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# Stress, song and survival in sparrows

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**The stress response—increases in circulating glucocorticoids following a stressor—is typically considered adaptive, but few studies address the fitness consequences of individual variation in stress response. Generally, due to negative consequences of prolonged elevation of glucocorticoids, animals should have a transient stress response just sufficient to cope with the stressor. In rodents, stress responsiveness is affected by early developmental experience, and hyper-responsiveness to stress is linked to morbidity and mortality. We assessed individual variation in stress responses in free-living song sparrows, *Melospiza melodia*, in relation to fitness-related measures including song and overwinter survival. Birds with greater increases in corticosterone 30 min following restraint stress were less likely to return to breed the following year. Stress responsiveness was also correlated with song complexity: males with fewer syllables in their song repertoires had greater stress reactivity. Our findings support the hypothesis that developmental stressors both impair song development and affect the adult stress response. Thus, individual variation in the stress response may relate to variation in fitness.**

**Keywords:** birdsong; stress response; survival

## 1. INTRODUCTION

In vertebrates, the endocrine response to stress involves up-regulation of the hypathalamo–pituitary–adrenal (HPA) axis. In response to a perceived stressor, the adrenal cortex increases circulating levels of glucocorticoid hormones (primarily corticosterone in birds) that have numerous effects, including mobilizing energy to allow the organism to cope with a stressor during this emergency life history stage (Wingfield *et al.* 1998). Because prolonged elevation of glucocorticoids can have detrimental effects (e.g. McEwen 1999), regulation of HPA function is critical. Optimal HPA function should thus involve regulation of both increasing and decreasing levels of glucocorticoids in order to cope with a stressor (Romero 2004).

Despite a general consensus that the stress response is adaptive, few empirical studies have addressed the fitness consequences of individual variation in acute stress response (Breuner *et al.* 2008). Variation in corticosterone levels has been linked to survival in marine iguanas (Romero & Wikelski 2001) and storks (Blas *et al.* 2007). However, the relationship between corticosterone levels and fitness may vary with life history stage (Bonier *et al.* 2009). Further clarifying this relationship requires examining individual variation in the stress response, and in particular the dynamics of HPA function (Romero 2004).

One important factor underlying individual variation in stress reactivity is early developmental exposure to stress. Prolonged perinatal stress can result in increased adult glucocorticoid secretion in mammals (Darnaudéry & Maccari 2008). There is now growing evidence in birds for a similar effect: prolonged stress or corticosterone treatment early in life results in a greater increase in corticosterone during acute stress later in adulthood (Pravosudov & Kitaysky 2006; Spencer *et al.* 2009). Thus, early developmental experience may have important epigenetic effects through modifying the adult stress response and thereby fitness.

We explored the relationships between the acute stress response, song complexity and overwinter survivorship in song sparrows (*Melospiza melodia*). In this species, song complexity has been linked to physiological condition (Reid *et al.* 2005; Pfaff *et al.* 2007), and reproductive success (Reid *et al.* 2004). Moreover, nutritional stress during development impairs growth of song-control brain regions (MacDonald *et al.* 2006). We hypothesized that if male sparrows sing less complex songs due to prolonged exposure to stress during development (Nowicki *et al.* 1998), then they should also exhibit altered stress responses in adulthood. To address this, we measured baseline and stress-induced levels of corticosterone in free-living sparrows, and related this to their song complexity and overwinter survival.

## 2. MATERIAL AND METHODS

We mist-netted 24 adult male song sparrows breeding near Newboro, Canada, in May and June 2007. We collected a blood sample (*baseline*) within 3 min of the bird entering the net, then placed the bird in a paper bag. After 30 min we collected a second blood sample (*stressed*; Clinchy *et al.* 2004), and released the bird. Blood was kept cool in the field, centrifuged later that day, and supernatant plasma kept frozen until assay. Plasma was assayed for total corticosterone using a specific and sensitive radioimmunoassay kit (ImmuChem 07-120103, MP Biomedicals, Orangeburg, NY, USA; Washburn *et al.* 2007) previously validated for song sparrows (Newman *et al.* 2008). All samples were measured in a single assay. Sensitivity of the assay was 12.5 ng ml$^{-1}$ and within-assay coefficients of variation were 9.6 and 3.9 per cent for low and high controls.

We recorded complete song repertoires of 18 of these males (as validated by Pfaff *et al.* 2007) using Marantz Professional Solid State PMD 671 recorders and Telinga Twin Science parabolic microphones (Uppsala, Sweden). We generated sound spectrograms in Syrinx 2.6h (J. Burt; www.syrinxpc.com) and counted the total number of song types (song repertoire size) following Pfaff *et al.* (2007) and total number of syllables (syllable repertoire size) following Stewart & MacDougall-Shackleton (2008). These two measures are highly correlated ($r = 0.7$), but nonetheless predict different aspects of male phenotype (MacDougall-Shackleton *et al.* 2009).

We monitored breeding success for the remainder of the 2007 season, and the subsequent year (2008). As an index of territory quality we monitored the number of offspring produced (see electronic supplementary material). Song sparrows are highly philopatric following their first breeding attempt. In our population, males typically nest within 30 m of their nest the previous year. Males

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who do not return from the wintering grounds are presumed to have died overwinter. We thus assessed whether the birds sampled above did, or did not return to breed the following year.

We used SPSS 16 to run multiple regressions to determine whether song and other variables significantly predicted corticosterone levels. To determine whether stress response predicted overwinter survival, we ran binary logistic regressions to identify variables that were significantly related to returning to breed in 2008.

Glucocorticoids act *via* two receptor types, with baseline and stress-induced levels acting through different pathways (Romero 2004). However, because we were primarily interested in individual variation in corticosterone regulation (independent of downstream effects), we assessed which variables were significantly related to the change in corticosterone (stress response). We then separately tested what variables were significantly related to absolute levels of baseline and stress-induced corticosterone.

## 3. RESULTS

The birds exhibited a stress response (increase in corticosterone over 30 min) comparable to that in other studies of breeding song sparrows (see electronic supplementary material, Clinchy *et al.* 2004; Newman *et al.* 2008).

### (a) *Stress response and song*

To test whether the stress response was related to song we ran backward and forward multiple regressions to determine which of the following variables significantly predicted the increase in corticosterone (stressed − baseline levels): song and syllable repertoire size, number of offspring in the first nest, body mass and date. Both forward and backward procedures yielded the same model, in which only syllable repertoire size significantly predicted stress response (overall model: $r^2 = 0.46$, $p = 0.01$). Specifically, birds with smaller syllable repertoires had greater corticosterone increases following restraint than birds with larger syllable repertoires (figure 1; standardized $\beta = -0.68$, $p = 0.01$). Baseline corticosterone was not related to any of these variables; stressed levels of corticosterone were predicted by syllable repertoire size (see electronic supplementary material).

### (b) *Stress response and survival*

All of the 24 birds for which we measured stress response survived through the 2007 breeding season. Eleven of these males returned to breed in 2008, similar to the typical adult return rate of 45–50% in this population (E. A. MacDougall-Shackleton 2009, unpublished data). Stress responses were highly predictive of which birds returned and which did not: birds that returned to breed in 2008 had significantly lower stress responses in 2007 than those that failed to return (figure 2; Wald = 4.96, $n = 24$, $p = 0.026$). Returning to breed in 2008 was also significantly predicted by stress-induced levels of corticosterone (Wald = 4.72, $n = 24$, $p = 0.03$). None of the other variables examined predicted survival in this sample (see electronic supplementary material). Thus, a greater stress response to a standardized stressor appears negatively related to survival in this species.

## 4. DISCUSSION

We found that (i) birds with less complex songs had greater stress responses, and (ii) birds with greater stress responses were less likely to return to breed the following year. Though observational, these findings suggest important links between courtship signals, the stress response and survival. Variation in corticosterone levels has been linked to survival in other taxa (Romero & Wikelski 2001; Blas *et al.* 2007). For example, increased juvenile stress responses are predictive of reduced survival to adulthood in storks (*Ciconia ciconia*; Blas *et al.* 2007). In humans, hyperreactivity to a cold pressor test predicts hypertension 45–57 years later (Wood *et al.* 1984). Our findings provide further evidence that stress responses can be predictive of morbidity and mortality.

Although we observed no direct correlation between song complexity and survival, such a relationship has

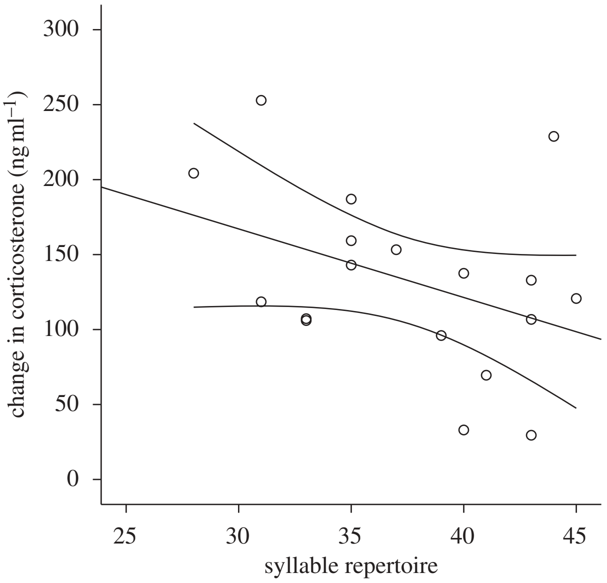

Figure 1. The relationship between song complexity (syllable repertoire size) and stress response (stress-induced baseline corticosterone levels). Lines indicate least-square linear regression (fitted for purposes of illustration) and 95 per cent confidence intervals.

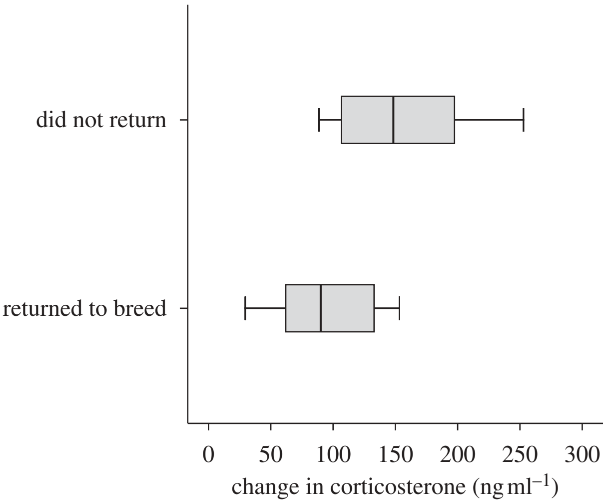

Figure 2. Probability of returning to breed in 2008, as a function of stress response (stress-induced baseline corticosterone levels), the previous year. Box plots indicate 10th, 25th, 50th, 75th and 90th percentiles.

been reported in a western subspecies of song sparrow (Reid *et al.* 2005). Further analyses are required, but our results suggest that the correlation between song and survivorship may result from both being correlated to a third variable. Perhaps, traits affected by stressors early in life, such as song or the stress response itself become correlated through development. Such developmental correlations may make song a predictor of a wide range of traits associated with direct and indirect benefits. Our study provides evidence of links between a sexually selected ornament, the stress response and survival in a free-living population and suggests that song may act as an indicator of developmental conditions and adult male quality.

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