

# Indicators of physiological stress and the elaboration of sexual traits in the collared flycatcher

László Zsolt Garamszegi,<sup>a</sup> Santiago Merino,<sup>b</sup> János Török,<sup>c</sup> Marcel Eens,<sup>a</sup> and Javier Martínez<sup>d</sup>

<sup>a</sup>Department of Biology, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, B-2610 Antwerp, Belgium, <sup>b</sup>Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, Madrid, Spain, <sup>c</sup>Department of Systematic Zoology and Ecology, Eötvös Loránd University, Budapest, Hungary, and <sup>d</sup>Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Alcalá, Madrid, Spain

Stress may have consequences for the evolution of condition-dependent sexual traits. For example, stress may be related to sexual traits through immune function, and sexual traits can reflect how individuals bear the costs of stress-mediated immunosuppression. However, male traits may be directly associated with stress, and such traits would then indicate stress tolerance. Here, we present initial results for the relationship between physiological stress estimated by the levels of heat shock proteins (HSP60 and HSP70) and heterophil/lymphocyte ratio and the elaboration of sexual traits, such as forehead and wing patch size and song features in the collared flycatcher *Ficedula albicollis*. Males producing longer and more versatile songs had significantly higher levels of HSP70, but other traits were unrelated to stress. In general, effect sizes for the relationship between stress and sexual traits had broad confidence intervals and varied between being small and medium effects. Immunoglobulin levels, leukocyte abundance, haemoparasite prevalence, male age, and date and time effects did not affect the relationship between stress and sexual traits. These preliminary results, serving a basis for further experimental studies indicate that the relationship between sexual traits and stress does not seem to be strong, but stress may partially constrain the expression of some sexual traits. *Key words*: bird song, blood parasites, collared flycatcher, heat shock proteins, immunity, sexual selection. [*Behav Ecol* 17:399–404 (2006)]

Animals should adapt to the short- or long-term adverse and unpredictable alterations in environmental conditions by behavioral and physiological means (Parsons, 1991). The combined physiological reactions to such stress factors are called stress response, which involves the reallocation of resources from nonvital functions to fundamental life-preserving processes (Charmandari et al., 2005). From an ecological point of view, stressful conditions and individual variation in physiological responses to different stress levels may set up evolutionary constraints for behavioral strategies and life-history trade-offs (Birkhead et al., 1999; Ilmonen et al., 2003; Merino et al., 1998; Moreno et al., 2002; Råberg et al., 1998; Sanz et al., 2004; von Schantz et al., 1999). Behaviors, such as sexual displays and parental care, may impose high costs requiring a high metabolic rate (e.g., Eberhardt, 1994; Grafe and Thein 2001; Grafe, 1996; Masman et al., 1989; Moreno, 1989; Moreno et al., 1991; Vehrencamp et al., 1989; but see also Ward et al., 2004) and can therefore be considered ecologically relevant stress (Råberg et al., 1998). Condition-dependent sexual traits, those that require resources for maintenance thus having the potential to signal the ability of individuals to cope with demands of resource allocation, are susceptible to the effects of physiological stress. Therefore, individual variation in stress responses represents a basis for the evolution of condition-dependent traits (Buchanan, 2000).

For example, due to the physiological trade-off between immune function and the elaboration of costly traits, stress-induced immunosuppression may play a role in the develop-

ment of secondary sexual characters (Buchanan, 2000; Råberg et al., 1998). Stress-induced immunosuppression may be adaptive when reallocating resources to essential activities, avoiding harmful metabolic products and autoimmune responses (see details in Råberg et al., 1998; Sheldon and Verhulst, 1996; von Schantz et al., 1999). Individuals may differ in how they manage stress-induced immunosuppression, which can be signaled through condition-dependent handicap traits, such as costly signals imposing metabolic stress (Buchanan, 2000). However, condition-dependent signals may also be affected directly by individual stress responses without the involvement of the immune system as stress may have consequences for signal development (Buchanan et al., 2003). In addition, individuals may also differ inherently in how they respond to stress in their entire life, and stress responsiveness may have some genetic basis (Merilä and Hoffmann, 1999). The individual variation in stress responsiveness is evolutionary relevant because the reaction of an animal to stress is a result of the balance between costs and benefits of stress response (Feder and Hofmann, 1999; Sørensen et al., 2003). Hence, male ornaments that are sensitive to stress may reliably reveal the status of the bearers' homeostatic systems.

Heat shock proteins (HSPs) are very important in stress resistance and thus in adaptation to the environment (Morimoto, 1991). The heat shock family of stress proteins is a group of highly conserved molecules that primarily function as molecular chaperones and thus plays key roles in coping with stress-induced denaturation of other proteins. The identified, ecologically relevant inducers of HSPs involve environmental stresses, such as abnormal temperature, toxins, UV radiations, parasites, physical activity, as well as genetic stresses, such as senescence, inbreeding, and deleterious mutations (Feder and Hofmann, 1999; Sørensen et al., 2003). Hence, HSP levels are widely used in ecological studies to detect the presence of

Address correspondence to L.Z. Garamszegi. E-mail: laszlo.garamszegi@ua.ac.be.

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physiological stress for most stressors (e.g., Martínez-Padilla et al., 2004; Merino et al., 1998, 2002; Morales et al., 2004; Moreno et al., 2002; Sanz et al., 2004; Tomás et al., 2005).

In the majority of birds, heterophil leucocytes are the second most numerous cells in circulation (Maxwell and Robertson, 1998). Their numbers increase during mildly or moderately stressful conditions. Heterophils respond to a stimulus during the early inflammatory phase, and they may also have sensitive and selective phagocytosing properties. The number of heterophils relative to the number of lymphocytes (H/L ratio) has proved sensitive to increased plasma corticosterone levels (Morici et al., 1997; Post et al., 2003). The H/L ratio has now become widely accepted as a reliable and accurate physiological indicator of the stress response in wild bird populations (Hörak et al., 1998; Moreno et al., 2002; Ots et al., 1998).

In this correlative study, as a preliminary effort, we explored the relationship between male ornamentation and estimates of physiological stress in the collared flycatcher *Ficedula albicollis*. We measured two plumage characteristics, namely, forehead patch size and wing patch size, and several features of song that all have the potential to be involved in sexual selection (see Garamszegi et al., 2004; Gustafsson et al., 1995; Török et al., 2003). In parallel, we estimated HSP levels together with H/L ratio to assess the rate of physiological and/or environmental stress. As immune function may mediate the relationship between stress and sexual signals via immunosuppression (Buchanan, 2000), we also estimated health status of individuals by immunoglobulin G (IGG) levels, leucocyte abundance, and prevalence of blood parasites. Given that several mechanisms may be in effect, in an initial correlative study, the direction of the general relationship between sexual signaling and stress indicators cannot be predicted a priori.

## METHODS

### Study species and field procedures

The collared flycatcher is a small insectivorous, socially monogamous hole-nesting passerine (Cramp and Perrins, 1994). Males arrive earlier than females at the breeding site and, by occupying nest-boxes or natural cavities, establish territories where they attract females by singing and displaying. Males sing strictly at the beginning of the breeding season, before pairing. During this period, they engage in intense territorial disputes, in which forehead patch size and wing patch size may be important (Pärt and Qvarnström, 1997; Török et al., 2003). Females visit several males before making their choice and prefer males based on their forehead patches and/or song characteristics (Garamszegi et al., 2004; Gustafsson et al., 1995; Potti and Montalvo, 1991). After pairing, extrapair copulations may occur frequently resulting in approximately 20% extrapair paternity among nestlings (Garamszegi et al., 2004; Michl et al., 2002; Sheldon et al., 1997). Extrapair copulatory decisions of females seem to be dependent on male plumage characteristics (Michl et al., 2002; Sheldon et al., 1997).

The fieldwork was carried out at Pilis Field Station near Budapest (47° 43' N, 19° 01' E), Hungary, where breeding plots for hole-nesting passerines were established in 1981. In the reproductive season of 2004, we recorded songs of 23 males with a Sony TCD-D8 DAT tape recorder connected to an MD 21 U microphone attached to a parabolic antenna. Recordings were made during the most active singing period (usually 0500–1000 h, but no later than 1200 h) and only in relatively good weather conditions. To stimulate males, prior to recording, they were exposed to a caged female for 5 min. We recorded only unpaired males: if a female was observed nearby during recording, the male was excluded from subse-

quent analyses. After removing the female stimulus, we recorded each male for at least 10 min to obtain a sample with sufficient songs, with minimal background noise. Birds were trapped within 1 h after recording. We then measured their body mass (to the nearest 0.1 g), tarsus length, width and height of the forehead patch, and lengths of white from the tip of primary coverts on the outer web of fourth to eighth primaries on the right wing (to the nearest 0.1 mm). Forehead patch size was estimated as patch width  $\times$  height, whereas wing patch size was calculated as the sum of the lengths of the measured white parts on the wing (see Török et al., 2003). Based on differences in adult and subadult plumages, we categorized male age as yearling or older. We obtained blood (about 80–100  $\mu$ l from brachial vein) with the aid of a standard, heparinized capillary tube. One drop of this blood was smeared on a slide for detection of blood parasites. Blood smears were immediately air dried and fixed with methanol. The rest of the blood had been left in the capillary tube and was later used for HSP and IGG analyses. The closed capillaries were stored in a cooling box at approximately 4°C until returning to our laboratory, where we centrifuged blood samples for 10 min at 10,000 rpm, and we separated serum and cell fractions. Samples were then frozen till further preparation (see below). This method has been shown to not affect HSP detection in other bird species (Tomás et al., 2004). We assumed that measured HSP levels reflected the HSP levels at the time of song recordings. This assumption is very likely to be the case because HSP-mediated stress response is a slow process (Burel et al., 1992; Lindquist, 1986). For birds, it takes many minutes to produce stress proteins, and one needs hours or days to detect this increase. The stress response is maintained until the stressor disappears. Thus, HSPs can be involved in the short-, medium-, or long-term response.

### Song recordings and spectrogram analysis

Male flycatchers usually sing 2- to 4-s-long strophes consisting of variable song elements, or so-called figures. We estimated song rate as the number of strophes/minute based on the available recordings (a previous study using repeated measures of unmanipulated birds in the same season has shown significant repeatability of song rate assessed in this manner [Garamszegi et al., 2004]). We selected 20 consecutive strophes with minimal background noise for further sonographic analyses. For this analysis, we used the computer program "Avisoft-SASLab Pro" (Specht, 1999) blindly with respect to the HSP and immunological data. The following variables were measured for each strophe: strophe length in seconds, number of figures, versatility (number of unique figures), complexity (number of unique figures/number of figures), and tempo (number of figures/second). In accordance with previous findings, these song parameters varied significantly among individuals (one-way ANOVAs:  $F_{22,437} > 2.406$ ,  $p < .0004$ ), thus we averaged these variables for each male. We used capture-recapture approach to estimate individual repertoire size by controlling for heterogeneous figure detection probability (Garamszegi et al., 2002). Applying this procedure, we first selected the capture-recapture model (Otis et al., 1978) that fits best the data and then used this model for repertoire size estimation (see Garamszegi et al., 2005).

### Estimates of stress

We determined HSP levels from the blood cellular fraction by means of a western blot. Samples of soluble proteins (35  $\mu$ g/well) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The total protein value

**Table 1**  
**Means ( $\pm$ SDs) of sexual traits and component loadings of song features from a principal component analysis in the collared flycatcher**

	Mean $\pm$ SD	Factor loadings		
		PCA1	PCA2	PCA3
Repertoire size	30.17 $\pm$ 11.54	0.224	<b>-0.881</b>	0.139
Song rate (1/min)	4.069 $\pm$ 1.387	-0.170	-0.057	<b>0.762</b>
Complexity (1/figures)	0.652 $\pm$ 0.087	-0.582	<b>-0.760</b>	-0.092
Tempo (1/sec)	3.487 $\pm$ 0.389	0.502	-0.010	<b>-0.724</b>
Strophe length (sec)	3.037 $\pm$ 0.507	<b>0.822</b>	0.132	0.487
Versatility	6.528 $\pm$ 1.005	<b>0.771</b>	-0.582	-0.038
Number of figures	10.54 $\pm$ 2.052	<b>0.990</b>	0.091	0.037
Explained variance		41.72%	24.57%	19.62%
Forehead patch size	72.86 $\pm$ 14.07	$r = -.173$ $p = .430$	$r = -.286$ $p = .186$	$r = .045$ $p = .837$
Residual wing patch size	420.6 $\pm$ 141.3	$r = .055$ $p = .803$	$r = -.033$ $p = .880$	$r = -.109$ $p = .622$

The most important loadings are in bold (values larger than 0.70 or smaller than -0.70). The amount of total variance accounted for by the three principal component axes is 85.91%. For plumage traits, Pearson's correlations with the principal components are given.  $n = 23$ . Repertoire size is estimated for the entire sample involving 20 strophes by using capture-recapture approaches (see main text), while versatility and complexity refer to a single strophe. Similarly, song rate is for the whole recording, while tempo is for one strophe.

has been found to be in the linear range of the antibody-antigen response for the species and antibodies studied. The primary monoclonal antibodies used were anti-HSP70 (clone BRM22, Sigma H-5147) diluted 1/5000 and anti-HSP60 (clone LK2, Sigma H-3524) diluted 1/1000. The peroxidase-conjugated secondary antibody was goat anti-mouse specific for Fc region (Sigma A-0168) at 1/6000 dilution. Protein bands were quantified using 1D image analysis software (Scion Image for Windows, Scion Corporation, Frederick, MD). Immunoreactivity of the bands was measured as arbitrary units using the formula: mean optical density  $\times$  area. To be able to compare HSP values of samples run in the two blots needed in this experiment, a standard (pooled cellular fraction from several individuals) was run in four wells in the two blots. Thus, individual values were recalculated to a common HSP value. For details see Moreno et al. (2002) and Tomás et al. (2004).

We estimated H/L ratio based on the analysis of blood smears (see details below).

### Immunoglobulin determination

In order to measure circulating levels of total IGG, the blood serum fraction was analyzed by means of a direct enzyme-linked immunosorbent assay, using anti-chicken antibodies conjugated with peroxidase (Sigma A-9046). The linear range of the sigmoid curve for this antibody-antigen response as well as the optimal serum dilution (1/5000) have been previously determined. Absorbances were measured using a plate spectrophotometer at  $\lambda = 405$  nm. For details see Martínez et al. (2003).

### Quantification of parasite prevalence and leukocyte profile

Fixed blood smears were stained with Giemsa for 45 min. Half the smear was scanned at 200 $\times$  magnification and was searched for large extracellular parasites, whereas small intracellular parasites were detected with oil immersion at 1000 $\times$  magnification. The prevalence of infection by blood parasites was generally low in the examined samples, thus we

have used a combined presence-absence index for analyses of all parasites found (*Trypanosoma* and *Haemoproteus*). We also counted white blood cells at 600 $\times$  magnification to obtain H/L ratio. Count of leukocytes was carried out in a part of the smear where cells are separated in a monolayer and crossing the sample from down to up to minimize differences in the thickness of the blood smear. The proportion of different types of leukocytes was assessed by examining a total of 100 leukocytes. White blood cell abundance was estimated by assessing the number of erythrocytes that are needed to detect 100 leukocytes.

### Statistical analyses

Means ( $\pm$ SDs) are given in Table 1. Variables were checked for normality and homoscedasticity. When necessary, statistical transformation was applied in order to meet the parametric criteria.

As we found many significant correlations between song traits, we reduced the number of the statistical tests performed with multiple song features by using principal component analysis. The advantage of using principal components is that this method provides measures of song structure and complexity that are uncorrelated with each other (Tabachnick and Fidell, 1996). We calculated three independent principal components. The component loadings and the explained variance for the principal component axes are presented in Table 1. In the subsequent analyses, we used the scores of these components, instead of the original variables.

Wing patch size is known to be age dependent (Török et al., 2003), which we also found in our data ( $t_{21} = 8.257$ ,  $p < .001$ ). Hence, wing patch size was standardized for age before analyses by bringing the age classes to a common mean of 0 and SD of 1. Plumage traits were not significantly related to song traits (Table 1).

To determine the strength and direction of the relationship between male sexual traits and measures of physiological stress, we estimated Pearson's correlations between these variables. Because we used several variables, we performed various statistical tests (see Table 2), which may raise Type I errors.

**Table 2**  
Effect size correlations ( $r$ ) and the associated 95% CIs for the relationship between male sexual traits and estimates of stress

	Effect size	95% CI		$p$
		Lower	Upper	
<b>HSP70</b>				
PCA1	0.454	0.051	0.730	.029
PCA2	-0.055	-0.457	0.366	.803
PCA3	0.211	-0.220	0.573	.333
Forehead patch size	-0.234	-0.589	0.197	.283
Wing patch size <sup>a</sup>	0.064	-0.358	0.464	.772
<b>HSP60</b>				
PCA1	0.152	-0.278	0.531	.490
PCA2	0.013	-0.401	0.423	.952
PCA3	0.016	-0.399	0.425	.943
Forehead patch size	-0.259	-0.606	0.172	.232
Wing patch size <sup>a</sup>	-0.355	-0.669	0.067	.097
<b>H/L ratio</b>				
PCA1	0.089	-0.336	0.484	.686
PCA2	-0.045	-0.449	0.374	.838
PCA3	0.137	-0.292	0.520	.534
Forehead patch size	-0.132	-0.516	0.296	.548
Wing patch size <sup>a</sup>	0.249	-0.182	0.560	.253

Effect size conventions:  $r = .10$ , small effect;  $r = .30$ , medium effect; and  $r = .50$ , large effect. The significance of the associated Pearson's correlation coefficients is also given.  $n = 23$  for all cases.

<sup>a</sup> Age standardized (see text).

The application of the conventional Bonferroni correction to adjust individual significance levels to the number of tests performed would require lowering  $p$  values to .0034 (at  $\alpha = 5\%$ ) for each particular test. However, this procedure would increase the risk of committing Type II errors enormously because for 80% power,  $n = 149$ – $1412$  would be needed if considering medium-small effect sizes (Cohen, 1988). Arising from the methodological constraints of the study of bird song, we had data for 23 males, which are similar to other behavioral studies. The use of Bonferroni correction would result in very high risk of not rejecting  $H_0$  when it is false (power = 0.007–0.058 from small to medium effect size). For similar

**Table 3**  
Multivariate linear model testing the relationship between PCA1 and estimates of stress while holding health status and potentially confounding variables constant as independent variables

Independent variables	Dependent variable: PCA 1		
	$F$	df	$p$
Full model	2.297	9,13	.084
HSP70	5.824	1,13	.031
HSP60	0.83	1,13	.379
H/L ratio	1.167	1,13	.300
IGG levels	0.631	1,13	.441
Parasite prevalence	1.731	1,13	.211
WBA	1.037	1,13	.327
Date	4.375	1,13	.057
Time	1.959	1,13	.185
Age	3.421	1,13	.087

IGG levels, prevalence of blood parasites, and abundance of white blood cells (WBA) were used to reflect health status, whereas date and time of song recordings (and physiological measurements) and male age were considered as potentially confounding variables.

reasons and also for mathematical and logical considerations, the Bonferroni correction has been criticized recently in the field of ecology and behavioral ecology (Moran, 2003; Nakagawa, 2004; Perneger, 1998). As a solution, Nakagawa (2004) suggested reporting effect sizes together with confidence intervals (CIs) for all potential correlations to allow the readers to judge the biological importance of the results. In this study, we adopted this approach, and used the software Comprehensive Meta Analysis (BioStat, 2000, <http://www.meta-analysis.com/>) to calculate the 95% CI of effect sizes.

We checked whether health status can modify the relationship between stress and sexual traits via stress-mediated immunosuppression. Additionally, we assessed the effect of potentially confounding factors that may generate apparent correlations between sexual characters and stress traits. For example, the time and the date of recordings may generate bias in the data as song displays, stress levels, and health status may potentially vary along a seasonal and/or daily axis. The expression of male sexual signals is generally thought to depend on individual age (Trivers, 1972), but HSP levels may also be affected by age due to age-specific mortality or survival (Feder and Hofmann, 1999; Tatar et al., 1997). Hence, we introduced sexual traits in different multiple linear models as a dependent variable, in which measures of stress, estimates, or health status and the considered confounding factors were included as independent variables.

## RESULTS

The effect sizes for the relationship between male sexual characteristics and measures of stress are given in Table 2. The largest effect sizes were found for HSP70 in association with the first principal component (PCA1) of song characteristics. This relationship was statistically significant. PCA1 involved large positive factor loadings for strophe length, the number of figures, and strophe versatility (see Table 1).

In general, effect sizes for the relationship between stress and sexual traits had broad CIs and varied between being small and medium effects (Table 2). The mean ( $\pm$ SD) unsigned effect size was 0.164 ( $\pm$ 0.129), while the mean ( $\pm$ SD) of the 95% CI was 0.792 ( $\pm$ 0.043).

When we controlled for the effect of health status and other potentially confounding factors in multiple linear models the patterns for the relationship between estimates of stress and sexual traits were unaffected. For example, the model for PCA1 revealed a significant slope for HSP70, but not for other variables (Table 3). Analogous models for the rest of the sexual traits provided results similar to what can be concluded from Table 2 (data not shown).

## DISCUSSION

Among the estimates of physiological stress and sexual traits investigated, we only found significant relationships between HSP70 levels and PCA1 reflecting strophe length, the number of figures, and strophe versatility. The effect size for the remaining relationships was small or intermediate with wide CIs (Table 2). Therefore, the relationship between stress and sexual traits does not appear to be strong in the collared flycatcher. Our results also suggest that if the relationship between stress and sexual traits exists, it is independent of health status.

There may be four different explanations why the relationship between stress and sexual signals was not found to be strong and thus went statistically undetected in our sample. First, the chosen plumage and song traits may be poor indicators of male quality as they are cheap to produce. For

example, the stress consequences of the production of the white patches on the plumage are minimal as they are relatively cheap to produce (Badyaev and Hill, 2000). We think that this is unlikely because many of the chosen characters in this species have been repeatedly shown to be dependent on condition and have an important function in female choice or male-male competition (Garamszegi et al., 2004; Gustafsson et al., 1995; Pärt and Qvarnström, 1997; Sheldon et al., 1997; Török et al., 2003). Second, the chosen stress variables may be poor indicators of stress. This possibility is in conflict with experimental studies, which showed that the manipulation of an environmental stress factor results in the change of HSPs and/or H/L ratio (e.g., Hórak et al., 1998; Ilmonen et al., 2003; Merino et al., 1998; Tomás et al., 2005). In addition, there is a good reason to assume that HSP levels have ecological importance. This reason is that all individuals cannot have high levels of these proteins because it involves costs paid in terms of fertility/fecundity, energy, development, survival, and/or immunosuppression (Sørensen et al., 2003). Accordingly, significant repeatability of HSP levels would indicate a role for consistent variation between individuals. Unpublished data in female pied flycatchers support this prediction (HSP60:  $R = .54$ , HSP70:  $R = .91$ ; Morales J, unpublished data). Third, different mechanisms shaping the relationship between stress and sexual traits may act in concert, and observed HSP levels or H/L ratio may simultaneously reflect both differences in stress suffered and individual differences in stress response. Hence, if both processes take place simultaneously, it may mask any simple direct relationship in a correlative study. With the available data, we cannot deal with this confounding possibility because only an experimental approach has the potential to discriminate between different mechanisms. Fourth, the relatively small sample size may have contributed to the large CIs and the general lack of significant relationships. Although our sample size is comparable to what one can reach in behavioral and physiological studies, this explanation is very likely. Therefore, future studies should rely on experimental approaches in which the current knowledge can be amalgamated. For example, experiments focusing on HSP levels and song length have the highest potential to discover some aspect of the mechanisms that mediate a relationship between sexual traits and stress response.

Based on correlative findings and on the preliminary nature of this study, at this moment, it remains difficult to make inferences regarding the causal mechanisms. At least, our results allow some careful speculation. As briefly mentioned in the introduction, there might be at least three potential mechanisms that can mediate relationships between stress and sexual traits. First, if HSP levels and H/L ratio reflect immediate stress levels that individuals experience, stress factors can be expected to elevate the levels of stress proteins and/or number of heterophil lymphocytes and to reduce simultaneously the investment in costly song or plumage traits. This mechanism predicts a negative relationship between estimates of stress and sexual traits. Although such tendency was detected for wing patch size at a  $p < .1$  level, plumage traits can hardly reflect immediate stress levels as they are developed well before the breeding season. Second, if stress variables are to reflect the ability of individuals to cope with stress, under the handicap theory, good quality males may be expected to have more elaborated sexual characters and better physiological condition as reflected by HSP or heterophil levels. Hence, this hypothesis predicts a positive relationship between sexual traits and stress variables. The association between PC1 and HSP70 levels may be a good example. Third, it may be also plausible that the signaling mechanism is at work via stress-induced immunosuppression. In this study, we did not find any evidence that variables reflecting health would play a role.

Yet, one should be careful in interpreting these null results because it remains likely that we did not measure the relevant immune trait (see Adamo, 2004). Importantly, other studies usually found a relationship between HSP expression and immune function (e.g., Merino et al., 1998, 2002; Morales et al., 2004; Sanz et al., 2004; Tomás et al., 2005).

Strophe length seems to be an important trait in the collared flycatcher. A previous experimental study showed that individual responses in terms of song rate reduction to an immune challenge depend on the characteristics of the song, including strophe length (Garamszegi et al., 2004). As the same characteristics were unaffected by parasitic infection, they may have the potential to reflect parasite resistance independent of the immediate parasite pressure. Here, we found that individuals producing longer (and more versatile) strophes also have higher levels of HSP70. Hence, they may have the physiological capacity to better resist against different stress factors in general, and parasite resistance is a part of this capacity. Such metabolic consequences of singing long strophes have important consequences for the cost of bird song, which is a controversial issue in behavioral ecology (review in Ward et al., 2004). Elevated levels of HSPs may mirror an entire set of physiological means by which individuals cope with the cost of singing and in which energy metabolism plays only a partial role.

In our initial efforts based on effect sizes and the associated CIs, we failed to detect strong relationships between sexual traits and estimates of stress in the collared flycatcher. Although at least some sexual signals may be associated with certain measures of stress response, there is no evidence for strong correlation in general. In particular, males singing longer and more versatile strophes have higher levels of HSP70, which may allow them to cope with the metabolic demands of costly songs. However, further experiments are needed to improve our understanding about the role of stress in sexual selection.

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