Original article

Fluctuating asymmetry as a biomarker of habitat fragmentation in an area-sensitive passerine, the Eurasian treecreeper (*Certhia familiaris*)

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A B S T R A C T

The research investigating fluctuating asymmetry (FA), a phenotypic proxy of developmental instability, as a potential early-warning biomarker of anthropogenic stress like habitat fragmentation has produced controversial results. We examined the influence of habitat fragmentation at the landscape-scale, divided into habitat amount and configuration, on feather length FA in the Eurasian treecreeper (*Certhia familiaris*), an area-sensitive old-growth forest passerine breeding in boreal forests that are currently under intense management. Our study included one tail and wing feather, measured in both sexes of the 14-day-old nestlings and adults. Habitat amount was measured as the proportions of suitable forest habitats and open unsuitable areas, mean patch isolation and nesting patch size whereas habitat configuration was measured as mean patch density, size and shape and edge density. We found only weak sex- and age-related associations between feather length FA and habitat fragmentation that explained just 4.9% of variance in FA. Habitat loss was associated with higher FA in males only while the habitats with low degree of configuration tended to be related to lower FA in adults only. From the biomarker perspective, FA may not thus hold a great promise as a strong and general indicator of habitat fragmentation.

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1. Introduction

Landscape modification and habitat fragmentation are of special concern to conservation biologists (Haila, 2002; Fazey et al., 2005), as these processes pose the two most important threats to global biodiversity among almost all the taxonomic groups studied (Sala et al., 2000; Foley et al., 2005). A major challenge is to identify vulnerable populations before irreversible demographic and genetic harm take place. This task is however commonly daunting, because traditional biomarkers (e.g. heat shock proteins and haemoglobin adducts) reliably reflecting the effect of such environmental factors are hard, expensive and time-consuming to measure in most field conditions and they may not be applicable across species (Lens and Eggermonth, 2008). There is thus an urgent need for a reliable, general and easy-to-use biomarker of health and well-being of individuals that can be applied to population-level in the field (Leung et al., 2003).

One of such biomarker of environmental stress suggested is fluctuating asymmetry (FA), i.e. random deviations from perfect bilateral symmetry, in different morphological and sexual traits (Leary and Allendorf, 1989; Clarke, 1995; Leung et al., 2003; Lens and Eggermonth, 2008). FA has widely been used as a pheno-

typic marker of developmental stability, an ability of individuals to undergo identical development on both sides of a bilaterally symmetrical trait(s) (Palmer and Strobeck, 1986; Møller and Swaddle, 1997). FA has been suggested to respond to various environmental and genetic stressors (Møller, 1999; Hoffman and Woods, 2003; Leamy and Klingenberg, 2005; Pertoldi et al., 2006) and to correlate with several life-history traits (Møller and Swaddle, 1997) and animal health (Knierim et al., 2007).

Studies associating habitat fragmentation with FA conducted on several animal species both at the individual- and population-level have produced inconsistent results. A recent review in birds found mixed results among studies reporting an association between FA and habitat fragmentation (Lens and Eggermonth, 2008). In amphibians, habitat loss has been found to increase FA in most studies (Sarre, 1996; Gallant and Teather, 2001; Wright and Zamudio, 2002), but not in all (Delgado-Acevedo and Restrepo, 2008). One study even found decreased FA in disturbed (logged) habitats in amphibians (Lauck, 2006). In insects, habitat fragmentation has been found to be unrelated to FA (Berggren, 2005; Henríquez et al., 2009) whereas in mammals, habitat fragmentation has been shown to increase FA (Wauters et al., 1996; Marchand et al., 2003).

Some of these inconsistencies may have arisen because few studies aimed to separate fragmentation-related effects on FA between sexes. This is potentially an important shortcoming when considering sexually dimorphic species, since the larger sex is generally expected to show higher environmental sensitiv-

ity (Clutton-Brock et al., 1985). Accordingly, recent studies have indicated that stress can inflict sex-dependent associations on FA (Badyaev et al., 2000; Serrano et al., 2008; Bonduriansky, 2009; Helle et al., 2010). Another factor producing variability into the current findings may be age or the life-history stage of the individuals studied, since both adults and juveniles have many times been examined simultaneously. For example, it may be much harder to identify FA to a specific environmental stressor in parents compared to nestlings when the developmental environment of parents is unknown. If FA turns out to be an age- or sex-specific measure of environmental stress, its utility as a universal biomarker would be reduced. Thus, more studies taking individual sex and age into account are clearly needed to verify the utility of FA as a phenotypic biomarker of habitat-related stress for conservation purposes.

Another potential reason for the heterogeneous associations reported between habitat fragmentation and FA may be due to the variety of definitions and measurements of habitat fragmentation used in the literature (Fahrig, 2003). In general, perhaps the most important shortcoming of the fragmentation literature has been the inability to separate, both at the conceptual and methodological level, the effects of habitat amount (removal of habitat) from habitat configuration (breaking apart of habitat) (Schmiegelow and Mönkkönen, 2002; Fahrig, 2003). Despite of being two fundamentally different but simultaneous processes, they are highly correlated in nature (Fahrig, 2003). Fahrig (2003) proposed that the number and size of habitat patches should represent the general effect of spatial habitat configuration, whereas patch isolation and the total amount of habitat the general effect of habitat amount. All these variables should be measured at the landscape not the patch level, because habitat fragmentation is a landscape-level process (McGarigal and Cushman, 2002) and included simultaneously in the analysis (Fahrig, 2003). In previous studies relating habitat fragmentation to FA, many different variables representing fragmentation have been used, but their association on FA have not been considered together. Because the effect of habitat amount and configuration on stress, and thus presumably on FA, may well be species-specific, it is currently hard to determine the effect of fragmentation on FA.

Our aim was to investigate how habitat fragmentation related to feather length FA in the Eurasian treecreepers (*Certhia familiaris*). We recorded the length of one tail and wing feather in sexed 14-day-old nestlings and adults aged over two growing seasons. According to Fahrig (2003), we divided habitat fragmentation into two components within a radius of 500 m, considered as landscape-level in this species. Habitat amount was measured as the proportions of forest habitats suitable for this species and open unsuitable areas, mean patch isolation and nesting forest patch size, whereas habitat configuration was measured as mean patch density, size and shape and edge density. The Eurasian treecreepers prefer old forests as breeding sites and are specialised to forage on tree-trunk arthropods (Suhonen and Kuitunen, 1991). It is an area-sensitive and obligatory woodland bird with a large breeding territory (Suorsa et al., 2005). This species is well-suited for our purposes, since habitat fragmentation has previously been related to the increased stress (i.e. corticosterone levels) and reduced body size and condition of nestlings (Suorsa et al., 2003a, 2004) and, for example, stress hormone levels have been related to nestling FA (Helle et al., 2010).

2. Materials and methods

2.1. Study area and design

We collected the data from a study area covering 1150 km^2 in central Finland (62° 37′N, 26° 20′E) during the summer of 2000. The
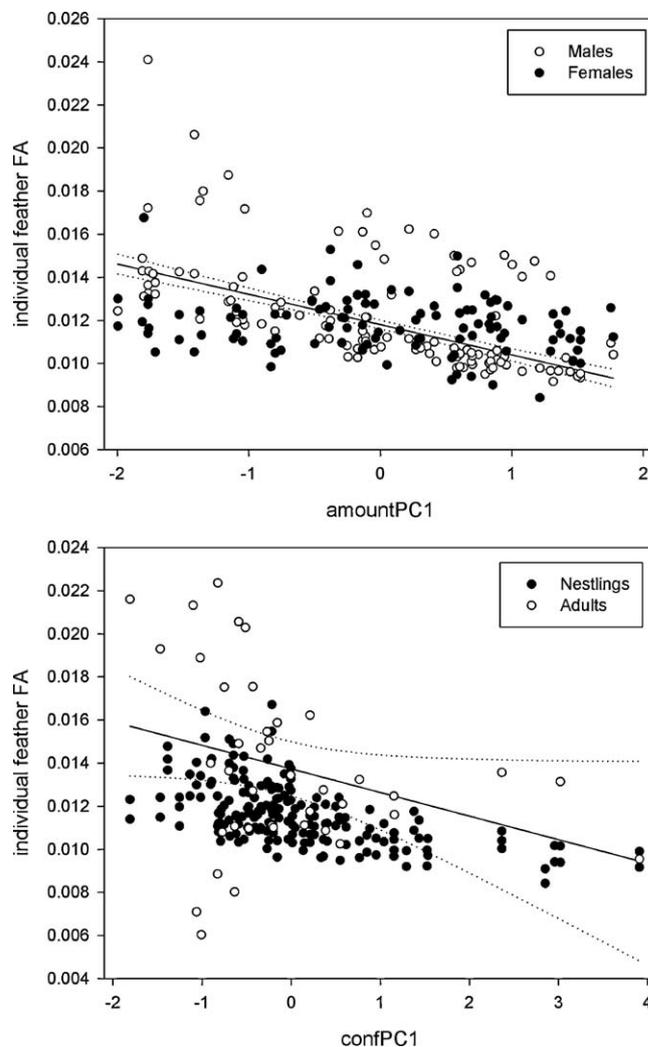
Fig. 1. Predicted values of individual FA in relation to (top panel) the first PC of habitat amount (amountPC1) and (bottom panel) of habitat configuration (confPC1). The regression lines (straight lines) and their 95% confidence intervals (dotted lines) are given for statistically significant associations only.

study area consists of a mixture of managed forest patches (71%), agricultural land (16%) and human-created forestless areas, such as clear-cuts (3%), roads etc. (5%) and other open habitats (5%). The study area is dominated by conifer forest of Norway spruce (*Picea abies*), with patches of mixed forest of Norway spruce, Scots pine (*Pinus sylvestris*) and deciduous trees (mainly birches, *Betula* spp.).

Because the treecreeper breeds in purpose-designed nest-boxes (Kuitunen, 1985), it is easy to provide standardized nest sites in differing landscapes and follow the progress of breeding in relation to habitat fragmentation. We mounted two identical wooden nest-boxes with narrow (2 cm width) entrances on tree trunks 1.5 m above the ground in each forest patch. We used two nest-boxes in a study patch (n = 229 patches), because treecreepers may breed twice during the breeding season and they prefer to change their nest location within a territory from the first to the second breeding. Nest-boxes were situated 30–50 m apart in the middle of forest patches because this species avoids nest sites close to edges (Kuitunen and Mäkinen, 1993). The study was designed so that only one treecreeper pair bred in each forest patch. 160 nest-boxes were occupied in the year 2000 and were considered territories. The forest patch areas occupied (ha) ranged from 0.1 to 71.1, with a mean (±s.d.) of 37.5 (±17.9). The first breeding often begins in the middle of April when the ground is still covered by snow. Offspring of the

first clutch leave the nest by the end of May. The potential second clutches are produced in the beginning of June and offspring leave the nest by the middle of July. We followed the course of breeding accurately from the onset of nest building to fledging by checking nest-boxes regularly.

Adult breeding birds were captured at the nests by mist netting and determined for sex and age. Only parents aged over two growing seasons were considered here ($n = 40$), as these local individuals had grown their feathers in the habitat studied (moulting takes place post-breeding from mid-June to early September and both tail feathers and primaries are moulted at the same time). The 9-day-old nestlings ($n = 432$) were blood sampled for 50–80 μl after a puncture of a tarsometatarsal vein to sex determination applying DNA molecular methods (Suorsa et al., 2003b). In order to measure FA, we removed the ninth primary wing feathers (second outermost) and the sixth rectrices (outermost) from the left and the right sides of the wing and the tail from both adults (wing mean ± s.e. mm = 44.1 ± 0.26, tail = 52.5 ± 0.29) and the 14-day-old nestlings (wing = 29.3 ± 0.11, tail = 23.0 ± 0.20) about to fledge.

2.2. Landscape data

We imported the land-use and forest data of each treecreeper territory into a Geographical Information System (GIS) and measured the landscape structure within a radii of 500 m (78.5 ha) around the midpoint of a nest-box pair, which was defined as a territory centre. This spatial scale includes the entire treecreeper territory (approx. 10 ha that corresponds roughly to a radii of 200 m) and its surrounding landscape. Our rationale for using the radius of 500 m was that, first, landscape-level inference should be based on scale(s) that enable the progressive division of habitats in terms of their area and arrangement on the landscape (McGarigal and Cushman, 2002) and, second, the foraging trips and home range of adults may exceed the size of their territory (H. Hakkarainen, unpublished). We used classified Landsat TM 5 satellite images (from 1995 to 1997) produced by the National Land Survey of Finland (NLS) as the land-use and forest-resource data (Vuorela, 1997). Pixels originally measuring 30 m × 30 m were resampled to measure 25 m × 25 m by NLS. Digital maps of roads and agricultural land, etc. were used to separate forested and non-forested land. Each pixel in the NLS classification can initially belong to one of 50 land-use and forest classes. In the case of forested areas, each pixel was classified according to its total timber volume (mainly applying 50 m³/ha intervals) and further according to the main tree species or mixed species composition. Clear-cuts were separated as distinct classes by allowing a maximum of 4 m³/ha for clear-cuts and 12 m³/ha for sapling stands (Vuorela, 1997). A forest patch was defined in hectares as a separate forested area containing more than 50 m³/ha. By using this criterion, a forest patch was clearly separable from the neighbouring open or less forested habitats in the field. We used spatial analysis program FRAGSTATS (McGarigal and Marks, 1995) to measure percentages of forest habitats (>50 m³/ha) and unforested habitat (etc. clear-cuts, fields, open mires), forest patch density (number per 100 ha), mean nearest-neighbour distance of patches (m), forest-open land edge density (m/ha), mean patch size (ha), mean shape index and the size of a nesting forest patch (ha). Descriptive statistics for the landscape variables is given in Table 1.

2.3. Measurements of FA

We photographed the feathers using a digital imaging system ChemiImage™ 4400 (Alpha Innotech Corporation, Catalina St. San Leandro, California). Images were captured in a MultiImage™ light cabinet (DE-400) using a zoom lens (F 1.2, 12 mm × 75 mm) and a white light tube as a light source. The instrument was controlled by

Table 1
Descriptive statistics for the landscape variables used in the analysis. All variables are measured within a radius of 500 m.

Landscape variable	Mean	Std	Min	Max
Proportion of unforested areas	28.53	13.33	7.30	60.40
Proportion of forest habitat	58.47	14.37	25.71	82.99
Mean nearest-neighbour distance of patches	34.94	13.69	0.00	75.00
Nesting forest patch size	38.17	17.21	0.13	65.06
Forest patch density	8.70	5.14	1.27	27.85
Edge density	107.48	18.85	63.61	151.90
Mean patch size	12.59	14.11	1.23	65.06
Mean shape index	1.77	0.53	1.24	4.72

an Alpha Erase™ software (version 4.0, 1998), which allows image processing and saving the image as an original file. The few feathers with visual cues about wear or damage that could have affected feather length were not measured. We took two independent measurements of feather lengths using Sigma Draw 5.0 image software. The length of the feathers was measured once in a random sequence (left, right or right, left) to 10^{-11} mm, after which the measurements were repeated to all feathers. The raw feather measurements were log transformed in order to remove any size-dependency of FA and making the detection of underlying developmental instability more powerful (Palmer and Strobeck, 2003). This transformation yields dimensionless FA values, as it describes FA as a proportion of the trait mean.

2.4. Statistical analyses

We applied linear mixed modeling approach using restricted maximum likelihood (REML) estimation to distinguishing individual FA from directional asymmetry (DA) and measurement error (ME) (Van Dongen et al., 1999). DA should not compromise the FA values, if due to a systematic bias (Van Dongen, 2006). Otherwise, the results based on traits showing DA should be interpret with care (Van Dongen, 2006). A prior screening of feather measurements showed no outliers. We started by examining the statistical significance of FA, the presence of DA and heterogeneity of ME. Separate models were fit for nestlings and parents and for both traits. In these models, side was included both as a fixed (estimating DA) and as a random factor (estimating individual FA) (Van Dongen et al., 1999). ME was incorporated by including the two measurements as a within-individual repeated-measures (Van Dongen et al., 1999). The potential heterogeneity pattern of this R matrix among sexes in both nestlings and parents and among brood number (1st or 2nd) in nestlings was examined and the R matrix having the smallest Akaike's information criteria (AIC) was selected (Littell et al., 2006). The significance of FA was obtained using a likelihood ratio test and DA using F tests, adjusting the denominator degrees of freedom by Kenward–Roger method (Littell et al., 2006). In both nestlings and adults and the traits studied, we found significant FA that was higher in adults (Table 2). In nestlings, we also found significant DA (Table 2). 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 was evaluated by examining the kurtosis of the distribution of the signed FA values (Palmer and Strobeck, 2003), but no strong indication for this was found (Table 2).

Association between habitat amount and configuration and individual unsigned FA values of both traits were analysed in a single model where trait-specific FA values were treated as repeated measures assuming a compound symmetry variance–covariance matrix (Van Dongen et al., 1999). Since we had measured just two traits, trait was treated as a fixed not a random factor (Van Dongen, 2006). Our model also included the age (nestling or adult) and

Table 2
Variance components of wing and tail feathers measurements of treecreeper adults ($n = 40$) and nestlings ($n = 432$). K denotes to kurtosis of the distribution of signed FA. The estimates of FA, DA and ME have been multiplied by 1000.

Trait	σ^2_{FA}			DA				σ^2_{ME}		
	Est.	χ^2_1	p	Est.	df	F	p	Heterogeneity	Est.	K
Adults										
Wing	0.36	173.9	<0.0001	−0.97	1,39.5	0.10	0.75	Males	0.0059	6.5
								Females	0.0170	
Tail	0.50	237.1	<0.0001	3.37	1,39	0.90	0.35	None	0.0051	4
Nestlings										
Wing	0.24	997.8	<0.0001	−2.80	1,431	13.6	0.0003	Males	0.0043	13
								Females	0.0059	
Tail	0.31	455	<0.0001	−7.40	1,432	69.2	<0.0001	1st brood	0.0250	5.4
								2nd brood	0.0360	

sex of an individual and their two-way interactions with habitat amount and configuration. Although biologically potentially interesting, three-way interactions could not be reliably incorporated into the analysis due to low number of adult males ($n = 22$) and females ($n = 18$). Brood number nested within age (because this association was estimated for nestlings only) was also included into the model, because there is preliminary information for its potential influence on nestling FA (Helle et al., 2010). Because we included nestlings from the same broods and some adults were their parents, territory identity was included as a random factor in the model and the denominator degrees of freedom of fixed terms were adjusted by Kenward–Roger method (Littell et al., 2006).

However, high multicollinearity among fragmentation variables, measured as variance inflation factors (VIFs) that ranged from 1.7 to 14.4, distorts the standard errors of regression coefficients and thus increases type II error rate and inflates the test statistics based on these coefficients (Graham, 2003). Therefore, we used principal component analysis (PCA) using correlation (ranging from |0.16| to |0.91|) matrix to reduce the number of predictors by constructing linear orthogonal principal components (Khattree and Naik, 2000). Using territory-wise ($n = 100$) records for habitat amount and configuration in two separate PCAs performed on standardized habitat variables (mean = 0, std = 1), we retained the first two principal components (PCs) that cumulatively explained 93.2% and 90.2% of the total variance in variables describing habitat amount and configuration, respectively. In terms of habitat amount, PC1 had an eigenvalue of 3.0 and it explained 74.0% of the total variance, whereas PC2 had an eigenvalue of 0.8 and it explained 19.2% of the total variance. In terms of habitat configuration, PC1 and PC2 had eigenvalues of 2.5 and 1.1 and they explained 63% and 27% of the total variance, respectively. We used orthogonal varimax rotation to make the component loadings interpretable as correlations between PCs and measured fragmentation variables (Table 3). Component loadings higher than |0.5| were considered as interpretable given the sample size (Budaev, 2010), meaning that these variables

Table 3
The first two principal components (PCs) extracted from the two separate principal component analyses of landscape variables measuring habitat amount and configuration. Interpretable component loadings (>|0.5|) are given in bold.

Landscape variable	PC1	PC2
PCA on habitat amount		
Proportion of unforested areas	**−0.933**	0.152
Proportion of old forest habitat	**0.943**	−0.271
Mean nearest-neighbour distance of patches	−0.209	**0.978**
Nesting forest patch size	**0.914**	−0.191
PCA on habitat configuration		
Forest patch density	**−0.851**	0.213
Edge density	−0.040	**0.990**
Mean patch size	**0.949**	−0.103
Mean shape index	**0.941**	0.242

have a meaningful correlation and thus interpretation with the PCs retained. That is, low proportion of unforested areas and high proportion of forest habitats as well as large nesting forest patch size loaded heavily on the PC1 of habitat amount, whereas large mean nearest-neighbour distance of forest patches showed excessive correlation with the PC2 of habitat amount (Table 3). High mean patch size and shape index but low forest patch density defines the PC1 of habitat configuration, whereas the PC2 of habitat configuration is defined by high edge density (Table 3).

Prior to the main analyses, we checked for the potential non-linearity (without interactions) of associations between habitat amount and configuration and FA. Second-order polynomials were considered only, because we would expect to see thresholds, i.e., a levelling-off or an increase of FA beyond a certain point of habitat amount and/or configuration. However, we found no evidence for such non-linearity ($F_{2,69.4-74.9} < 1.97$, $P > 0.15$). Likewise, no evidence was found for interaction between habitat amount and configuration ($F_{4,73.2} = 1.93$, $P = 0.11$).

Statistical inference was based on a full maximal model in order to obtain the most accurate point estimates and to control for proper type I error rate (Harrell, 2001). Initial inspection of the model residuals showed several large residuals, a rather common phenomenon in FA studies (Knierim et al., 2007), indicating a non-normal distribution with a long tail to the right. We thus used Gamma distribution and log link function to model residuals (Zuur et al., 2010) and model parameters were estimated using residual pseudo-likelihood. Moran's I was used to assess the extent of spatial autocorrelation of model residuals, averaged for individuals and territories. No evidence for spatial autocorrelation was found under the assumption of randomization and binary weights ($z = -0.95$, $P = 0.34$). R^2 statistics reported here are based on maximum likelihood ratios of the models compared (Magee, 1990). All analyses were conducted with SAS statistical software version 9.2.2 (SAS Institute Inc, Cary, North Carolina, USA).

3. Results

For PCs summarizing variables of habitat amount, we found that association between PC2 (AmountPC2) and FA differed between adults and nestlings, but not between traits or sexes (Table 4). That is, the PC2 of habitat amount showed a non-significant positive trend with FA in nestlings (β (95% CIs) = 0.057 (−0.020, 0.135), $e^\beta = 1.06$, $F_{1,77.7} = 2.16$, $P = 0.15$) while a non-significant negative trend was found in adults (β (95% CIs) = −0.343 (−0.789, 0.104), $e^\beta = 0.71$, $F_{1,34} = 2.43$, $P = 0.13$). The association between the PC1 of habitat amount (AmountPC1) and FA tended to differ between sexes, but not between traits or ages (Table 4). The PC1 of habitat amount was negatively associated with FA in males (β (95% CIs) = −0.135 (−0.267, −0.004), $e^\beta = 0.87$, $F_{1,23.0} = 4.53$, $P = 0.044$, Fig. 1), but unrelated to FA in females (β (95% CIs) = 0.001 (−0.132,

Table 4
The association of age (nestling or adult), trait (primary or rectrice), sex, brood number and the first two principal components (PCs) describing habitat amount (amountPC1, amountPC2) and configuration (confPC1, confPC2) and selected two-way interactions on the FA of individuals. Variance of FA was related to territory identity, fitted as a random factor ($\chi^2 = 2.76$, df = 1, $P = 0.048$). Likelihood ratio test $R^2 = 4.9\%$, including the fixed terms shown in the table.

	$df_{num, den}$	F	P
Age	1, 455.8	0.15	0.70
Trait	1, 467.1	5.05	0.025
Sex	1, 452.8	0.37	0.54
Brood number (age)	1, 200.9	0.19	0.66
AmountPC1	1, 272.2	0.27	0.60
AmountPC2	1, 417.1	2.44	0.12
AmountPC1 × age	1, 455.6	2.67	0.10
AmountPC2 × age	1, 451.5	4.85	0.028
AmountPC1 × sex	1, 451.4	2.80	0.095
AmountPC2 × sex	1, 441.7	0.03	0.87
AmountPC1 × trait	1, 467	0.35	0.55
AmountPC2 × trait	1, 467	1.29	0.26
ConfPC1	1, 284.3	3.84	0.051
ConfPC2	1, 300.6	0.17	0.68
ConfPC1 × age	1, 446.8	4.62	0.032
ConfPC2 × age	1, 453.3	0.44	0.51
ConfPC1 × sex	1, 448	0.92	0.34
ConfPC2 × sex	1, 447.1	1.27	0.26
ConfPC1 × trait	1, 467.1	0.00	0.96
ConfPC2 × trait	1, 467.1	0.02	0.88

0.134), $e^\beta = 1.00$, $F_{1,96.5} = 0.00$, $P = 0.99$, Fig. 1). This means that in males low amount of habitat was significantly associated with higher FA, although it explained only 1.3% of variation in FA.

The PC1 summarizing the variables of habitat configuration (ConfPC1) had no independent association with FA, but its association varied between adults and nestlings but not between traits or sexes (Table 4). In adults, the PC1 of habitat configuration tended to be negatively associated with FA (β (95% CIs) = −0.326 (−0.709, 0.057), $e^\beta = 0.72$, $F_{1,34} = 3.00$, $P = 0.09$, Fig. 1), whereas in nestlings there was a weak positive and clearly non-significant association between the two (β (95% CIs) = 0.014 (−0.091, 0.119), $e^\beta = 1.01$, $F_{1,76.2} = 0.07$, $P = 0.79$, Fig. 1). That is, habitats with low degree of configuration tended to be related to low FA in adults. The PC2 summarizing the variables of habitat configuration (ConfPC2) was not associated with FA, irrespective of age, trait or sex (Table 4). Territory identity also explained variation in FA, suggesting that unmeasured territory properties, differences in individual quality and/or heritability of FA may also be responsible of variation in FA (Table 4).

4. Discussion

Our results suggest rather weak associations between habitat fragmentation and feather length FA in the Eurasian treecreepers studied. These associations seemed to further be sex- and age-specific. In males only, low amount of habitat was associated with higher FA, whereas in adults only high mean patch size and low patch density indicating low degree of habitat configuration tended to be associated with lower FA.

Despite the statistically significant associations between FA and habitat fragmentation reported here and elsewhere, there are several points that might downplay the potential importance of FA as valuable biomarker of habitat fragmentation. First, FA may not be sensitive enough biomarker (Leung et al., 2003). A recent review on the utility of FA as a biomarker of anthropogenic stress in birds including habitat fragmentation concluded that, on average, just 9% of the variation of FA was explained by environmental stress (Lens and Eggermonth, 2008). For other taxa, this information seems not to be regularly reported. Only exception is the study by Berggren (2005) who reported that as much as 23% of the variance of FA was

accounted by habitat isolation (a measure of habitat amount). In the current study, variation in habitat amount explained merely 1.4% of variance in males' feather length FA while the full model of habitat fragmentation allowing for varying slopes between the traits, sexes and ages explained a total of 4.9% of variance in FA. These values correspond rather well to those commonly found in ecological and evolutionary studies, including FA (Møller and Jennions, 2002). Therefore, at least in birds, the predictive power of FA to reliably signal the environmental change of current interest may be rather limited, as several other, often unmeasured biotic and abiotic factors are likely to produce the most variation of FA.

Second, FA may not be a general biomarker, but rather to be specific to the sex and/or the life-history stage of an individual (Lens and Eggermonth, 2008). This conclusion is supported by the current data, because low amount of habitat was associated with higher FA in males only. This finding is in line with the general notion of the higher sensitivity of males on adverse environmental conditions in bird species where the males are the larger sex (Råberg et al., 2005) like in the treecreepers (Suorsa et al., 2003b). This effect is commonly attributed to the greater nutritional demands of larger males (Clutton-Brock et al., 1985) and it fits to our previous finding of a stress hormone-related increase of FA that was documented for male nestlings only in this population (Helle et al., 2010). In addition, our results suggest that adults and nestlings may have responded differently to habitat fragmentation, as low degree of habitat configuration tended to be related to lower FA in adults only. However, the reason for this difference remains unclear. For example, we might have expected stronger associations among nestlings, since the habitat measured here was the only environment yet experienced by the developing young. On the other hand, it is unclear to what degree the observed directional asymmetry (DA) in the feathers of nestlings (Table 1) and the fact that these feathers were still growing (see Section 2) impacted these results. Instead in adults, some previous studies have suggested that FA may be fixed already early in life (Møller, 1996; Stige et al., 2005). On the other hand, side-specific compensatory growth for instance may level-off past developmental shocks, making FA a valid indicator of very recent or present stress events (Kellner and Alford, 2003; Stige et al., 2006). Because we have no information on the longitudinal variation of individual feather length FA in treecreepers, this issue remains speculative at best in this species. The developmental origins of FA in the species studied thus needs to be understood before FA can be taken as a useful biomarker of environmental stress.

Third, another potential shortcoming of FA as a biomarker is that its association with individual fitness is currently unclear (Møller, 1997; Lens et al., 2002; Clarke, 2003). This means that although FA would reliably reflect environmental stress, its usefulness as an "early warning system" would be diminished if this does not translate into between-individual differences in survival and reproductive success in a predictable manner (Leung et al., 2003). In treecreepers, we do not currently know whether FA is related to survival and reproductive success in this population. We might not, however, expect very high covariation between the FA of feathers and fitness, because important fitness-related traits like feathers in birds due to flying ability may be more strongly buffered against environmental perturbations than traits not directly related to fitness (Clarke, 2003).

There are some limitations in our study that may have reduced our ability to detect associations between habitat fragmentation and FA. The aim of this study was to investigate these questions at the landscape-level, because habitat fragmentation is a landscape-level process. Although the radius of 500 m used here likely represents a 'landscape' from the perspective of the treecreepers, we may have missed some aspects of the ecologically important scale. In other words, the landscape-level scale approach used here

may not have fully captured the process of habitat degradation (i.e., quality) relevant for this species (Mortelliti et al., 2010). For example, smaller scales have been found to be more important for forest patch occupancy in this species (Suorsa et al., 2005).

In conclusion, more field research is needed to determine whether FA can be considered as a biologically meaningful and sensitive enough biomarker of habitat fragmentation. At least in area-sensitive bird species like the Eurasian treecreeper, habitat fragmentation does not seem to inflict consistent or strong associations to FA it to be considered as reliable and general biomarker of fragmentation-related stress. Unless the sex in dimorphic species and/or the age of an individual are easily available during sampling in the field and the developmental origins of FA of the study species well-known, FA as a sole biomarker should be used with a great caution.

Acknowledgements

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