Developmental stress, social rank and song complexity in the European starling (*Sturnus vulgaris*)

K. A. Spencer[1*], K. L. Buchanan[2], A. R. Goldsmith[1] and C. K. Catchpole[3]

[1]*School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK*
[2]*School of Biosciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3TL, UK*
[3]*School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK*
[*] Author for correspondence (karen.spencer@bristol.ac.uk).

Bird song is a sexually selected trait and females have been shown to prefer males that sing more complex songs. However, for repertoire size to be an honest signal of male quality it must be associated with some form of cost. This experiment investigates the effects of food restriction and social status during development on song complexity in the European starling (*Sturnus vulgaris*). Birds that experienced an unpredictable food supply early in life produced a significantly smaller repertoire of song phrases than those with a constant food supply. Social status during development was also significantly correlated with repertoire size, with dominant birds producing more phrase types. This study therefore provides novel evidence that social as well as nutritional history may be important in shaping the song signal in this species.

Keywords: repertoire size; corticosterone; bird song; sexual selection; dominance; signalling

1. INTRODUCTION

The nutritional stress hypothesis (Nowicki *et al.* 1998) proposes that song complexity in birds is a reliable indicator of male quality, because the underlying neural pathway controlling song production and learning develops when young birds are likely to be at risk of experiencing nutritional stress. Songbirds learn their songs in the first few months of life when the song control pathway in the forebrain is still developing (Nottebohm 1999). Thus any stress that interrupts development or learning may be reflected in the song signal in adult life. Recent tests of this hypothesis have gone some way towards showing a causal link between developmental stress, song learning and song complexity (Buchanan *et al.* 2003; Nowicki *et al.* 2003; Spencer *et al.* 2003).

Many birds are social foragers, allowing more efficient foraging and greater individual energetic gains. This is certainly true for starlings during the non-breeding season and during early independence (Feare 1984). During this activity, dominance hierarchies are established and maintained through a series of pairwise interactions. These aggressive interactions can provoke large increases in stress hormones—corticosterone in birds (Wingfield &

Silverin 2002)—in both dominant and subordinate birds (Creel 2001). Dominant birds gain an advantage over subordinates through increased access to food and enhanced survival. However, there is mixed evidence from several studies on a wide range of species, which have failed to find a consistent relationship between social rank and stress hormone levels (Creel 2001).

The acquisition of a high dominance rank has been shown significantly to depress maze learning in mice (Barnard & Luo 2002). It is therefore possible that dominance could significantly affect song learning if such stress is applied during the development of the song-learning nuclei in the brain. If an individual pays a cost for attaining a high social status, such as increased metabolic rate (Hogstad 1987; Bryant & Newton 1994), then this may have a negative effect on song learning. However, subordinate birds may also suffer food limitation due to continual displacement if they sing. This might be compounded by a reduction in song output in such lower-ranked birds (Otter *et al.* 1997), having a negative impact on song learning owing to reduced auditory feedback (Brainard & Doupe 2000).

In the following experiment, 48 juvenile starlings were held in captivity in four groups of 12 animals. Two groups were held under an unpredictable food supply, with the other two under *ad libitum* supply (note: the same birds as were used in Buchanan *et al.* (2003)). In each group, the dominance hierarchy was quantified and the effects of social rank and nutritional stress on song complexity were investigated.

2. METHODS

Fledgling starlings ($n = 48$) were caught via a decoy trap in south Somerset, UK (*ca.* 35–50 days of age) and brought into outdoor aviaries during May 2001. Birds were housed in four mixed sex groups of 12 randomly assigned individuals in adjacent cages (1.8 m × 0.9 m × 1.8 m high). These groups were maintained throughout the experiment and randomly allocated to either a control or unpredictable food supply treatment. Control birds therefore experienced an *ad libitum* food supply. The 'unpredictable' group had their food hopper removed for 4 h each day on a random schedule between 09.00 and 16.00. After the end of all treatments all birds were kept on an *ad libitum* uninterrupted food supply.

The immune function of the birds was tested during the treatment period using a cell-mediated immune challenge. This involved an injection of phytohaemagglutinin (PHA) into the wing web (Lochmiller *et al.* 1993). The response (swelling) to the challenge was tested 5 h after a 50 µl suspension of PHA was injected into the wing using a spessimeter. No control injection of saline was injected into the left wing (Smits *et al.* 1999).

In March 2002, the song of individual male birds was recorded. In total, 15 males were recorded, six from the unpredictable group and nine controls. Each male was recorded for 1 h on two separate occasions. We estimated the repertoire size of each male by determining the number of different phrase types within 20 full song bouts (more than 5 s; Eens 1997).

To determine the social rank of each individual, an aviary was observed from a hide for two 1 h sessions over a two-week period during November 2001 (birds aged between 185–200 days). The order in which aviaries were observed was randomized. During each observation session the number of times that an individual displayed dominant behaviour to another and displaced its opponent was recorded. The dominance hierarchy and social rank of each bird within each cage were quantified according to Martin & Bateson (1986) using a dominance matrix. Two measures of social status were used in this study: social rank and the actual number of birds that an individual dominated within its cage.

One week before the end of the experiment, birds were blood sampled for corticosterone production (and testosterone levels in males), using a capture–handling–restraint method. Corticosterone and testosterone concentrations were measured after extraction of 20 µl aliquots of plasma in diethyl ether, by radioimmunoassay (as described in Buchanan *et al.* 2003).

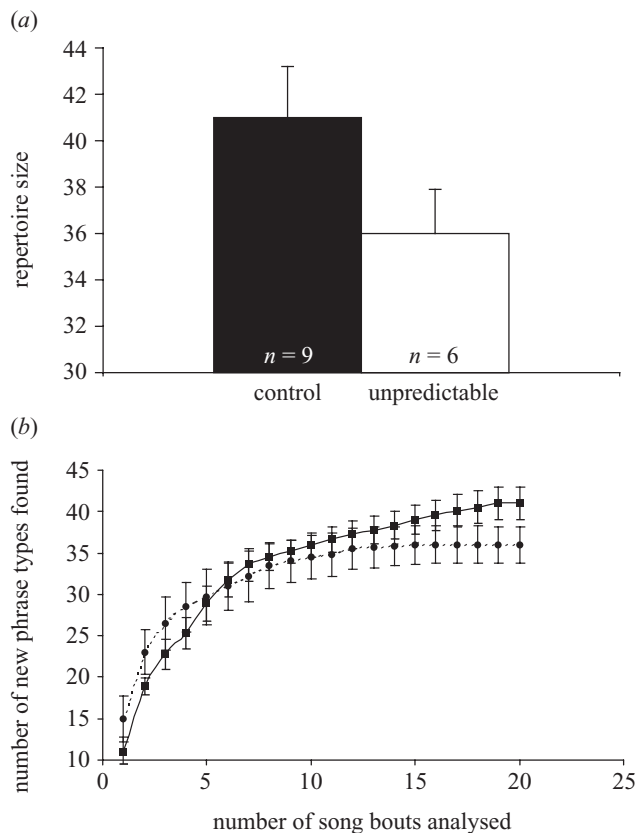For further details see electronic Appendix A available on The Royal Society's Publications Web site.

(a)

(b)

Figure 1. (a) The effect of experimental treatments on repertoire size in European starlings ($p = 0.01$). (b) Cumulative plot showing the mean ($\pm$ s.e.) number of new phrase types found in song analysis. Circles, unpredictable; squares, control.
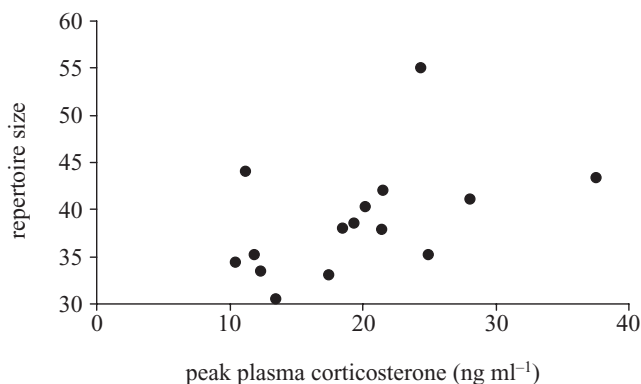

Figure 2. Relationship between peak corticosterone responses in starlings during development and adult song repertoire size ($p = 0.009$).

3. RESULTS

There was a significant effect of our experimental procedure on the repertoire size of male starlings in this study. Males that had experienced an unpredictable food supply in early life showed significantly smaller repertoires (ANCOVA: $F_{1,7} = 12.13$, $p = 0.010$; figure 1a,b). Basal hormone levels (corticosterone and testosterone) did not show any relationship with repertoire size ($p > 0.600$); however, peak levels of corticosterone were significantly positively related to the number of different phrase types in a male's repertoire ($F_{1,7} = 12.99$, $p = 0.009$; figure 2).
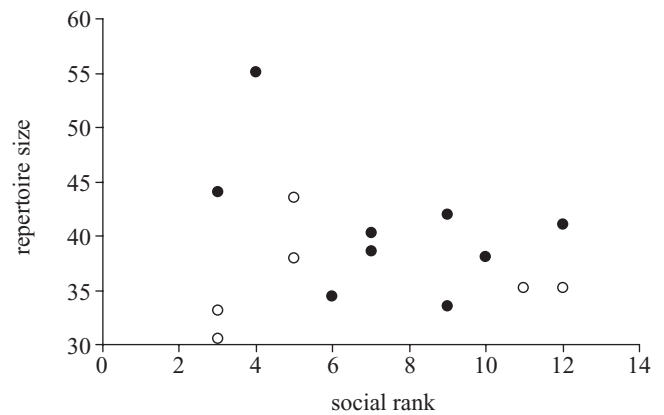

Figure 3. The effect of social rank during development on repertoire size in European starlings ($p = 0.014$). Removal of the outlier maintains significance of the relationship ($p = 0.044$). Rank 1 represents the most dominant; rank 12 the least. Open circles, unpredictable; filled circles, control.

Birds that exhibited higher body masses at the end of the experimental period were also more likely to exhibit larger repertoires ($F_{1,7} = 24.24$, $p = 0.002$), but the strength of this relationship differed depending upon which treatment a bird had undergone, with controls showing a steeper relationship (interaction: $F_{1,7} = 10.76$, $p = 0.013$). The results also indicate that birds which mounted a larger cell-mediated immune response produced a larger phrase repertoire ($F_{1,7} = 23.03$, $p = 0.002$).

When we consider social rank (within each cage), birds that held a more dominant rank were more likely to produce larger repertoires ($F_{1,7} = 10.48$, $p = 0.014$; figure 3). The same relationship was also seen when we considered the actual number of birds that an individual dominated within its hierarchy ($F_{1,7} = 5.49$, $p = 0.052$). Removal of an outlier in both analyses, which seemed to have a large influence on the data, did not affect these results (rank: $F_{1,7} = 6.02$, $p = 0.044$; number of birds: $F_{1,7} = 5.68$, $p = 0.048$). Social status was not related to individual mass, immune response, testosterone levels or corticosterone levels ($p > 0.336$).

4. DISCUSSION

It is clear from our results that developmental stress, in the form of an unpredictable food supply, can have significant deleterious effects on song learning and repertoire size. This work builds on a previously published study indicating that song bout length and output can be affected in this way (Buchanan *et al.* 2003). The mechanism underlying this effect is unclear. Spencer *et al.* (2003) have shown that both food restriction and elevated corticosterone levels during development can have significant effects on song complexity. Starlings in the unpredictable group here showed a trend towards elevated corticosterone levels (Buchanan *et al.* 2003), and this suggests a role for corticosterone. Because our experimental birds maintained a higher body mass than controls, it is unlikely that an energetic deficit occurred during the experimental treatment period. It is worth speculating whether the changes in repertoire size may be associated with changes in brain morphology, as recently demonstrated in the swamp sparrow (*Melospiza georgiana*) (Nowicki *et al.*

2003). Unfortunately in this study we do not have the data to address this issue.

This study also provides evidence that social rank during the sensitive song-learning period can have significant effects on repertoire size. Dominant birds produced larger repertoires independent of experimental treatment. Our results suggest that birds of lower social rank may incur a cost potentially due to limited access to food or reduced ability to practise their song. Subordinate birds are usually heavier than dominants (Witter & Swaddle 1995), but we found no relationship between mass and social status, suggesting that social rank may affect song by impinging on song practice. However, we have no data concerning how social rank affects singing rates in our groups and more empirical work is needed to test this hypothesis. In a previous study on starlings (Eens 1997), a similar significant relationship between repertoire size and dominance was also found. This suggests that repertoire size could be a signal of male quality and that social rank during development is an important factor mediating the song signal.

One potential problem with our study is that each group of birds was able to forage in a relatively small area, compared with that encountered by birds in a wild situation. This may have exaggerated the frequency and intensity of the aggressive interactions because in a cage environment the dispersal distance of a supplanted bird is much reduced. Although our study suggests that social as well as developmental history may be important in shaping an individual's song signals, care must be taken in their interpretation until more empirical studies are undertaken. One empirical study that has investigated the relationship between dominance and song traits in the wild (Lambrechts & Dhondt 1986) found no relationship between dominance and repertoire size. Our data should therefore be treated as preliminary, and future experimental work should concentrate on the link between developmental stressors (such as social rank), song traits and fitness in the wild.

Our study may also provide evidence for an enhanced stress response signalling high male quality through a larger repertoire (figure 2). Immune response also showed a significant positive relationship with repertoire size. This measure of phenotypic quality has already been linked to song complexity in this species and may also be a reliable indicator of male quality (Duffy & Ball 2002). The lack of any relationship between testosterone and repertoire size may indicate that androgens are less important in modulating this particular aspect of song (Duffy & Ball 2002). However, this may be due to the timing of the blood sampling, at the end of the breeding season.

This study is, to our knowledge, the first to show the effects of developmental stress on repertoire size in a bird with a sexually selected song structure. It has also uncovered a possible link between social status during development and song quality. What is required now is a more thorough investigation into the many potential developmental stressors that a juvenile bird may encounter and the mechanisms controlling song development, if we are to understand fully the role of stress in determining song as an honest signal of quality.

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