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

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**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 & Eggermonth 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 specializes 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 covers 1150 km$^2$ in central Finland (62°37′ N, 26°20′ E) and 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 ($^{125}$I); 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 ($F_{3,155} = 0.44$, $p = 0.72$), and our main result (§3) did not depend on the kit number (interaction between kit number, sex and corticosterone: $F_{6,149} = 1.06$, $p = 0.39$), 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$^{-1}$, 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 nestling 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 CHEMIIMAGE 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$^{-11}$ mm 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

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.)

| trait | length (mm) | $\sigma^2_{FA}$ est. | $\chi^2_1$ | $p$ | DA d.f. | $F$ | $p$ | $\sigma^2_{ME}$ heterogeneity | est. | $K$ | $r$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| wing | 30.0 | 0.182 | 708.0 | <0.0001 | 1,164 | 4.34 | 0.039 | between broods | 0.005 | 3.01 | 0.636 |
| tail | 24.6 | 0.192 | 267.1 | <0.0001 | 1,166 | 15.8 | <0.0001 | between broods | 0.026 | 16.76 | 0.636 |

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.

| | $\beta$ (95% CIs) | d.f.$_{num, den}$ | $F$ | $p$ |
|---|---|---|---|---|
| corticosterone level (C) | −0.021 (−0.086, 0.043) | 1,159 | 0.93 | 0.34 |
| trait (T) | 0.059 (0.007, 0.111) | 1,162 | 4.96 | 0.027 |
| sex (S) | −0.004 (−0.052, 0.045) | 1,158 | 0.02 | 0.88 |
| brood number (B) | −0.044 (−0.092, 0.004) | 1,172 | 3.29 | 0.072 |
| C × T | — | 1,160 | 0.02 | 0.89 |
| C × S | — | 1,157 | 5.88 | 0.017 |
| C × B | — | 1,168 | 0.15 | 0.70 |
| C × T × S | — | 1,160 | 0.05 | 0.82 |
| C × S × B | — | 1,171 | 0.30 | 0.59 |
| C × T × B | — | 1,180 | 0.81 | 0.37 |

heterogeneity pattern of ME for both traits separately. In these models, side was included both as a fixed (estimating DA) and as a random factor (estimating FA) (Van Dongen *et al.* 1999). ME was incorporated into the models by including the two measurements as a within-individual repeated measures and the heterogeneity pattern of this $r$-matrix among sexes and brood number having the smallest Akaike's information criteria was chosen (Littell *et al.* 2006). The significance of FA was obtained using a likelihood ratio test and of DA using $F$-test, adjusting the denominator degrees of freedom by the Kenward–Roger method (Littell *et al.* 2006). Because we included nestlings from the same broods, brood identity was also included as a random factor in these models.

From these models, we obtained unbiased-individual FA values using empirical Bayes estimates of the random slope of the side (Van Dongen 2000). The presence of antisymmetry (distributional bimodality or platykurtosis of differences between the left and right sides) was evaluated by examining the kurtosis of the distribution of the signed FA values (Palmer & Strobeck 2003). We also calculated the hypothetical repeatability ($r$), which indicates how well unsigned FA values estimate the expected underlying amount of between-individual variation in developmental instability (Whitlock 1998). Finally, associations between corticosterone level and individual unsigned FA values of both traits were analysed in a single model where within-individual trait-specific FA values were treated as repeated measures (Van Dongen *et al.* 1999). As we had two traits, a trait was treated as a fixed not a random factor (Van Dongen 2006). Nestling sex and brood number (first or second) were included as fixed factors, as well as their two- and three-way interactions with corticosterone level. No stepwise model reduction was applied, because such methods dramatically increase the type I error rate (Mundry & Nunn 2009). Analyses were conducted with SAS statistical software v. 9.2 (SAS Institute Inc, Cary, NC, USA).

## 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), $F_{1,96.5} = 6.95$, $p = 0.01$), whereas in females FA was unrelated to corticosterone level ($\beta$ (95% CIs) = −0.017 (−0.046, 0.013), $F_{1,62.7} = 1.32$, $p = 0.26$; figure 1). FA also differed between traits, FA of wing feathers being 0.06 units higher than that of tail feathers (table 2). Using corticosterone values not corrected for autolysis did not change these results. There was still a significant interaction between corticosterone level and sex ($F_{1,159} = 6.44$, $p = 0.012$), as males showed a positive association between FA and corticosterone ($\beta$ (95% CIs) = 0.004 (0.001, 0.007), $F_{1,96.7} = 6.91$, $p = 0.01$), 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).
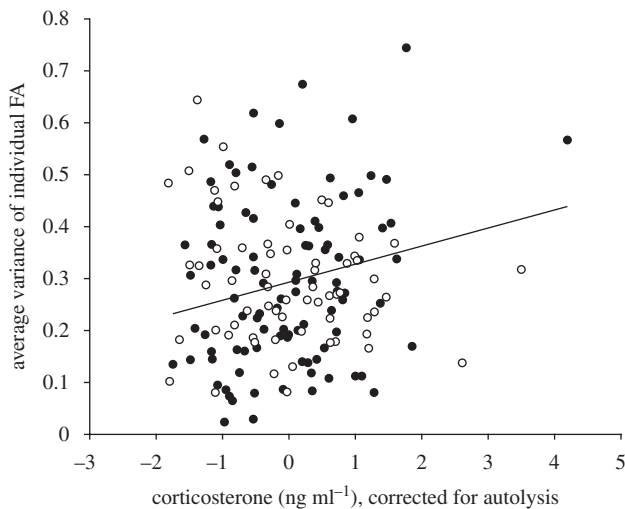
Figure 1. Association between autolysis-corrected corticosterone level and average individual FA in male (filled circles and solid line) and female (open circles) treecreeper nestlings.

## 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.* 2003*b*) 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.* 2003*b*) 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 between stress and FA have rarely been studied and documented. The examples include a fallow deer (Serrano *et al.* 2008) and a fly (Bonduriansky 2009). We cannot nevertheless infer causality or overrule the possibility that corticosterone levels merely better reflect stress in males than in females.

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