Distress calls may honestly signal bird quality to predators

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In predator–prey interactions, both interactors may benefit from sharing information about prey vulnerability. We examined the relationship between calls used to discourage close predators (distress calls) and the health condition of the caller to test whether these signals are reliable indicators of prey quality. The structure of calls from captured lesser short-toed larks *Calandrella rufescens* was related to their body condition and T-cell-mediated immunocompetence. Birds in better nutritional and immunological condition utter harsher calls (i.e. they spread the call energy over a wider range of frequency) than birds in poorer conditions. Hence, the harshness of distress calls seems honestly to signal the health status of prey and thus their ability to escape, on which the predator might base its optimal foraging choice. Previous studies have investigated the honesty of songs that have evolved via sexual selection, but this is the first study, to our knowledge, that demonstrates a relationship between individual quality and a vocalization primarily shaped by natural selection.

Keywords: birds; prey quality; distress calls; honest signal; immunocompetence; body condition

1. INTRODUCTION

Predators tend to optimize their food intake by attacking the most vulnerable prey, to reduce the time and energy expended during capture (Pole *et al.* 2003). This, in turn, dynamically determines adaptive responses in prey, such as the evolution of signals that convey information on their escape ability (Bergstrom & Lachmann 2001). Several pursuit-deterrent signals have been identified in different taxa (Caro 1995), which may alter a predator's behaviour simply by signalling of awareness (perception advertisement). There is little evidence, however, on signals conveying information about the prey's ability to escape, for example, its health (quality advertisement), and thus on the honesty of prey signalling (Caro 1995; Leal 1999).

Cresswell (1994) showed that singing performance of flushing skylarks (*Alauda arvensis*) was related to the probability of being seized by a predator, suggesting that lark song would be a condition-dependent pursuit-deterrent signal. We investigated the structure of distress calls, the ultimate alarm vocalizations used when individuals are

confronted or captured by a predator, in conditions of utmost danger (Högestedt 1983). These calls are related to the attempts to escape, and birds may also emit them when they are caught by humans (Jurisevic & Sanderson 1998). We studied the lesser short-toed lark *Calandrella rufescens*, a ground-nesting passerine that usually emits bouts of noisy calls when flying away from the ground as a response to the close presence of humans or predators. To test whether lark distress calls convey reliable information on signaller quality, we investigated the relationships between the spectro-temporal structure of these calls and two measures of current health status (body condition and T-cell-mediated immune response), which are related to fitness components such as survival, susceptibility to parasites, and reproductive performance (see reviews in Alonso-Alvarez & Tella 2001; Tella *et al.* 2001, 2002).

2. METHODS

The study was carried out in Fuerteventura (Canary Islands, Spain) in November 2003. Birds were captured using spring-released nets in the late afternoon. We then measured their T-cell-mediated immune response overnight, using the phytohaemagglutinin (PHA) test (following Smits *et al.* (1999)), as PHA responses of other small passerines (*Passer domesticus*) are stronger at night and remain stable after 6 h of treatment (Navarro *et al.* 2003). Briefly, the injection of PHA as a mitogen results in local activation and proliferation of T cells, followed by increased expressions of major histocompatibility complex molecules and, in turn, swelling of the skin at the point of injection. This is a standard estimate of cellular immunocompetence, measured as the skin swelling after injection with PHA. Larks were injected subcutaneously with 10 µl of 5 mg ml⁻¹ phytohaematogglutinin-P (Sigma L-8754) in phosphate-buffered saline (PBS) in the centre of the right wing patagium. Three measurements (to the nearest 0.01 mm) of patagium thickness were taken, just prior to and ca. 12 h (11.6 ± 1.4) after injection, using a pressure-sensitive micrometer (Baxlo Precision 3000). Measurements were highly repeatable (intra-class correlations: pre-injection $r = 0.93$, $p < 0.001$, post-injection $r = 0.98$, $p < 0.001$), so average measures were used to calculate the swelling. All birds were handled in a similar way and spent the night in the same dark room in separate cotton bags. Immediately before releasing birds the following morning, we measured body mass (± 0.1 g) and wing length (± 0.5 mm) as the best estimators of body size in this species (J. L. Tella, unpublished data). We thus obtained an index of body condition as the residuals of the linear regression of log(body mass) on log(wing length) ($r = 0.45$, $p = 0.003$). Finally, a drop of blood was extracted for molecular sexing.

Birds were recorded during release from 8.00 to 10.00; they were gently moved from their bag to the ground, where they uttered bouts of calls while flying away. Recordings were made in open habitat in conditions of low background noise (no wind or close singing birds), when individuals were 10 m or less from the recorder. We used a Sony TC-D8 DAT recorder and a Sennheiser ME67 microphone. Sound analyses were carried out with Avisoft SASLab Pro by R. Specht (Berlin), performing a fast Fourier transform (sampling frequency 22 050 Hz, FFT length 512, time resolution 8.9 ms, frequency resolution 43 Hz, Window Function: Bartlett; high-pass filter: cut-off frequency 900 Hz). We measured the following parameters (figure 1):

- (i) call rate (number of calls uttered per second);
- (ii) number of calls uttered in the first 40 s after release;
- (iii) call duration (D);
- (iv) mean carrying frequency (FM, the frequency with the greatest energy, measured on the average spectrum);
- (v) duration of a pulsed-unit (DP, measured at the end of the signal);
- (vi) pulse rate (number of pulses per call duration);
- (vii) call harshness (HAR, measured as the frequency range in which the signaller concentrate 50% of the call energy).

The latter parameter is evaluated as the distance between the upper (75%) and lower (25%) quartiles in the pulse carrying the greatest call energy. Harshness measurements might be affected by the distance

Figure 1. Distress call of a randomly chosen lesser short-toed lark. (a) Waveform, (b) mean power spectrum, (c) sonogram and (d) power spectrum of pulse 2. Numbers in the waveform indicate the pulsed-units of calls. See § 2 for acronyms.

between the microphone and the emitter, owing to sound attenuation. However, harshness values of the first call produced (when the distance to the bird was 1 m or less) were highly correlated with the mean harshness of the whole set of calls uttered by each individual ($r = 0.70$, $p < 0.001$, $n = 40$), suggesting that our harshness measurements were not seriously affected by degradation.

Information on health status and distress calls was obtained from 41 individuals (25 males, 16 females). All birds uttered calls after release ($n = 408$ calls, 9.95 ± 0.94 s.e. calls per bird). Analyses were done using individual mean values of call duration, harshness, pulse duration, carrying frequency and pulse rate, while only one value was available for the other two acoustic variables. All variables were normally distributed (Kolmogorov–Smirnov, $p > 0.10$), and thus parametric tests were applied. We summarized the seven acoustic variables with a Principal Components Analysis (PCA) on standardized data. We used the scores of the first three principal components as dependent variables in multiple regression analyses, with immune response, body condition, wing length, sex and time elapsed from injection to measuring PHA response as predictors. By applying Bonferroni correction for the three multiple regression models conducted, a model was considered significant when its p -value was less than 0.017.

3. RESULTS

Call duration, harshness, pulse duration, carrying frequency and pulse rate varied significantly between individuals (one-way ANOVAs: $F_{39,366} > 2.3$, $p < 0.001$). PCA gave three components with an eigenvalue of greater than 1 that explained 68% of the variability of the dataset. Duration of a pulsed unit and pulse rate showed the highest correlation with PC1 scores, PC2 was positively correlated with mean carrying frequency and negatively correlated with call rate, whereas call harshness alone provided the major (negative) loading on PC3 (table 1). No significant relationships resulted among immune response, body condition and wing length (p -values: 0.54–0.92).

Table 1. Results of principal component analysis (PCA) of acoustic variables.

(Significant correlations in bold ($p < 0.05$).)

	PC1	PC2	PC3
call rate	−0.13	−0.84	0.32
number of calls uttered ^a	−0.02	0.04	0.08
call duration	0.72	0.12	−0.41
mean carrying frequency	0.01	0.93	−0.02
duration of a pulsed unit	−0.84	0.07	−0.41
pulse rate	0.80	0.12	0.08
call harshness	−0.12	0.21	−0.88
eigenvalue	1.90	1.66	1.22
percentage of total variance	27.10	23.67	17.38

^a In the first 40 s after release.

A multiple regression ($R^2 = 0.23$, $F_{2,38} = 5.5$, $p = 0.0077$) showed that PC3 (call harshness factor) was positively related to both the immune response (partial $r = -0.38$, $t = 2.6$, $p = 0.014$) and body condition (partial $r = -0.35$, $t = 2.3$, $p = 0.024$) of individuals. Potentially confounding effects resulted which were non-significant (sex, $p = 0.84$; time elapsed from injection to measuring PHA response, $p = 0.76$; wing length, $p = 0.61$). When using actual values instead of derived PCA scores, the relationship between call harshness, immune response and body condition was stronger ($R^2 = 0.32$, $F_{2,38} = 8.76$, $p < 0.001$), as was the case when excluding four detected statistical outliers ($R^2 = 0.52$, $F_{2,34} = 18.14$, $p < 0.001$) and even those extreme values (greater than 0.3) of immune response ($R^2 = 0.29$, $F_{2,32} = 6.40$, $p < 0.005$) (figure 2). Individual mean call harshness ranged between 0.96 and 2.51 kHz (12–32% of the signal frequency range).

Immune response, body condition, sex, body size and time elapsed had no effect on PC1 and PC2 (p -range: 0.20–0.73).

4. DISCUSSION

Song production is considered to be energetically costly, and constrained by the nutritional and immune condition of individuals (Gil & Gahr 2002). In contrast to most previous studies (see Alonso-Alvarez & Tella 2001), T-cell-mediated immune response and body condition emerged as two independent measures of health status in *C. rufescens*, and both were positively related to call harshness. Therefore, birds in prime condition spread the call energy over a wider range of frequency than birds in poorer health. Noisy signals covering a wide frequency range express aggression and are supposed to ensure a more effective defence against a potential predator, which is scared by bouts of harsh sounds, whereas pure-tone calls are associated with friendly and non-aggressive vocalizations (Morton 1982). Harsh distress calls may not only discourage the predator from chasing the prey (Cresswell 1994), but also startle the predator into loosening its grip when the prey is caught, thereby giving the caller a chance to escape (Driver & Humphries 1969). Bird quality, however, was not correlated either with the number of calls uttered or with call rate. When prey and

Figure 2. Relationships between distress call harshness and (a) T-cell-mediated immune response, and (b) body condition of lesser short-toed larks. Lines correspond to simple regressions (dotted line excluding immune response of greater than 0.3 mm). See § 3 for multiple regression results.

predator are so close, the chance of calling might be very brief and selection is thus likely to act on the quality of one, or a few, calls rather than on their number. In fact, bird health also seems to be advertised by the harshness of only the first distress call given after release ($R^2 = 0.27$, $F_{2,37} = 6.7$, $p = 0.005$).

In summary, the harshness of distress calls seems honestly to signal the health status of prey and thus their ability to escape, given that predator escape performance of birds has been shown to be affected by their nutritional condition and presence of disease (Lindström *et al.* 2003). By receiving these calls, a potential predator might optimize its foraging, choosing to focus on the easiest prey to catch. This could explain why birds that fall prey to a predator have lower immunocompetence (Møller & Erritzøe 2000). Therefore, we assume that predators of *C. rufescens* (mainly raptors, but also carnivores) can perceive variation in call harshness of as great as 1.5 kHz centred on a carrying frequency of 2.6–4.7 kHz. Accordingly, this bandwidth falls within, and represents a substantial proportion of, the hearing range of diurnal raptors (0.25–10 kHz; Jurisevic & Sanderson 2000) and some felids (0.45–64 kHz in *Felis catus*; Fay 1988).

To our knowledge, this is the first study demonstrating a relationship between individual quality and a vocalization primarily shaped by natural selection, given that previous studies investigated the honesty of bird songs that evolved via sexual selection (see reviews in Gil & Gahr 2002; Slater 2003). Further research on this subject should be encouraged, because the study of distress vocalizations could become a supplementary way to evaluate individual general status and encompass more traditional essays.

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