

Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*)

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Bird song is a sexually selected male trait where females select males on the basis of song quality. It has recently been suggested that the quality of the adult male song may be determined by nutritional stress during early development. Here, we test the 'nutritional-stress hypothesis' using the complex song of the European starling. Fledgling starlings were kept under experimental treatment (unpredictable short-term food deprivations) or control conditions (*ad libitum* food supply), for three months immediately after independence. We measured their physiological and immune responses during the treatment and recorded song production during the following spring. Birds in the experimental group showed increased mass during the treatment and also a significantly suppressed humoral response compared with birds in the control group. There was no difference between the groups in the cell-mediated response. Next spring, males in the experimental group spent less time singing, sang fewer song bouts, took longer to start singing and also sang significantly shorter song bouts. These data support the hypothesis that both the quality and quantity of song produced by individual birds reflect past developmental stress. The results also suggest the 'nutritional-stress hypothesis' is best considered as a more general 'developmental-stress hypothesis'.

Keywords: song learning; corticosterone; stress; female choice; sexual selection; bird song

1. INTRODUCTION

Sexual signals advertising male quality evolve when they act as honest indicators of male quality (reviewed in Andersson 1994). Bird song is a well-known sexual trait, as male songbirds sing to establish territories and to attract a mate (Catchpole & Slater 1995). In a range of bird species, females have been shown to prefer males that produce quantitatively more song, or males that produce song which is qualitatively more complex in structure (for a review see Searcy & Yasukawa 1996). There is some evidence that females obtain direct benefits from mating with a male which produces song at a faster rate (Greig-Smith 1982). It is much less clear what benefits females obtain from mating with males with particular song structure. Several reasons for the evolution of preferences for complex song have been suggested (Catchpole & Slater 1995; Collins 1999), including the possibility that certain song structures may indicate male genotypic or phenotypic quality (Hasselquist *et al.* 1996; Buchanan & Catchpole 2000).

Nowicki *et al.* (1998) recently suggested that song quality could honestly reflect male quality by indicating how males respond to early developmental stress. As the brain nuclei associated with song learning grow and develop their synaptic connections during very early development, adult birds may be constrained in the quality of the signal they can produce by their early rearing conditions (Catchpole 1996; Buchanan 2000; Doutrelant *et al.* 2000). Young birds that suffer early developmental stress would be unable to compensate for the detrimental effects

of under-nourishment, and would continue to signal their poor early developmental conditions through simplified song structures (Nowicki *et al.* 1998). The assumption of this 'nutritional-stress' hypothesis is that variation in brain development translates into variation in song learning and in the resulting song complexity, which is used by females in mate choice decisions. So far, this hypothesis has been supported by observational data from a long-term study of the great reed warbler, *Acrocephalus arundinaceus* (Nowicki *et al.* 2000). This study demonstrated that nestling great reed warblers in the best condition (as measured by feather growth), produced the largest song repertoires as first-year adults. Although these observational data support the nutritional-stress hypothesis, such a relationship could be due to genetic measures of parental quality. For example, paternal repertoire size is positively correlated with provisioning rate in male sedge warblers, *A. schoenobaenus* (Buchanan & Catchpole 2000). If song repertoire size is a heritable trait, then paternal feeding rates and offspring song complexity could covary without invoking the nutritional-stress hypothesis. However, recent experimental work on the song sparrow (*Melospiza melodia*) has demonstrated that nutritional stress during early development detrimentally affects both the accuracy of song learning in the male (Nowicki *et al.* 2002a) and song preferences in the female (Nowicki *et al.* 2002b). This work confirms that song learning in this species is affected by early development conditions, with consequences for female preferences and the matching of local dialects. Song learning is also important for determining song complexity in species with highly variable song repertoires (Nottebohm 1999), and in many species song complexity is related to male attractiveness. Furthermore, the development of the song control nuclei

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in the brain has been shown to correlate with repertoire size in both interspecific (Székely *et al.* 1996) and intraspecific studies (Airey *et al.* 2000). The nutritional-stress hypothesis may therefore also be able to explain the evolution of complex song repertoires, as honest signals of quality.

To test the nutritional-stress hypothesis directly, we examined the effects of early developmental stress on song production in the European starling (*Sturnus vulgaris*). Starlings have complex songs and a considerable body of work has shown that they sing both to establish ownership of the nest site and to attract a mate (Feare 1984; Eens *et al.* 1991). Experimental studies have shown that female starlings are attracted to nest sites where more song is produced (Eens *et al.* 1991; Mountjoy & Lemon 1991). In addition, both song bout duration and repertoire size (which are highly correlated) have been found to relate to female choice (Mountjoy & Lemon 1996; Eens 1997), with females preferring males that produce more complex song. As captive male starlings produce more different types of song phrases when housed with a female than when housed with males (Eens *et al.* 1993), this suggests that males may change their behaviour to signal their repertoire size. Starlings are open-ended learners, as they continue to add to their song repertoire throughout life and also show seasonal plasticity and age-related development of the song control nuclei (Bernard *et al.* 1996; Bernard & Ball 1997). Both repertoire size and the volumes of the higher vocal centre (HVC) and the *robustus archistriatalis* (RA) in the brain have been shown to increase with age in male starlings (Bernard *et al.* 1996). In addition, the volumes of the HVC and RA nuclei have been shown to correlate with song bout duration (Bernard *et al.* 1996) suggesting that female choice has implications for male neural development. The brain nuclei controlling song learning and production have been shown to develop during the post-fledging period in the starling (Casto & Ball 1994). Specifically the RA shows a significant increase in size between 50 and 100 days of age in the male starling, a process that is thought to occur in conjunction with innervation of the RA by the HVC (Casto & Ball 1994). For this reason we hypothesized that the neural development associated with song learning in male starlings would be particularly susceptible to developmental stress during this time.

The principal aim of the study was to test experimentally whether the quantity and quality of song produced by adult starlings were determined by early nutritional stress. A second aim was to try to determine the mechanism by which such effects might occur. The development of the song nuclei could be detrimentally affected by increased corticosterone levels as a result of increased stress, as corticosterone can affect neural development and function (Brown 1994; Buchanan 2000). Corticosterone is likely to be produced in response to a range of stressors that young birds might encounter during the post-fledging period and could therefore be a mechanism for mediating stress effects. Alternatively, under nutritional stress detrimental effects might be mediated through changes in male condition due to resource restriction or reallocation to different physiological tasks. For this reason we also set out to measure male condition, corticosterone production and

immune function, in response to experimental nutritional stress.

2. METHODS

(a) *Experimental design*

Fledgling starlings ($n = 48$) were brought into outdoor aviaries during May 2001. The birds were caught on the same day using a decoy trap in south Somerset, UK, and were all within approximately three weeks of fledgling at 35–50 days old. The birds were housed in adjacent cages (1.8 m × 1.8 m × 0.9 m) in four mixed-sex groups of 12 randomly assigned individuals (between six to nine males in each) within visual and acoustic contact of each other. These groups were maintained throughout the experiment. The cages were randomly allocated to two experimental treatments, control and unpredictable food supply (two cages each). All the starlings were maintained on a diet of commercially produced turkey starter crumbs (BOCM Pauls, UK) administered in a food hopper, which was always kept filled. The control group therefore experienced an *ad libitum* food supply. The unpredictable food supply group were maintained on the same diet, but once a day the food hopper was removed from the cage for 4 h (*ca.* 25% of daylight hours). The start of this deprivation was randomized, but always occurred between 0900 and 1600. At the start of each food deprivation any spilt food was removed by sweeping the floor of the cage. To control for any possible effects of disturbance, the control cages were also entered, but the food was not removed. Across the corridor of the aviary (1 m) four adult male starlings were housed as song tutors. These birds were maintained in this arrangement throughout the experiment. In addition, as all the cages were outdoors, the experimental birds would also have heard and seen wild starlings singing. As all the experimental birds were within three weeks of fledging when captured for the experiment, most of their song exposure and song learning would have occurred during the experimental food deprivation as well as the months following the end of the treatment period.

Every 10 days during the treatment period there was a probe day for monitoring the mass of the birds, during which the experimental birds experienced no food deprivation. In this way the mass of the birds in the control group could be compared with the mass of the birds in the unpredictable food supply group, without the influence of food deprivations on the day of weighing (Cuthill *et al.* 2000). The birds were caught in succession and weighed (to the nearest 0.01 g). The birds were maintained on the experimental treatments from 4 June until 23 August 2001 and so they were 115–130 days old at the end of the treatment period. After the end of the treatments all the birds were kept on an uninterrupted food supply.

The immune function of the birds was tested during the treatment period by using both a cell-mediated and a humoral immune challenge. The cell-mediated challenge was done in mid-July and the humoral test in late July. At the start of the humoral test blood was also drawn to test the haematocrit levels of the birds. This was done by taking 50 µl from the brachial vein into a heparinized capillary tube. The blood was spun for 10 min at 14 000g in a benchtop Joann A14 centrifuge. The lengths of the plasma fraction and of the compacted red cell fraction were measured to calculate the percentage of red cell volume.

The cell-mediated response was tested by using an injection of phytohaemagglutinin (PHA) into the wing web. PHA is a plant lectin which promotes a hypersensitivity reaction and has

been used extensively to investigate immune function (Lochmiller *et al.* 1993). The thickness of the right wing web was measured before injection using a pressure-sensitive spessimeter (Alpa s.r.l., Milan). Subsequently, 50 μl of a suspension of PHA (Sigma L-8754) (0.45 mg in 50 μl) was injected into this wing web. A control injection of phosphate buffered saline (PBS) in the left wing, to control for any swelling as a result of the injection, was not done as this has previously been shown to be unnecessary (Smits *et al.* 1999). The peak response occurs between 5–24 h post-injection and so the thickness of the right wing web was measured using the spessimeter at 5 and 24 h after injection, after which time the swelling subsided.

The humoral response was tested by using intraperitoneal injections of sheep red blood cells (SRBC) (Deerenberg *et al.* 1997). A blood sample (100 μl) was drawn before the start of the immune challenge to test for the presence of heterologous antibodies which would cross-react with the SRBC antigens. The SRBC in Alsever's solution (TCS Microbiol Ltd, Claydon, UK) were washed three times in $1 \times$ PBS and resuspended in $1 \times$ PBS to form a 2% solution. Five hundred microlitres of the 2% solution were injected intraperitoneally and 7 days later 100 μl of blood was drawn to test for the presence of antibodies during the primary response. Two weeks after the first injection, an identical second injection was given and 5 days after this, a second blood sample was taken to test for the presence of antibodies during the secondary response. The blood samples were spun in a benchtop centrifuge at 14 000g for 15 min and the plasma removed, heat-treated at 56 °C in a water bath for 30 min and stored at –20 °C for further analysis. The blood samples were tested within three weeks of being drawn. Each plasma sample (20 μl) was serially diluted across a V-form microtitre plate (Greiner Labortechnik) in PBS. The SRBC solution (20 μl of a 2% solution) was then added to each of the wells of the tray and the tray incubated at room temperature for 1 h. Haemagglutination is apparent when the antibodies in the plasma are present in sufficient quantity to form a film of blood cells. The most dilute titre of plasma capable of maintaining this reaction was noted.

One week before the end of the experimental period, the birds were all blood sampled for corticosterone production, by using a capture–handling–restraint method, which has been used across a range of species (Wingfield 1994). The procedure involved capturing the birds in groups of three from their cage and taking a blood sample (100 μl) within 3 min, with three people blood sampling, to determine basal plasma corticosterone concentrations. Each bird was then kept in a small cloth bag and a further sample taken at 10 min after capture. This method of sampling is a standardized way of measuring both basal (3 min) and elevated (10 min) corticosterone production. Using this technique, corticosterone production in the starling has been shown to rise steadily until 10 min after capture, after which time it declines (Maddocks *et al.* 2001). For this reason, elevated corticosterone is referred to as 'peak corticosterone' production. After the birds were released, the aviary was not visited for at least 2 h to allow the corticosterone levels of the other birds to return to the baseline.

At the end of the song recording period (mid-May 2002) all the birds were caught and blood sampled for testosterone production. At this time the birds were also weighed.

(b) Song analysis

In March 2002 the song output of individual male starlings was recorded as follows. In the morning of the first recording

day a male was moved into a separate outdoor cage the same size as their housing cage in a separate outdoor aviary. The recording cage also contained a nestbox. To stimulate the male to sing, a female was housed in an identical cage across the aviary corridor, 1.5 m away and therefore within visual and acoustic contact. The song output of the male was recorded twice for 1 h: once in the afternoon of the day on which the male was moved and once the following morning. The start of the first recording was always at least 3 h after the male was moved to this aviary. The order of recording was such that it alternated daily between the two treatment groups. The recordings were made using a Sony DAT Walkman (TCD-D8) and a Sennheiser K6 microphone body, with a Sennheiser (MKE 2-60 Gold C) sub-miniature microphone attachment, mounted on top of the nestbox. From the recordings the following measures were taken: (i) the total amount of time spent singing; (ii) the number of song bouts; (iii) the latency to start singing after the start of the recording; and (iv) the duration of each song bout. Song bouts were defined as continuous song and were separated from each other by at least 1 s (Eens 1997). These measures were averaged over both recording periods. Birds that did not sing during either recording attempt were not included in the song analysis.

(c) Hormone assays

Blood samples (100 μl) were collected in heparinized capillary tubes after puncture of the brachial vein with a 25 gauge needle, centrifuged and the plasma stored at –20 °C for later hormone assay. Corticosterone concentrations were measured after extraction of 20 μl aliquots of plasma in diethyl ether, by radioimmunoassay (Wingfield *et al.* 1992; Maddocks *et al.* 2001) using anti-corticosterone antiserum (code B21-42, Endocrine Sciences, Tarzana, CA) and [1,2,6,7-³H]-corticosterone label (Amersham, UK). The extraction efficiency was 80–90%. The assay was run with 50% binding at 141 pg per tube, and the detection limit (for 7.3 μl aliquots of extracted plasma) was 0.45 ng ml⁻¹. Testosterone concentrations were measured in plasma samples by direct radioimmunoassay by using anti-testosterone antiserum (code 8680-6004, Biogenesis, UK) and ¹²⁵I-labelled testosterone (code 07-189126, ICN, UK) (Parkinson & Follett 1995). The assay was run with 50% binding at 7.4 pg tube⁻¹, and a detection limit of 0.04 ng ml⁻¹ for the 10 μl plasma volumes that were run (in duplicate) in the assay.

(d) Statistical analysis

The data were analysed by using MINITAB v. 13 (Minitab Inc., State College, PA, USA). In each case a general linear model ANOVA was constructed to test the effects of the experimental manipulations on mass on each probe day and on each measure of song production. Treatment group was added to each model as a factor, with date of recording, mass on 10 August 2001 (mass at the end of the treatment period), basal and peak corticosterone titres, testosterone titres, PHA response, SRBC response and haematocrit all added as covariates. Stepwise deletion of non-significant terms was performed until the minimal adequate model was found (Zar 1984). The model residuals were checked for normality and homoscedasticity at each step.

3. RESULTS

All of the birds gained in mass after their capture. The birds exposed to an unpredictable food supply were

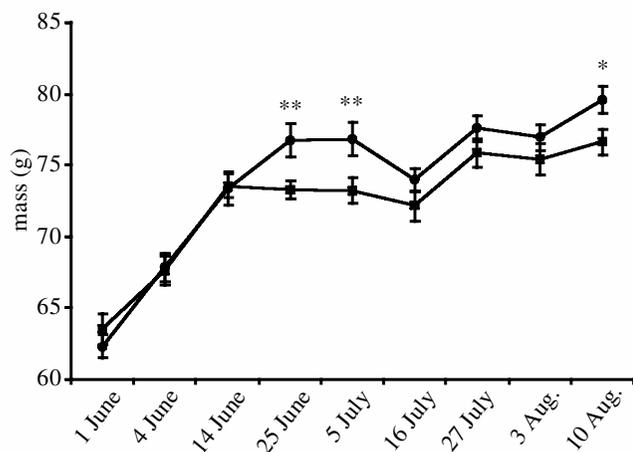


Figure 1. Mass of the experimental and control birds throughout the treatment period \pm s.e.m. Significant differences between the groups are indicated * $p < 0.05$, ** $p < 0.01$. Circles, unpredictable food supply; squares, control group.

heavier than control birds for most of the duration of the treatment period (figure 1). There was a drop in body mass on 16 July after the birds had been rehoused in a new aviary. The experimental birds were significantly heavier than the control birds on three of the probe days (25 June: $F_{1,43} = 7.50$, $p = 0.009$; 5 July: $F_{1,45} = 7.91$, $p = 0.007$; 10 August: $F_{1,45} = 6.62$, $p = 0.013$). Males were also significantly heavier than females throughout. There was a significant interaction between sex and treatment group on 25 June ($F_{1,42} = 10.50$, $p = 0.002$) such that females in the unpredictable food supply group were of lower mass than the males in that treatment.

There was no effect of treatment on basal corticosterone levels (figure 2a). There was an effect of treatment group on peak corticosterone titre, with birds in the unpredictable food supply group having higher corticosterone titres (figure 2a). Although this relationship was marginally non-significant ($F_{1,40} = 3.10$, $p = 0.086$), this was true while controlling for a significant effect of date ($F_{1,40} = 9.52$, $p = 0.004$). There was also a sex effect, with males showing higher peak corticosterone levels than females ($F_{1,40} = 6.22$, $p = 0.017$). There was no effect of treatment group on testosterone titre, measured in late spring at the end of the treatment period (seven months after the end of the treatment period: $F_{1,41} = 0.08$, $p = 0.784$; figure 2b), nor was there any significant effect of treatment group on mass as measured at this time ($F_{1,43} = 0.07$, $p = 0.787$).

Birds in the unpredictable food supply group produced a significantly lower humoral immune response than birds in the control group ($F_{1,45} = 5.51$, $p = 0.023$; figure 3). There was also a tendency for males to produce a lower secondary response, although this was marginally non-significant ($F_{1,45} = 3.11$, $p = 0.085$). There was no significant difference between the experimental groups in haematocrit ($F_{1,11} = 0.31$, $p = 0.588$).

There was no effect of treatment on PHA response measured either at 5 h ($F_{1,46} = 1.19$, $p = 0.280$) or 24 h ($F_{1,46} = 0.01$, $p = 0.961$) post-injection.

We obtained song recordings from 15 males, nine in the control group and six in the unpredictable food group.

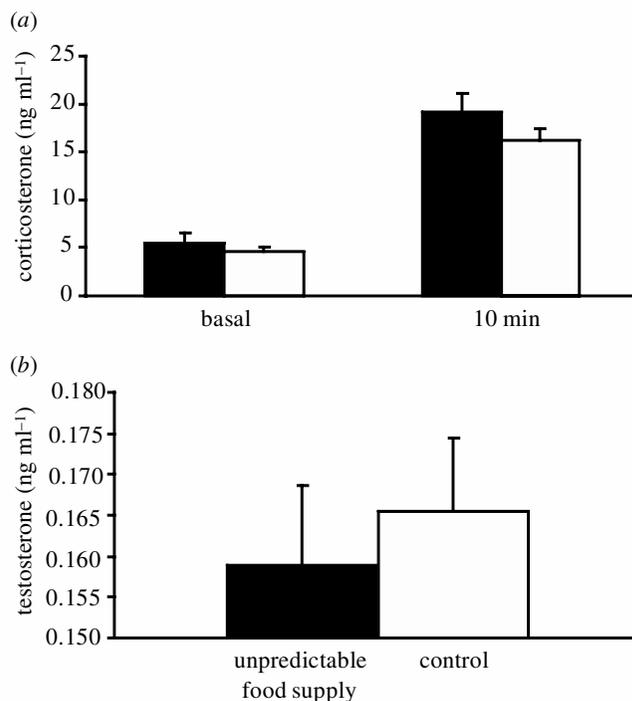


Figure 2. The hormone production of birds in the two treatment groups; unpredictable food supply (black) and control group (white). (a) Corticosterone (ng ml^{-1}) levels sampled within the treatment period, but not during a food deprivation, within 3 min of capture (basal) and 10 min of capture. (b) Testosterone (ng ml^{-1}) levels sampled during the spring following the end of the treatment period.

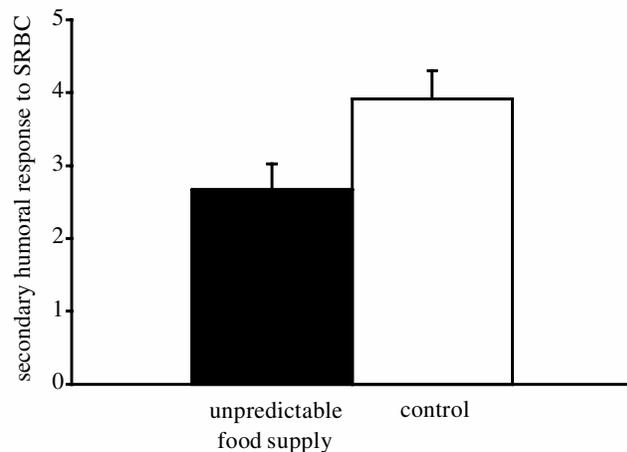


Figure 3. The humoral response of all birds to injection of SRBC in both treatment groups.

The variables describing significant variation in the measures of song production are shown in table 1. Males in the unpredictable food supply group spent significantly less time singing (table 1; figure 4a). There was also a significant effect of the date of recording, such that birds recorded earlier in the season spent longer singing.

Males in the unpredictable food supply group took longer to start singing after the start of recording (table 1; figure 4b). There was also a significant effect of basal corticosterone titre, whereas a marginally non-significant interaction between treatment group and basal corticosterone titre was constrained into the model. Overall, corticosterone titre had a negative effect on the latency to

Table 1. The variables explaining significant variation in the measures of song production.

song production	variable	<i>F</i>	d.f.	<i>p</i>
time spent singing	treatment group	6.69	1,12	0.024
	date of recording	4.53	1,12	0.055
latency to start singing	treatment group	7.90	1,11	0.017
	basal corticosterone	5.63	1,11	0.037
	treatment group × basal corticosterone	4.05	1,11	0.069
number of song bouts	treatment group	15.61	1,8	0.004
	mass	12.96	1,8	0.007
	basal corticosterone	10.45	1,8	0.012
	treatment group × mass	14.90	1,8	0.005
	basal corticosterone × date	10.46	1,8	0.012
mean song bout duration	treatment group	7.21	1,11	0.021
	PHA (5 h)	8.27	1,11	0.015
	treatment group × PHA (5 h)	9.03	1,11	0.012

start singing, such that birds with low corticosterone levels took longer to start singing. This was more pronounced in the unpredictable food supply group.

Males in the unpredictable treatment group sang significantly fewer song bouts (table 1; figure 4c), and there was also a negative effect of mass and a positive effect of basal corticosterone titre on the number of song bouts sung per hour. There were also significant interactions between treatment group and mass and between basal corticosterone and date. The interaction between mass and treatment group meant that within the control group the relationship between the number of song bouts and mass was negative. By contrast, mass had little effect on the number of song bouts within the unpredictable food group.

Males within the unpredictable food supply group sang significantly shorter song bouts (table 1; figure 4d), whereas there were also significant effects of PHA response at 5 h post-injection, as well as a significant interaction between treatment group and PHA response. Overall, birds that mounted a larger PHA response sang longer song bouts. Within the control group the relationship between PHA response and song bout duration was positive, whereas this effect was not apparent within the unpredictable food supply group.

Song bouts less than 5 s in duration are considered to be incomplete (Eens *et al.* 1991) and so we also considered the effect of the experimental treatment only on song bouts longer than 5 s. Using this definition of song bout, the effect of treatment group on the number of song bouts was marginally non-significant ($F_{1,12} = 4.29$, $p = 0.060$), although the effect of treatment group on song bout duration remained significant ($F_{1,7} = 6.48$, $p = 0.038$), with males in the unpredictable food supply group singing significantly shorter song bouts. There was also a significant three-way interaction between treatment group, 5 h PHA response and mass ($F_{1,7} = 5.82$, $p = 0.047$); both the relationships between PHA response and song bout duration and between mass and song bout duration were positive in the control group and negative in the unpredictable food supply group.

4. DISCUSSION

Although female choice for increased song complexity has been demonstrated in many species (Catchpole &

Slater 1995; Searcy & Yasukawa 1996), the reasons for such preferences are unclear. The original nutritional-stress hypothesis (Nowicki *et al.* 1998) proposed that song learning could be detrimentally affected by early developmental stress. Females would therefore choose males according to quantitative or qualitative aspects of song production because these males can indicate through their songs that they have not suffered early developmental stress effects. Recent results confirm that early developmental stress can detrimentally affect the accuracy of song copying from a tutored song (Nowicki *et al.* 2002a). The results presented here provide further support for this hypothesis and confirm the possible role of early developmental stress in controlling both the quantity of song produced and song complexity. All the quantitative measures of song production (amount of time spent singing, number of song bouts and latency to start singing) were affected by the unpredictable food treatment. In addition, males in this treatment group also sang shorter bouts. This measure is particularly relevant as female starlings have been shown to prefer males that produce longer song bouts (Eens *et al.* 1991; Mountjoy & Lemon 1996; Gentner & Hulse 2000). Females also prefer males with larger repertoires, and bout duration and repertoire size are strongly positively correlated (Eens 1997). Song complexity is clearly used by females in mate choice decisions and our results suggest it may be an honest indicator of early developmental stress.

The mechanism for the effects of developmental stress on song production is unclear. Birds in the unpredictable food supply group were significantly heavier than birds provided with *ad libitum* food, as has been found previously in studies of mass regulation in starlings (Witter *et al.* 1995; Cuthill *et al.* 2000). Furthermore, this increase in body mass does not appear to be solely a result of increased food consumption and may be in part caused by changes in individual activity levels or digestive efficiency (Bednekoff *et al.* 1994; Cuthill *et al.* 2000). It therefore seems unlikely that the birds in the unpredictable food treatment were subject to energetic restriction. Peak corticosterone levels were found to be higher in the birds in the unpredictable food supply group, although not significantly so. However, the statistical power for this test was only 10.3%, and thus there may well have been a biologically meaningful difference between the groups, which

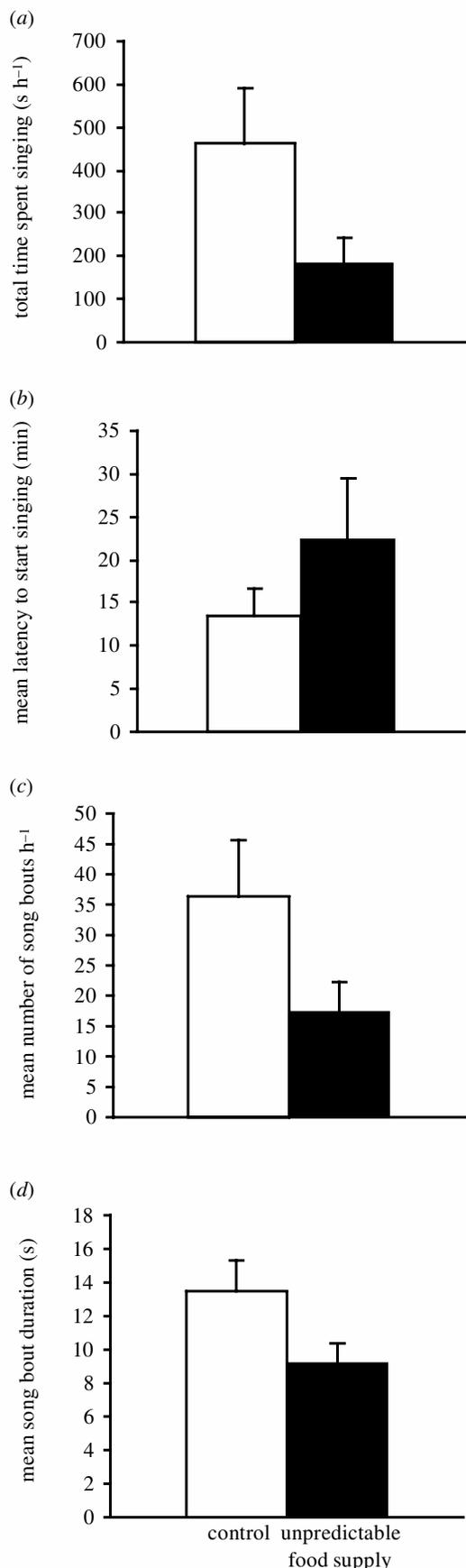


Figure 4. The song production of males in the two treatment groups. (a) Mean total time spent singing h^{-1} ; (b) mean latency to start singing; (c) mean number of song bouts h^{-1} and (d) mean song bout duration. Complete and incomplete song bouts included.

we were not able to detect with this sample size. Corticosterone is known to have adverse effects on neural development (Brown 1994; Buchanan 2000) and could therefore be a potential mechanism allowing developmental stress in the brain to be expressed through song production. Our measures of corticosterone production during the treatment period were restricted to one point measure, taken on a probe day when no food deprivation occurred, and therefore they may not have been sufficient to pick up shorter term changes which might coincide with the food removal. Further sampling during the food deprivations and in the months after the end of the treatments would have been useful in clarifying the role of corticosterone.

The original nutritional-stress hypothesis (Nowicki *et al.* 1998) was based on the assumption that developmental stress would affect the song control brain nuclei which develop early in life (Nottebohm 1999). In our experiment the treatments were started three weeks post-fledging, by which time the brain nuclei contributing to song learning should have been well-developed (Nottebohm 1999). However, as starlings are open-ended learners, their neural development continues not only post-fledging but also throughout the following months (Bernard *et al.* 1996; Nottebohm 1999). Indeed, data suggest that there are substantial increases in the volume of the RA between 50–100 days post-hatching in the male European starling and that this period may coincide with innervation of the RA by the HVC (Casto & Ball 1994). It therefore seems plausible that sufficient neural growth and synaptic development continued during the treatment period for the unpredictable food supply to have had a detrimental effect on neural development and consequently on song production. An alternative possibility is that developmental stress affects the processing and/or storage of the information during the learning process, without the involvement of morphological changes in the brain nuclei. Indeed, there is limited evidence for a direct link between brain nuclei development and repertoire size in species that show seasonal changes in neural development (Brenowitz *et al.* 1991; Smith *et al.* 1995).

As the unpredictable food treatment suppressed the humoral response to SRBC, this suggests that developmental stress caused differential resource allocation to different physiological processes. For example, under stressful, unpredictable conditions it may prove adaptive to draw resources away from less essential functions such as song and also to bear the cost of immunosuppression (Buchanan 2000). Corticosterone has been shown both to be generally immunosuppressive (Buchanan 2000) and to be involved in the reallocation of resources to different parts of the immune response (Braude *et al.* 1999). There was no effect of our treatments on the cell-mediated response to PHA injection, but birds producing larger PHA responses during the treatments sang significantly longer song bouts some seven months later. This correlation suggests that both song bout duration and the cell-mediated response are indicators of aspects of male phenotypic or genetic quality. This covariation between immunity and song quality is in agreement with the recent finding that in the European starling song rate is correlated with cell-mediated immunity, whereas song bout duration is correlated with humoral immunity (Duffy & Ball 2002). This covariation between immune function

and song quality is also in line with the immunocompetence handicap hypothesis, suggesting that song production may act as an indicator of immunocompetence (Folstad & Karter 1992). However, there is little evidence from this study to support the direct influence of testosterone, as predicted in the original hypothesis.

We found no evidence that testosterone levels were a significant predictor of any of the song variables, nor did we find that testosterone production was affected by the treatments. This may be due in part to the fact that the testosterone levels were measured at the end of the breeding season, when the birds were becoming photorefractory. However, Duffy & Ball (2002) also found no relationship between either song rate or song bout duration and testosterone levels, in this case measured within days of the song recordings. This suggests that although the complexity of song may be affected by the action of testosterone on initial or seasonal brain development (Bernard & Ball 1997), levels of testosterone during the breeding season are less important for controlling the aspects of song quality measured in this study.

Many reasons have been suggested for female preference for males that produce more song, including advertisement of territorial resources (Radesäter & Jakobsson 1989) or of male provisioning abilities (Greig-Smith 1982). This study has demonstrated experimentally, for the first time, to our knowledge, that song output can reflect past levels of environmental and physiological stress in a species with a sexually selected song structure. It therefore provides strong support for the nutritional-stress hypothesis (Nowicki *et al.* 1998), suggesting that complex repertoires could have evolved as indicators of previous physiological stress. The results also support the recent finding that both the accuracy of song learning (Nowicki *et al.* 2002a) and female choice (Nowicki *et al.* 2002b) are affected by early nutritional stress in the song sparrow. Indeed there is some indication that these effects are mediated through direct effects on the song control nuclei in the brain (Nowicki *et al.* 2002a). It is worth noting that if such effects were also expressed in the female brain, this might explain the changes demonstrated in female mate choice in relation to developmental stress reported by Nowicki *et al.* (2002b). Our results demonstrate that females within the unpredictable food supply group differed from males in their response to the food deprivations, both in terms of their mass and their corticosterone production. Unfortunately, we have no information on how this translated into investment in neural development.

Our results also suggest that the mechanism for the effects on male song output may occur through the detrimental effects of corticosterone on neural development or function, although the nature and extent of hormonal involvement in this process is currently unclear. The results, however, support the recent findings that both nutritional stress and direct administration of corticosterone to nestling zebra finches (*Taeniopygia guttata*) adversely affects song learning (Spencer *et al.* 2003), also suggesting that corticosterone may be involved in the mechanism. Recent results from a study of brain development in the song sparrow confirm that early nutritional stress detrimentally affects the development of the brain nuclei involved in song learning and song production

(Nowicki *et al.* 2002a). The mechanism for this effect could be either through direct energetic restriction, through elevated corticosterone levels or another unidentified stress-related physiological route. This raises the possibility that the 'nutritional-stress hypothesis' would be better extended to a more general 'developmental-stress hypothesis' to include other environmental effects that young birds encounter such as low temperature, parasitism or disturbance, which are likely to have major deleterious effects upon developing birds (Buchanan *et al.* 1999). Certainly corticosterone is known to have adverse effects on neural development and function when chronically elevated (Buchanan 2000). When the costs of song production and learning are more fully understood we may move closer to an explanation of how song may act as an honest indicator of male quality.

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