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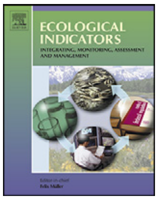

Feather growth bars as a biomarker of habitat fragmentation in the Eurasian treecreeper

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ABSTRACT

Habitat fragmentation poses a major threat to animal species worldwide. However, a lack of accurate and easy-to-collect biomarkers can hamper the identification of populations vulnerable to fragmentation. Ptilochronology, the measurement of growth bar length from feathers, has been shown to respond to individual nutritional status and may thus serve as a useful biomarker of habitat fragmentation in birds. We examined the influence of habitat fragmentation on feather growth bar length in the free-living Eurasian treecreeper (*Certhia familiaris*), an area-sensitive old-growth forest passerine breeding in boreal forests under intense management. We found no clear indication that feather growth bar length was associated with habitat fragmentation. However in young treecreepers, high amount of edge and large number of patches tended to be related to shorter growth bar length. Our results thus provide no strong support for the utility of ptilochronology as an important biomarker of habitat fragmentation in this species.

1. Introduction

Habitat fragmentation is of special concern to biologists (Fazey et al., 2005), as it poses an important threat to global biodiversity among almost all the taxonomic groups studied (Foley et al., 2005). A major challenge is to identify vulnerable populations before irreversible demographic and genetic harm takes place. Thus there is an urgent need for reliable, general and easy-to-use biomarkers of health and well-being of individuals that can be applied in the field (Leung et al., 2003). One such potential phenotypic biomarker of environmental stress in birds is ptilochronology, the measurement of growth bars from feathers, since it does not require destructive sampling or specialised equipment to characterise (Lens and Eggermont, 2008). Growth bars consist of alternating dark and light bands, pairs of which are formed in a 24 h cycle during moult as demonstrated under controlled conditions (Brodin, 1993). Ptilochronology is primarily considered a measure of nutritional stress in birds (Grubb, 2006).

Previous studies have shown that birds living in high-quality environments, measured by indicators related to patch size, food abundance and individual survival, have wider growth bars than those in poorer quality areas (Yosef and Grubb, 1992; Carlson, 1998; Brown et al., 2002). Although some of these studies have considered habitat amount, they have not used a landscape scale nor have they explicitly considered the process of fragmentation, which includes both habitat loss and changes in habitat configuration (Fahrig, 2003). Since habitat fragmentation has been shown to reduce invertebrate abundance (Burke and Nol, 1998; Zanette et al., 2000), it would be expected to shorten the growth bars of birds living in fragmented habitats. To the best of our knowledge, there is only one study that has previously explicitly examined the association between habitat fragmentation and ptilochronology. Stratford and Stouffer (2001) showed that in two Amazonian bird species individuals found in fragmented landscapes had shorter growth bars than those captured in continuous habitats. However, this study did not examine a comprehensive set of habitat variables simultaneously (Fahrig, 2003), leaving the utility of ptilochronology as a biomarker of habitat fragmentation elusive.

Our aim was to investigate whether ptilochronology could be used as a biomarker of habitat fragmentation in the Eurasian treecreeper (*Certhia familiaris*, hereafter treecreeper). The treecreeper is a small arboreal passerine with a large breeding territory (Suorsa et al., 2005), breeds preferably in old forests and is specialised in foraging on tree-trunk arthropods (Suhonen and Kuitunen, 1991). The effects of habitat fragmentation on adult treecreepers are currently unknown, but in treecreeper nestlings habitat fragmentation has been related to the stress response and to reduced body condition (Suorsa et al., 2003, 2004).

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2. Methods

We sampled birds during 4 years (2000–2002 and 2006) in our study site covering 1150 km^2 in central Finland (centered on 62°37′N, 26°20′E), which is subjected to intensive commercial forestry. Adult birds were trapped with mist nets near nest boxes during their breeding season in June. We sampled a total of 125 adult treecreepers (2000 $n = 28$, 2001 $n = 45$, 2002 $n = 29$, and 2006 $n = 36$). All birds were measured, sexed, aged [age of two calendar years (2cy) or older (+2cy)] and banded after which we plucked the outermost left and right retrices (r6) from each individual. We only included individuals that had been sampled in our study area during the previous breeding season in order to be able to identify the landscape in which the feathers had been moulted (mid June to late July, P. Suorsa, unpublished data). In addition to using naturally moulted feathers we also included feathers that had been grown to replace those removed by us in the previous year because both types of feathers were regrown at the same time.

Feathers were photographed with a Panasonic FZ18 digital camera next to a length standard in a dark room with a light source shining from a shallow angle. We measured growth bar width using ImageJ software (Abramoff et al., 2004) so that the width of one growth bar was taken as the distance between the midpoints of two adjacent dark bands. In contrast to previous studies (e.g. Grubb, 1989; Gienapp and Merilä, 2010), we measured the widths of all visible growth bands individually. This enabled us to consider larger number of bands (mean per individual: 25, range: 7–50) and improved the accuracy of growth bar measurements by incorporating a longer time period per individual. For each growth bar we also measured the distance from the start of the barbed part of the feather, which described the locations of individual growth bars, as well as the length of the entire feather [mean (SD): 46.6 mm (5.0)]. The repeatability of growth bars measured as an intra-class correlation (Lessells and Boag, 1987) was $r = 0.70$ ($n = 32$, $F_{33,30} = 5.21$, $p > 0.0001$). All growth bars were measured without prior knowledge of the territory characteristics from which the feathers had been collected.

Landscape variables were determined from two classified Landsat TM5 images with a resolution of 25 m (Tomppo et al., 2008) taken in 2002 and 2005 to describe the landscape for feathers collected in 2000–2002 and 2006, respectively. For calculating landscape metrics we classified the landscape into non-forest (water, fields and other open areas, and forests under 50 m^3/ha) and forest (over 50 m^3/ha). We used a threshold of 50 m^3/ha because treecreepers have been observed to only nest in forests over this density (H. Hakkarainen, unpublished results). We used Fragstats (McGarigal et al., 2002) to calculate landscape metrics of which we selected five important ones (Fahrig, 2003): forest area, mean nearest neighbor distance, mean patch size, number of patches and amount of edge (Table 1). All landscape variables were calculated within a radius of 500 m, which should correspond better to the area that treecreepers utilise after their breeding season than their breeding territory of 200 m (Suorsa et al., 2005).

We used principal component analysis (PCA) on the five landscape variables outlined above in order to reduce the number of predictors by constructing linear orthogonal principal components (Khattree and Naik, 2000). We retained the first two principal components (PCs), which cumulatively explained 73.8% of the total variance. This variable reduction was done in order to avoid potential multicollinearity and over-fitting (Harrell, 2001), since we were also interested in including some biologically important interactions (see Section 4). We used orthogonal varimax rotation to make the component loadings interpretable as correlations between PCs and habitat variables (Table 1). Component loadings higher than 0.5 were considered as interpretable given the sample size (Budaev, 2010). These loadings indicate that PC1 was associated with forest area, mean nearest neighbor distance, mean patch size and number of patches. PC2 was mostly associated with edge amount and number of patches (Table 1).

We used a general linear mixed model (Littell et al., 2006) to analyse the effects of landscape variables, measured as PC1 and PC2 on growth bar width. As fixed factors we also included sex, the age when the feather was grown, the side from which each feather was collected, the distance of each growth bar from the start of the barbed area and feather length. On the basis of a priori biological expectations, interactions between PCs with age and sex were also considered (Lens and Eggermont, 2008). Our model included three random factors: the side from which the feather had been removed nested within individual identity, territory identity since numerous territories were included more than once and study year. Likelihood ratio tests with mixture distributions were used to determine the significance of random terms (Bolker et al., 2009). The Kenward–Rogers method was used to calculate the degrees of freedom of fixed effects and to estimate parameter estimates and their standard errors (Littell et al., 2006). Since our goal was to estimate and test the associations between the predictors and the outcome as accurately as possible, our statistical inference was based on a global. This model provides the most accurate point estimates and their errors, and thus accurate p-values for statistical inference (Harrell, 2001). However, our biological conclusion remained the same had we used model selection based on small sample AIC (Burnham and Anderson, 2002): the base model including age-by-habitat interactions showed the best fit to the data (results not shown). All analyses were conducted with SAS statistical software version 9.22 (SAS Institute, Cary, NC, USA).

3. Results

We found that the association between growth bar width and habitat fragmentation depended on individual age, but not on sex (Table 2). When we analysed our data separately for age classes, we found that young (2cy) individuals showed a weak negative association between PC2 and growth bar width [estimate (SE): −0.071 (0.039), $F_{1,54.52} = 3.40$, $p = 0.07$], while PC1 did not show a relationship [0.031 (0.046), $F_{1,55.61} = 0.47$, $p = 0.50$]. In older (+2cy) individuals neither PC1 [−0.040 (0.029), $F_{1,38.93} = 1.91$, $p = 0.18$] nor PC2 [−0.050 (0.034), $F_{1,48.43} = 2.19$, $p = 0.15$] showed a statistically significant association. By using log likelihood values to compare the model used and a model without the principal components or their associated interactions, we found that 12.7% of total variance was explained by the landscape characteristics studied. Variance in growth bar width was further explained by individual and territory

Table 1
The first two principal components (PCs) extracted from the principal component analyses of landscape variables measuring habitat loss and configuration. Interpretable component loadings (>0.5) are given in bold.

Landscape variable	Mean ± SD	PC1 (53.1% variance explained)	PC2 (20.7% variance explained)
Amount of forest habitat (ha)	44.6 ± 10.6	**0.79**	−0.29
Mean nearest neighbor distance (m)	31.2 ± 11.3	**−0.82**	−0.27
Mean patch size (ha)	9.7 ± 12.9	**0.84**	−0.31
Number of patches	8.6 ± 5.1	**−0.59**	**0.57**
Amount of edge (m)	10550.8 ± 1926.2	−0.03	**0.88**

Table 2
The results of the general linear mixed model explaining feather growth bar width in relation to habitat fragmentation and individual feather characteristics.

Independent variable	$\mathrm{df}_{\mathrm{num,den}}$	Estimate ± SE	F/χ^2	p
Fixed effects				
PC1	1, 83.3	0.093 ± 0.046	0.11	0.74
PC2	1, 89.6	−0.052 ± 0.043	2.98	0.09
Feather length (mm)	1, 186.7	0.047 ± 0.007	50.36	<.0001
Growth bar location (mm)	1, 3426.0	0.156 ± 0.007	457.46	<.0001
Side (reference = left)	1, 142.3	−0.011 ± 0.033	0.11	0.74
Sex (reference = male)	1, 207.4	−0.015 ± 0.042	0.13	0.72
Age (reference = 2cy)	1, 286.1	0.087 ± 0.042	4.38	0.04
PC1 × Age (reference = 2cy)	1, 228.0	−0.113 ± 0.045	6.44	0.01
PC2 × Age (reference = 2cy)	1, 361.6	0.079 ± 0.039	4.17	0.04
PC1 × Sex (reference = male)	1, 206.2	−0.055 ± 0.041	1.76	0.19
PC2 × Sex (reference = male)	1, 132.7	−0.072 ± 0.049	2.10	0.15
Random effects				
Year	1	0.026 ± 0.023	37.94	<.0001
Side (individual ID)	1	0.052 ± 0.008	308.4	<.0001
Territory ID	1	0.038 ± 0.011	31.55	<.0001

identity and by study year. Feather length and the location of each growth bar on the feather were also related to growth bar width, but growth bar width did not differ between left and right sides (Table 2).

4. Discussion

Our results did not show a clear and consistent association between habitat fragmentation and growth bar width in treecreepers. In young treecreepers only, a higher amount of edge and larger number of patches tended to be related to shorter growth bar length. Our results thus differ from those of Stratford and Stouffer (2001) who showed that birds captured in fragmented landscapes had shorter growth bars than those found in continuous habitats. However, this difference may be explained by several methodological differences. Most importantly, Stratford and Stouffer (2001) were unclear whether individuals captured in fragments had moulted in that particular landscape. Also, they used only one estimate of fragmentation (fragment size) as compared to our approach where we included five variables describing fragmentation, thus capturing much more of the important landscape variability (Fahrig, 2003). In addition they did not consider the age or the sex of individuals, which might have confounded their results (Grubb et al., 1991) because feather growth rates have previously been found to differ between age classes (Kern and Cowie, 2002) and sexes (Gienapp and Merilä, 2010).

Ptilochronology is considered to be an indicator of nutritional condition in birds (Grubb, 2006), meaning that in order for fragmentation to affect growth bar width it would need to affect food availability. Our results thus indirectly suggest that habitat fragmentation may not have affected the nutritional condition of treecreepers. Treecreepers have been shown to locally deplete food abundance during the breeding season (Jäntti et al., 2001), suggesting that food resources are limited during the postnuptial moult. There is currently no direct evidence linking habitat fragmentation to food availability in treecreepers, although small forest nesting patch size has been found to be related to the stress response and to lowered body condition in treecreeper nestlings (Suorsa et al., 2003, 2004). Therefore it is possible that breeding birds shifted the cost of nutritional stress on their nestlings and therefore no effect on adult treecreepers was found.

Studies have shown that natural forests contain a more abundant and diverse set of invertebrates than managed ones in boreal areas (Pettersson et al., 1995) and that the amount of invertebrates is higher in large fragments than in small ones (Burke and Nol, 1998; Zanette et al., 2000, but see Sekercioglu et al., 2002). However, any decrease in food availability due to small fragment size could be offset by an increase in food abundance in edge habitats (Helle and Muona, 1985). Although our results did not show a clear association between habitat fragmentation and growth bar width, we did find that territory identity strongly influenced feather growth even when individual identity and yearly variation were controlled for. This suggests that there likely were unmeasured differences in territories in terms of food availability, which could be due to habitat quality characteristics not directly affected by habitat fragmentation such as trunk density and forest age (Suorsa et al., 2003).

Easily measurable biomarkers are useful tools in identifying vulnerable populations before demographic and genetic harm takes place. Our results do not favor the use of ptilochronology as a sensitive and reliable bioindicator of habitat fragmentation in the treecreeper. However, the association between habitat fragmentation and feather growth bar width has received very little attention and we suggest that similar studies in different species using feathers moulted in a known landscape and taking age and sex into account should be conducted before more general conclusion can be drawn.

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