Fluctuating asymmetry in relation to corticosterone levels is sex-dependent in Eurasian treecreeper (Certhia familiaris) nestlings

Samuli Helle\textsuperscript{1,*}, Petri Suorsa\textsuperscript{1}, Esa Huhtá\textsuperscript{2} and Harri Hakkarainen\textsuperscript{1}

\textsuperscript{1}Section of Ecology, Department of Biology, University of Turku, 20014 Turku, Finland
\textsuperscript{2}Kolari Research Unit, Finnish Forest Research Institute, 95900 Kolari, Finland
\*Author for correspondence (sayrhe@utu.fi)

Fluctuating asymmetry (FA) has been widely used as a stress-related phenotypic marker of developmental instability. However, previous studies relating FA to various stressful conditions have produced inconsistent results and we still lack quantitative individual-level evidence that high FA is related to stress in wild vertebrate species. We studied how baseline plasma levels of corticosterone predicted FA of wing and tail feathers in free-living Eurasian treecreeper (Certhia familiaris) nestlings. We found a sex-specific association between corticosterone levels and FA: high corticosterone levels were related to an increased FA in male but not in female nestlings. These results suggest that in treecreepers, FA may correlate with individual stress hormone levels, male developmental trajectory being potentially more sensitive to stress than that of the female.

Keywords: birds; developmental instability; stress

1. INTRODUCTION

Fluctuating asymmetry (FA) has been advocated as a potential estimate of developmental instability, i.e. the inability of individuals to undergo identical development on both sides of a bilaterally symmetrical trait, which can bear fitness costs (Palmer & Strobeck 1986; Møller & Swaddle 1997). It has been proposed that various genetic and environmental factors distort symmetrical morphological development of traits, resulting in higher FA of the phenotype. These factors include variation in genetic variability, in- and out-breeding (Leamy & Klingenberg 2005; Pertoldi et al. 2006), and adverse environmental conditions during development; such as high temperature, food shortage, chemical pollution, parasitism and habitat disturbances (Møller 1999; Hoffman & Wood 2003; Lens & Eggermont 2008).

Despite its promise as a useful phenotypic marker of developmental instability, many aspects of FA research still remain controversial and understudied (Van Dongen 2006). There is currently little direct evidence at the individual level that elevated stress increases FA. The most convincing evidence for such a link comes from poultry, where experimentally elevated corticosterone levels led to higher FA (Eriksen et al. 2003; Satterlee et al. 2008). While, for example, food shortage generally increases the level of baseline glucocorticoids (Wingfield et al. 1998), only one study has reported that birds exposed to experimental food restriction had both increased baseline corticosterone level and higher FA (Pravosudov & Kitaysky 2006). More importantly, we are not aware of any research directly correlating stress hormones to FA in wild vertebrate species.

We investigated whether elevated baseline plasma levels of the primary avian stress hormone, corticosterone, were associated with higher FA in Eurasian treecreeper (Certhia familiaris) nestlings. Our analysis included two traits, wing and tail feathers, and both males and females were assessed. This species is well suited for studying the effects of environment-induced stress on FA, as habitat characteristics and food supply have previously been related to individual corticosterone levels in treecreeper nestlings (Suorsa et al. 2003a, 2004).

2. MATERIAL AND METHODS

(a) Model species and study design

The Eurasian treecreeper is a 9 g arboreal passerine that prefers old forests and specialises in searching for tree-trunk arthropod food (Suorsa et al. 2005). Treecreepers may breed twice during the breeding season but within the same home range. The study area (39°N, 26°E) consists of a mixture of managed forest patches (where nest boxes were situated), agricultural land and other human-created forestless areas.

During the summer of 2000, 9 day old nestlings (n = 165) were blood sampled for 50–80 μl after a puncture of a tarsometatarsal vein (Suorsa et al. 2003a), to measure plasma corticosterone levels using four radioimmunoassay kits (Biotrak rat corticosterone (125I); Amersham, UK). The amount of blood sampled from nestlings did not allow us to determine the inter- and intra-assay coefficient of variation (CV) for the kits used. The inter- and intra-assay CV given by the manufacturer were 5.9 per cent and 5 per cent, respectively. As the kit number was unrelated to individual FA (F(1,155) = 0.44, p = 0.72), and our main result (§3) did not depend on the kit number (interaction between kit number, sex and corticosterone: F(2,149) = 1.06, p = 0.39), we are confident that the corticosterone values used here are valid. The handling of nestlings did not mount an acute elevation of corticosterone levels (Suorsa et al. 2003a). These corticosterone levels (mean ± s.d. = 31.2 ± 12.0 ng ml\textsuperscript{-1}, min = 10.1, max = 81.1) were corrected for autolysis (Suorsa et al. 2003a) by using studentized residuals from the regression of corticosterone on the total handling time of plasma (from bleeding to storing of plasma) as estimates of nestling corticosterone levels. The extracted blood cells of the same samples were also used for nesting sex determination applying DNA molecular methods (Suorsa et al. 2003b).

For FA measurements, we removed the ninth primaries and sixth rectrices from the left and right sides of the wing and tail of the 14 day old nestlings about to fledge. Feathers were photographed using a digital imaging system (CHEMAGRAPH 4400 (Alpha Innotech Corporation, Catalina St San Leandro, CA). The length of the feathers was measured once in a random sequence (left, right or right, left) to the nearest 10 μm using SIGMA DRAW 5.0, after which the measurements were repeated independently for all feathers.

(b) Statistical analyses

We applied linear mixed models with restricted maximum likelihood estimation to distinguishing FA (random differences between the left and right sides) from directional asymmetry (DA, a non-zero mean difference between the left and right sides) and measurement error (ME) and to examine associations between individual FA and corticosterone levels (Van Dongen et al. 1999). A prior screening of feather measurements showed no obvious outliers. We started by assessing the statistical significance of FA and DA and the
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Table 1. Decomposing the variation of wing and tail feather measurements of treecreeper nestlings ($n = 165$) into fluctuating asymmetry (FA), directional asymmetry (DA) and measurement error (ME). ($K$ denotes kurtosis of the distribution of signed FA and $r$ the hypothetical repeatability.)

<table>
<thead>
<tr>
<th>trait</th>
<th>length (mm)</th>
<th>$\sigma^2_F$</th>
<th>$\chi^2$</th>
<th>$p$</th>
<th>d.f.</th>
<th>$F$</th>
<th>$p$</th>
<th>$\sigma^2_M$</th>
</tr>
</thead>
<tbody>
<tr>
<td>wing</td>
<td>30.0</td>
<td>0.182</td>
<td>708.0</td>
<td>&lt;0.0001</td>
<td>1,164</td>
<td>4.34</td>
<td>0.039</td>
<td>between broods: 0.005 3.01 0.636</td>
</tr>
<tr>
<td>tail</td>
<td>24.6</td>
<td>0.192</td>
<td>267.1</td>
<td>&lt;0.0001</td>
<td>1,166</td>
<td>15.8</td>
<td>&lt;0.0001</td>
<td>between broods: 0.026 16.76 0.636</td>
</tr>
</tbody>
</table>

Table 2. Association of corticosterone level, trait, sex and brood number and their selected interactions on the unsigned FA of treecreeper nestlings ($n = 163$). CIs, confidence intervals.

<table>
<thead>
<tr>
<th>$\beta$ (95% CIs)</th>
<th>d.f.</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>cortex. level (C)</td>
<td>-0.021 (-0.086, 0.043)</td>
<td>1,159</td>
<td>0.93</td>
</tr>
<tr>
<td>trait (T)</td>
<td>0.059 (0.007, 0.111)</td>
<td>1,162</td>
<td>4.96</td>
</tr>
<tr>
<td>sex (S)</td>
<td>-0.004 (-0.052, 0.045)</td>
<td>1,158</td>
<td>0.02</td>
</tr>
<tr>
<td>brood number (B)</td>
<td>-0.044 (-0.092, 0.004)</td>
<td>1,172</td>
<td>3.29</td>
</tr>
<tr>
<td>C × T</td>
<td>—</td>
<td>1,160</td>
<td>0.02</td>
</tr>
<tr>
<td>C × S</td>
<td>—</td>
<td>1,157</td>
<td>5.88</td>
</tr>
<tr>
<td>C × B</td>
<td>—</td>
<td>1,168</td>
<td>0.15</td>
</tr>
<tr>
<td>C × T × S</td>
<td>—</td>
<td>1,160</td>
<td>0.05</td>
</tr>
<tr>
<td>C × S × B</td>
<td>—</td>
<td>1,171</td>
<td>0.30</td>
</tr>
<tr>
<td>C × T × B</td>
<td>—</td>
<td>1,180</td>
<td>0.81</td>
</tr>
</tbody>
</table>

3. RESULTS

Both traits showed significant FA, but also DA (table 1). DA should not compromise the FA values used here, if owing to a systematic bias (Van Dongen 2006). Signed FA values also showed high leptokurtosis (table 1). A closer look revealed that leptokurtosis appeared to be due to a single individual in each case showing clearly outlying FA values. We thus removed these individuals from further analysis. This resulted in markedly reduced kurtosis of the signed FA ($-0.49$ and 0.48 in wing and tail feathers, respectively), providing no evidence for antisymmetry (Palmer & Strobeck 2003). High values of hypothetical repeatability, $r$, suggest substantial between-individual variation of developmental instability in the traits studied (table 1).

The analysis of unsigned FA values showed a sex-related association between corticosterone and FA (table 2). In males, a high corticosterone level was associated with high FA ($\beta$ (95% CIs) = -0.046 (0.011, 0.080), $F_{1,62.7} = 6.91, p = 0.012$), whereas in females FA was unrelated to corticosterone level ($\beta$ (95% CIs) = -0.017 (-0.046, 0.013), $F_{1,196.5} = 6.44, p = 0.012$). As males showed a positive association between FA and corticosterone ($\beta$ (95% CIs) = 0.004 (0.001, 0.007), $F_{1,96.7} = 6.91, p = 0.011$), while females showed none ($\beta$ (95% CIs) = -0.002 (-0.004, 0.001), $F_{1,63} = 2.13, p = 0.15$). These results also remained unaltered when the potential size dependency of FA using log transformation was taken into account (Palmer & Strobeck 2003), or when the two individuals with outlying FA values were included in the analysis (results not shown).
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4. DISCUSSION

Our results provide, to our knowledge, the first reported quantitative association between stress hormone levels and FA in a free-living vertebrate species. In treecreepers, high baseline corticosterone levels were related to an increased FA of both wing and tail feathers in male but not in female nestlings. Sexual size dimorphism of treecreeper nestlings (Suorsa et al. 2003b) may provide an answer to why only larger and heavier males showed a corticosterone-related increase in FA. Male development owing to higher energetic demand (e.g. parental provisioning rate is higher in male-biased broods, Suorsa et al. 2003b) is probably more sensitive to environmental stressors like food restriction, resulting in a stronger association (or faster response to) between corticosterone levels and FA. Such sex-related associations are probably more sensitive to environmental stressors like food restriction, resulting in a stronger association (or faster response to) between corticosterone levels and FA. Such sex-related associations

Figure 1. Association between autolysis-corrected corticosterone level and average individual FA in male (filled circles and solid line) and female (open circles) treecreeper nestlings.

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