher T-cell mediated immune response and larger relative bursa size, while controlling for phylogenetic relationships and potentially confounding factors, such as body size, coloniality, and migration. We also checked whether the relationship between immunity and song complexity depended on age, by using T-cell response of nestlings and adults, given that previous studies have reported different selection pressures for cell-mediated immune response acting on chicks and adults (Martin et al. 2001; Tella et al. 2002).

#### MATERIALS AND METHODS

T-cell mediated immune response was measured as the response to a challenge with PHA by APM., J. Blount, P. Christie, J. Fair, M. Martín-Vivaldi, and J. Soler. Birds were injected with 0.05 ml of 0.2-mg PHA in one wing web and 0.05 ml of physiological water in the other wing web at premarked sites. The dose of PHA used in this study is similar to that used in other studies of free-living or captive birds

(e.g., Lochmiller et al. 1993; Saino et al. 1997b; González et al. 1999; Soler et al. 1999). Before injection, the thickness of the patagium in both wings was measured using a spessimeter, with an accuracy of 0.01 mm. A second measurement was taken six hours after injection in adults and 24 h after injection in nestlings. We measured adult immune response after six hours because studies of temporal change in T-cell immune response showed no further change after six hours (Goto et al. 1978; Navarro et al. 2003). Previous studies have demonstrated that this measure has a very high repeatability (exceeding 0.90), as shown by three independent measurements of both wing webs (Møller et al. 2001). We used the increase in thickness of the wing injected with PHA minus the increase in thickness of the wing injected with physiological water as a measure of the intensity of the immune response. We collected T-cell response data in the field during the breeding seasons of 2000–2001. For each species, on average 9.50 adult (SE = 1.70; range = 1–61,  $n = 40$ ) and 36.67 nestling (SE = 21.15; range = 1–1073,  $n = 42$ ) T-cell response tests were performed. Data for swallows and martins were from Møller et al. (2001), for wild *Taeniopygia guttata* from Ewenson et al. (2001), and for *Junco hyemalis* from Casto et al. (2001). Tella et al. (2002) showed that cell mediated immune response from different studies independent of exact methods were highly repeatable and consistent in magnitude.

We obtained information on the size of the bursa of Fabricius from post mortem examination of dead birds brought to a taxidermist (JE) between 2 February 1994 and 12 October 2001. The mass of this organ was measured on a balance to the nearest 0.1 g. The relative size of the bursa was significantly repeatable ( $R = 0.74$ ,  $F_{71,619} = 29.32$ ,  $P < 0.001$ ). The bursa of Fabricius regresses during sexual maturity (Glick 1983; Toivanen and Toivanen 1987) and this may potentially cause bias; however, the date of sampling was not related significantly to relative bursa size (Spearman rank order correlation,  $P > 0.40$ ). We used bursa mass data for 424 individuals of 33 species.

We primarily obtained information about song type complexity from Read and Weary (1992). We intended to use song repertoire size as the measure of long-term song complexity, and syllable repertoire size to reflect short-term song complexity, because previous comparative studies demonstrated that these estimates are subjects of sexual selection (Read and Weary 1992; Møller et al. 2000). However, available song data from the literature were limited: in our dataset on immune response we found information on song type repertoire size and syllable repertoire size for 23 and 19 species, respectively. Our preliminary calculations indicated that to detect an explanatory effect by immune response of 10% of the variance in song complexity at 80% power, a sample size of 73 is needed. Hence, we were conscious that a test of our predictions using these variables was likely to suffer from insufficient statistical power.

Therefore, to increase sample size, as a surrogate of syllable repertoire size we used our measure for short-term syllable repertoire size by analyzing sonagrams in handbooks (Cramp and Perrins 1985–1994; Poole et al. 1993–2002). This analysis was done by Garamszegi without knowledge of the immune response data. Sonagrams for male adver-

tisement territorial songs were selected for 57 species (mean  $\pm$  SE =  $2.491 \pm 0.133$  songs; range = 1–5) to estimate syllable repertoire size. Syllables were defined as continuous figures (or groups of figures) separated by noticeable time intervals or frequency switches in the spectrograms. The availability of sonagrams for different species may vary due to typographical space limits and differences in study intensity resulting in potential biases. To control for these confounding effects, we calculated the average syllable repertoire size/length of the analyzed songs. Providing references (Cramp and Perrins 1985–1994) and keys for unit definition, we asked two ornithologists independently to estimate song complexity for a subset of birds, without providing information about the purpose of the study. The repeatability of song complexity calculated among the three observers (including Garamszegi's measurements) was very high ( $R = 0.98$ ,  $F_{10,22} = 159.9$ ,  $P < 0.0001$ ). In many species more than one song was analyzed for syllable repertoire size, allowing us to determine within-species repeatability of measurements, which also appeared to be very high (for a larger dataset:  $R = 0.76$ ,  $F_{93,139} = 9.866$ ,  $P < 0.0001$ ). This is important because handbooks often present sonagrams for different populations of the same species, and individuals from different populations are more likely to differ than individuals from the same population. We examined the reliability of our estimate by testing the prediction that a positive relationship should exist between variables designated to reflect similar measures of song complexity. Our relative syllable repertoire size measurement was significantly positively correlated with syllable repertoire size as reported by Read and Weary (1992; for a larger sample, Pearson's  $r = 0.438$ ,  $n = 93$ ,  $P < 0.001$ ; for the present dataset, Pearson's  $r = 0.593$ ,  $n = 17$ ,  $P = 0.012$ ). Therefore, in addition to song type repertoire size from Read and Weary (1992) we used our relative syllable repertoire size assessment, under the assumption that this is an appropriate and reliable estimate of short-term song complexity that can be measured with high repeatability. A song type repertoire size scored as infinity by Read and Weary (1992) was assigned an arbitrary value of 1000.

We controlled for body size, migration, and coloniality, because these variables are related to bird song and measures of immune defense, and may potentially be confounding (Read and Weary 1992; Côte and Poulin 1995; Møller and Erritzøe 1996; Møller et al. 2001; Tella et al. 2002). We used our own body mass measurements to reflect body size. We obtained information on migration and coloniality from handbooks (Cramp and Perrins 1985–1994). Colonial species were given a score of 2; semicolonial species a score of 1, and solitary species a score of 0. Migration was scored as 1 for migratory species, and 0 for resident species. These scores represented increasing levels of coloniality and migration. Because we intended to control for confounding effects applying a multivariate approach based on independent linear contrasts (Harvey and Pagel 1991), we treated these variables as continuous, since intermediate states between character states are biologically meaningful. The complete dataset is given in the Appendix.

After Felsenstein (1985), for a comparative analysis to be feasible, song complexity measures, T-cell mediated immune

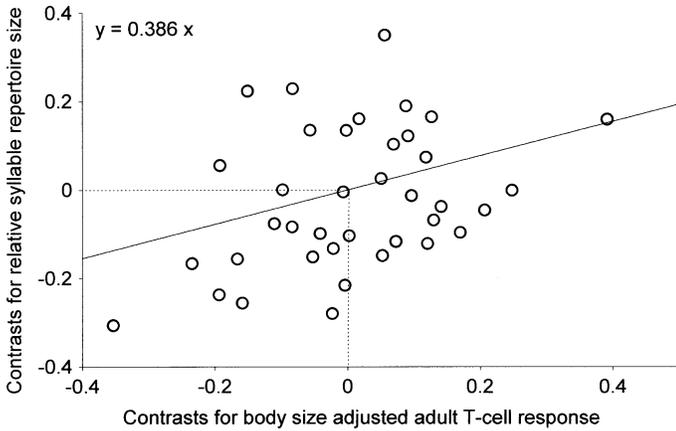


FIG. 1. Regression of short-term song complexity (number of unique syllables within songs/song length) on T-cell mediated immune response, after removing allometric effects by using residuals after controlling for body mass. Datapoints are phylogenetically independent linear contrasts ( $N = 38$ ). The line and equation are from linear regression forced through the origin.

response, the mass of the bursa of Fabricius, and body mass were  $\log_{10}$ -transformed.

We controlled for similarity among species due to common descent by linear regression analysis based on statistically independent contrasts (Felsenstein 1985; Harvey and Pagel 1991) using Phenotypic Diversity Analysis Programs (PDAP; Garland et al. 1993). Our phylogenetic hypothesis was based on a composite phylogeny based on that reported by Sibley and Ahlquist (1990) and combined with additional information (Sheldon et al. 1992; Blondel et al. 1996; Arnaiz-Villena et al. 1998; Cibois and Pasquet 1999; Grapputo et al. 2001). We assumed that branch lengths were equal in the calculations of contrasts. Linear contrasts were normally distributed with the exception of migration and coloniality. For these two later variables, we calculated parametric regressions based on the ranks of these contrasts. With this approach we obtained normality and simultaneously reduced the importance of extreme datapoints.

We only present the results based on phylogenetically independent contrasts. However, similar results were obtained using species as independent datapoints.

RESULTS

A partial correlation between adult T-cell response and bursa size controlling for body mass revealed that the two components of immune function were not significantly related to each other (partial  $r = 0.079$ ,  $df = 23$ ,  $P = 0.708$ ). Entering migration and coloniality as potentially confounding variables into the analysis did not affect this relationship (partial  $r = 0.069$ ,  $df = 21$ ,  $P = 0.754$ )

In a small sample adult T-cell response was not significantly related to contrasts for song type repertoire size when controlling for allometry ( $r^2 = 0.089$ ,  $F_{2,17} = 0.833$ ,  $P = 0.452$ ; slope [SE] for body mass = 0.266 [0.319],  $P = 0.415$ ; slope [SE] for song type repertoire size = -0.050 [0.049],  $P = 0.315$ ). Using a larger sample, a similar regression for short-term song complexity revealed that relative syllable repertoire size was positively and significantly associated

TABLE 1. Size of bursa of Fabricius and T-cell mediated immune response of adults (dependent variables) in relation to song type repertoire size, relative syllable repertoire size, body mass, coloniality, and migration (independent variables) in multiple linear regression models, forced through the origin, based on statistically independent contrasts.

Independent variable	Slope (SE)	<i>t</i>	<i>P</i>
<b>Adult T-cell response</b>			
Model: $r^2 = 0.115$ , $F_{4,15} = 0.487$ , $P = 0.746$			
Song type repertoire size	-0.058 (0.056)	-1.021	0.323
Coloniality	0.001 (0.002)	0.455	0.656
Migration	-0.001 (0.002)	-0.560	0.584
Body mass	0.200 (0.375)	0.535	0.601
Model: $r^2 = 0.252$ , $F_{4,34} = 2.884$ , $P = 0.037$			
Relative syllable repertoire size	0.341 (0.144)	2.370	0.024
Coloniality	0.001 (0.001)	1.142	0.261
Migration	-0.001 (0.002)	-0.938	0.354
Body mass	0.481 (0.236)	2.038	0.049
<b>Bursa of Fabricius</b>			
Model: $r^2 = 0.476$ , $F_{4,12} = 2.728$ , $P = 0.080$			
Song type repertoire size	0.046 (0.058)	0.784	0.448
Coloniality	-0.002 (0.002)	-0.708	0.492
Migration	0.001 (0.002)	0.916	0.378
Body mass	0.381 (0.138)	2.764	0.010
Model: $r^2 = 0.667$ , $F_{4,28} = 5.616$ , $P = 0.002$			
Relative syllable repertoire size	0.456 (0.154)	2.959	0.006
Coloniality	0.001 (0.002)	0.613	0.545
Migration	-0.001 (0.001)	-0.339	0.737
Body mass	0.716 (0.215)	3.322	0.002

with adult T-cell immune response (Fig. 1;  $r^2 = 0.215$ ,  $F_{2,36} = 4.933$ ,  $P = 0.013$ ; slope [SE] for body mass = 0.512 [0.234],  $P = 0.035$ ; slope [SE] for relative syllable repertoire size = 0.350 [0.149],  $P = 0.019$ ). When controlling for coloniality and migration as potentially confounding effects in multiple regressions, these results remained unchanged (Table 1).

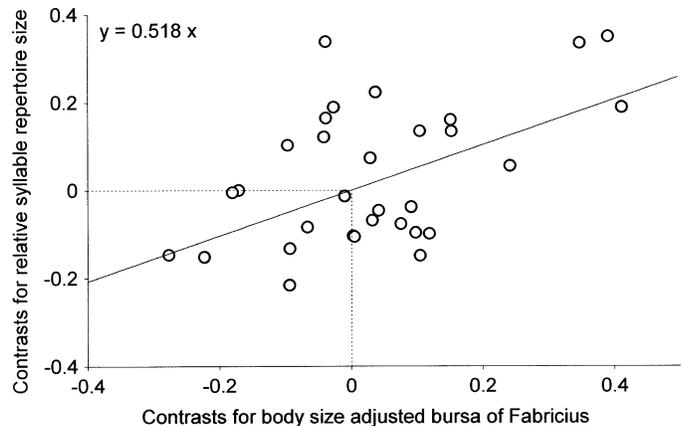


FIG. 2. The relationship between short-term song complexity (number of unique syllables within songs/song length) and the mass of the bursa of Fabricius after adjusting for allometry by using residuals after controlling for body mass, based on phylogenetically independent linear contrasts ( $N = 32$ ). The line and equation are from linear regression forced through the origin.

There was no significant relationship between contrasts of bursa of Fabricius and song type repertoire size ( $r^2 = 0.417$ ,  $F_{2,14} = 5.013$ ,  $P = 0.023$ ; slope [SE] for body mass = 0.809 [0.263],  $P = 0.008$ ; slope [SE] for song type repertoire size = 0.030 [0.050],  $P = 0.566$ ). Relative syllable repertoire size was significantly related to relative bursa mass (Fig. 2;  $r^2 = 0.436$ ,  $F_{2,30} = 11.61$ ,  $P < 0.001$ ; slope [SE] for body mass = 0.765 [0.189],  $P < 0.001$ ; slope [SE] for relative syllable repertoire size = 0.455 [0.150],  $P = 0.005$ ). Again, similar effects emerged when migration and coloniality were held constant (Table 1).

To test for age-dependent effects of T-cell immune response on short-term song complexity, we introduced adult and nestling T-cell response as independent variables in the same model, together with body mass, migration, and coloniality, and used relative syllable repertoire size as the dependent variable in a stepwise multiple regression. The overall model was significant ( $r^2 = 0.269$ ,  $F_{2,24} = 4.416$ ,  $P = 0.023$ ) and included adult PHA response (slope [SE] = 0.514 [0.183],  $P = 0.010$ ) and body mass (slope [SE] =  $-0.513$  [0.272],  $P = 0.072$ ). A similar analysis for song type repertoire size did not reveal any significant effect.

#### DISCUSSION

Here we have demonstrated that two measures of host immune defense, the relative size of bursa of Fabricius and T-cell mediated immune response, were related to an estimate of short-term song complexity, and that this was independent of several potentially confounding factors.

When studying interspecific patterns of bird song, it is important to address the possibility of biases, because a comparability problem may arise if measures of song variability in different species are not estimates of the same phenomenon (Krebs and Kroodsma 1980). For instance, repertoire size is a widely used measure to quantify the complexity and variety of bird song, although the use of estimates of repertoire size across species is a controversial issue (MacDougall-Shackleton 1997). The unit problem emerges from differences in terminologies and definitions of song elements applied in different species, whereas the sampling problem deals with the difficulty of detecting all repertoire components in a given record (Krebs and Kroodsma 1980). In comparative studies of bird song aiming to uncover evolutionary patterns, the unit problem combined with the fact that different species organize their vocalizations in different ways may lead to comparability problems (Krebs and Kroodsma 1980; MacDougall-Shackleton 1997). Most authors assume that this problem is solved in their comparative framework hoping that any heterogeneity present in the dataset will cause random noise (Gil and Gahr 2002). However, such heterogeneity caused by the comparability problem will render findings conservative. Comparative evidence showing biologically relevant associations indicates that repertoires in different species are comparable (Read and Weary 1992; Møller et al. 2000).

Here we used our own estimate to characterize song complexity. Variables that are designated to describe a similar biological phenomenon may be expected to be positively correlated, thus different estimates of song complexity should

be comparable. Our finding that relative syllable repertoire size based on information in handbooks and that of Read and Weary (1992) are significantly positively correlated confirms this prediction. With our simple but highly repeatable estimate for short-term song complexity, we assumed that we eliminated the unit problem, which could have been an important source of incomparability. In our analysis, a single observer analyzed the available sonograms applying the same unit (syllable) definition criteria to all species involved. The use of relative (this study) or absolute (Read and Weary 1992) syllable repertoire size restricted the focus to within-song variability. These estimates may represent ecologically relevant measures of song variability as perceived by receivers, because they are usually exposed to small fractions of the singers' whole repertoires (Hiebert et al. 1989). Although relative and absolute syllable repertoire size may differ by definition, because they refer to different time units, there was a strong correlation between these traits. In addition, we observed that both measures were similarly related to polygyny (L. Z. Garamszegi, unpubl. data), in which sexual selection is presumed to be more intense than under social monogamy. Consequently, we suggest that relative syllable repertoire size is a reliable substitute for Read and Weary's (1992) short-term song complexity, and that the former provides larger sample sizes for comparative studies.

We found that song type repertoire size was unrelated to various components of the immune system. However, these phenomena are most parsimoniously explained by the low statistical power of the tests involved, rather than having any biological meaning, and hence we do not discuss these null results further. On the other hand, we found that both adult T-cell mediated immune response and the size of the bursa of Fabricius were associated with relative syllable repertoire size. These findings are in accordance with the Hamilton-Zuk hypothesis (1982), suggesting that parasites may enhance sexual selection by favoring female preference for genetic disease resistance, thus costly song traits evolved to reveal health status of males. However, the opposite causal relationship between trait expression and immune defense may also be at work, since sexual selection favoring trait expression may have given rise to increased parasite virulence, which would then select for more efficient immune defense in highly sexually selected hosts (Møller 1997). The comparative data presented here do not permit us to discriminate between these alternatives. To shed more light on the exact mechanism that generates an evolutionary relationship between sexual characters and pathogens, future investigations should, for example, determine genetic correlations between trait expression and immune defense. Hypotheses associated with parasite-mediated sexual selection for indirect fitness benefits of female choice predict the existence of such a genetic correlation. However, the alternative mechanism for sexual selection increasing the risk of multiple infections and thus parasite virulence by frequent body contact of host individuals does not require genetic linkage between resistance and ornamentation.

Our results do not support the hypothesis that predicts a negative interspecific association between ornamentation and immune function. According to this hypothesis of immunocompetence handicaps by resource allocation, species in-

vesting more in the expression of a particular trait by elevating testosterone levels or metabolic investment should simultaneously suffer from immuno-suppression. As support for this mechanism Møller et al. (1998) found that male birds have relatively smaller immune defense organs than females, but only among adults, and this may be caused by the ornamented sex being more sensitive to immuno-suppression. However, the present study implies that immuno-suppressive mechanisms cannot hold for songs; although contrary to morphological characters, behavioral traits such as songs are strongly testosterone dependent (e.g., Balthazart 1983; Catchpole and Slater 1995). Selection driven by parasites and female preference may thereby have favored mechanisms that allow species to simultaneously develop strong immune systems and maintain testosterone and resource-dependent ornaments. We note that recent studies aiming to investigate intraspecific interactions between testosterone, immune function, and song performance conflict in their conclusions (Weatherhead et al. 1993; Duffy and Ball 2002; Garamszegi et al. 2003).

Different parasite species can vary in their virulence and abundance within host species in an age-specific way, because nestlings with a poorly developed immune system have to cope with a different parasite fauna than adults (Noble and Noble 1976; Cox 1982). T-cell mediated immune response in nestlings was not related to short-term song complexity, while T-cell response of adults showed a clear pattern. We found that the relative size of the bursa, which regresses before sexual maturity, was also associated with syllable repertoire size. Clearly, selection pressures due to parasitism differ between nestlings, juveniles, and adults. If evolution of complex song occurs in species with high parasite pressure, this should result from sexual selection acting on adults. Factors determining differences in immune function may remain after the disappearance of the bursa of Fabricius, because it is the production of memory cells in yearlings that determines the immune performance of adult birds (Møller and Erritzøe 2001).

Different components of the immune system constitute an effective defense machinery against pathogens. This system may also be evolutionarily favored by sexual selection, as each component was found to be independently related to song complexity in this study. Thus, parasites may be a general driving force affecting host physiology (e.g., Wakelin 1996), ecology (Møller and Erritzøe 1996, 1998), life history (Martin et al. 2001; Tella et al. 2002) and sexual selection (Møller et al. 1999).

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## APPENDIX

Song type and relative syllable (syllable repertoire size/song length) repertoire size, mean T-cell response (mm) of adults and nestlings, mass of the bursa of Fabricius (g), body mass (g), coloniality, and migration in birds.

Species	Repertoire size		T-cell immune response		Bursa of Fabricius (g)	Body mass (g)	Coloniality	Migration
	Song type	Relative syllable	Adult	Nestling				
<i>Alauda arvensis</i>		5.80		0.38	0.05	38.6	0	1
<i>Carduelis cannabina</i>		2.73	0.15		0.05	18.6	1	0
<i>Carduelis carduelis</i>		2.80	0.07			14.0	0	0
<i>Carduelis chloris</i>	22	0.89	0.14	0.83	0.02	28.6	1	0
<i>Corvus corone</i>		0.77		2.14	0.66	530	0	0
<i>Corvus frugilegus</i>		0.57		2.69	0.76	430	2	0
<i>Corvus monedula</i>		0.91		2.05		234	2	0
<i>Delichon urbica</i>		6.50	0.44	2.19		18.3	2	1
<i>Emberiza cirius</i>	3	0.56	0.16			22.8	0	1
<i>Emberiza citrinella</i>	2	1.18	0.12	0.63	0.05	26.9	0	0
<i>Emberiza schoeniclus</i>	20	2.25	0.15			19.4	0	1
<i>Erethacus rubecula</i>	1000	3.11	0.30	1.24	0.03	18.1	0	0
<i>Ficedula hypoleuca</i>	1000	2.43	0.20	0.45		14.0	0	1
<i>Fringilla coelebs</i>	3	2.00	0.20	0.73	0.04	22.7	0	0
<i>Galerida theklae</i>		3.00	0.32			37.0	0	0
<i>Garrulus glandarius</i>		2.11	0.61		0.28	162.0	0	0
<i>Hippolais icterina</i>		3.23	0.23		0.02	12.5	0	1
<i>Hippolais polyglotta</i>		1.88	0.09			10.1	0	1
<i>Hirundo daurica</i>		2.40	0.13	0.94		19.1	0	1
<i>Hirundo rustica</i>	1000	3.60	0.22	1.27	0.03	18.9	2	1
<i>Junco hyemalis</i>	5		0.35			21.0	0	0
<i>Lanius excubitor</i>		0.55		1.30	0.04	65.6	0	0
<i>Luscinia megarhynchos</i>	220	1.54	0.15			20.7	0	1
<i>Luscinia svecica</i>		1.12		0.43		18.0	0	1
<i>Motacilla alba</i>		2.57		1.05	0.05	22.6	0	1
<i>Motacilla cinerea</i>		0.67		1.49		17.6	0	0
<i>Muscicapa striata</i>		2.44	0.10	1.40	0.02	15.0	0	1
<i>Myiarchus cinerascens</i>	2			0.96		27.2	0	1
<i>Oenanthe leucura</i>		4.29	0.35	0.16		35.0	0	0
<i>Parus caeruleus</i>	5	1.43	0.13	0.54	0.03	10.8	0	0
<i>Parus cristatus</i>	1	1.43	0.27		0.03	10.9	0	0
<i>Parus major</i>	3	2.00	0.18	0.63	0.03	16.9	0	0
<i>Passer domesticus</i>	1	1.00	0.23	1.33	0.04	28.2	2	0
<i>Passer montanus</i>	1	1.71	0.29	2.81	0.03	22.4	2	0
<i>Petronia petronia</i>		2.00	0.24			31.7	1	0
<i>Phoenicurus ochruros</i>		2.88		0.93		16.4	0	0
<i>Phoenicurus phoenicurus</i>	200	2.29	0.13	1.13	0.02	14.3	0	1
<i>Phylloscopus bonelli</i>	5	1.00		0.34		7.2	0	1
<i>Phylloscopus trochilus</i>	31	2.08	0.14		0.01	8.8	0	1
<i>Pica pica</i>		3.25	0.81	1.56	0.47	220	0	0
<i>Prunella modularis</i>	4	6.00	0.21		0.05	20.8	0	0
<i>Pyrrhocorax graculus</i>		1.00		1.66		245	1	0
<i>Pyrrhula pyrrhula</i>		1.14	0.14		0.02	24.5	0	0
<i>Riparia riparia</i>		2.80	0.51	0.99	0.02	14.0	2	1
<i>Saxicola rubetra</i>		4.00		0.21		16.9	0	1
<i>Saxicola torquata</i>		4.00		0.64		15.3	0	0
<i>Serinus serinus</i>		6.44	0.12	0.55		12.0	1	0
<i>Sialia mexicana</i>		0.68		0.76		29.5	0	0
<i>Sitta europaea</i>		0.86		1.38	0.03	22.7	0	0
<i>Sturnus vulgaris</i>	49	2.00		1.23	0.12	69.0	2	1
<i>Sylvia atricapilla</i>		5.64	0.13	0.93	0.04	17.0	0	1
<i>Sylvia borin</i>		4.71	0.28		0.02	19.4	0	1
<i>Sylvia cantillans</i>		4.43		0.6		10.8	0	1
<i>Sylvia communis</i>		5.50	0.15	0.51	0.02	14.7	0	1
<i>Sylvia curruca</i>		3.63	0.27		0.03	11.9	0	1
<i>Sylvia melanocephala</i>		5.73	0.15	0.74	0.03	11.5	0	0
<i>Sylvia undata</i>		6.00		0.35		10.8	0	0
<i>Tachycineta bicolor</i>	2.6	1.67	0.18	0.37		20.0	0	1
<i>Troglodytes troglodytes</i>	21	2.56	0.13	0.63	0.05	9.4	0	0