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Parental and genetic factors rather than nesting forest patch size affect physiological traits of offspring in an area-sensitive passerine: an experimental study

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Our understanding of the effects of habitat loss on individual performance is limited due to a lack of experimental studies that take the potential genetic and parental effects producing phenotypic variation into consideration. To assess the relative role of habitat loss on offspring phenotype while controlling for the confounding effects of genetic and parental variation we performed a partial cross-fostering experiment using the Eurasian Treecreeper *Certhia familiaris*. The experiment was carried out by swapping half the nestlings in a brood between small and large nesting forest patches to determine the effect of nesting forest patch size on five nestling traits reflecting morphological size, body condition, physiological stress and inflammation status. There was no effect of nesting forest patch size on the offspring traits examined. Instead, we found evidence of genetic and early parental effects on all traits except inflammation status, as well as parental effects after cross-fostering for all of the measured offspring traits. Our results suggest that genetic and parental effects should be taken into account when making inferences about species' responses to habitat loss.

Keywords: body condition, *Certhia familiaris*, cross-fostering, habitat loss, inflammation, physiological stress, Treecreeper.

Habitat loss due to forestry and agriculture has been identified by the International Union for Conservation of Nature Species Survival Commission (Baillie *et al.* 2004) as the most pervasive threat to bird species worldwide. Our understanding of the fundamental processes behind the effects of habitat loss, which includes both decreasing patch size and increased isolation of patches (Fahrig 2003), is still limited due to at least three reasons: (1) previous studies have mainly been conducted at the population level, (2) there have been relatively few experimental studies and (3) potential genetic and parental effects producing phenotypic variation have not been taken into consideration. The last point is particularly important, as the response of individuals to their environment is strongly determined by their genotype and investment by their parents.

Previous studies have generally shown negative effects on birds of habitat loss. However, most have examined the effects of habitat loss at the population level, although individual-level processes provide a mechanistic means to understand the effects of habitat loss on populations (Bowers & Dooley 1999). Previous individual-level studies on the effects of habitat loss in birds have demonstrated negative impacts on food supply (Burke & Nol 1998, Zanette *et al.* 2000), survival (Robinson *et al.* 1995, Doherty & Grubb 2002, Ruiz-Gutiérrez *et al.* 2008, Europe: Kurki *et al.* 2000) and reproductive success (Hinam & St. Clair 2008, Laaksonen *et al.* 2004, but see Pasinelli *et al.* 2008). Assessing the extent to which habitat loss influences offspring traits is especially important because recent studies have shown that the conditions that individuals encounter during their early

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development have long-lasting consequences on subsequent life-history traits (Lindström 1999, Monaghan 2008) and thus on population growth rate (Pelletier *et al.* 2007).

Due to the practical difficulties of conducting landscape-level experiments, studies investigating the consequences of habitat loss on individuals and populations have mainly been correlative in nature (McGarigal & Cushman 2002). However, some experimental studies have been conducted showing, for example, that habitat loss decreases bird species richness in Australian plantation forests (Lindenmayer *et al.* 2009), alters community structure in old boreal forests in Canada (Schmiegelow *et al.* 1997) and increases physiological stress of offspring in managed boreal forests in Finland (Suorsa *et al.* 2004). Until more experimental studies are conducted at the individual level, our understanding of how habitat loss affects individuals and populations will continue to be poor.

Most importantly, previous studies concerning habitat loss have not taken genetic background into consideration, even though it is a key component producing phenotypic variation among individuals (Falconer & Mackay 1996) and could have acted as a confounding factor in previous studies. Similarly, parental effects arising from shared parental investment (Kruuk & Hadfield 2007) have not previously been taken into consideration. Partial cross-fostering experiments, where half of the brood is swapped with offspring from a different nest, provide a practical way to determine experimentally the relative roles of environmental, genetic and parental effects on individual trait variation. However, previous cross-fostering experiments designed to estimate, for example, the heritability of traits, have treated environmental variation as a nuisance factor to control for variation arising from siblings sharing the same developmental environment in addition to their genes (Falconer & Mackay 1996). Furthermore, these studies have commonly manipulated clutch size as a proxy of environmental variation instead of explicitly considering the effect of habitat characteristics on offspring traits (e.g. Merilä 1996).

Our aim was to determine experimentally the effect of habitat loss, measured as nesting forest patch size, on five traits of nestling Eurasian Treecreepers *Certhia familiaris* (hereafter Treecreeper) while taking into account potential genetic and parental effects. We considered offspring traits reflecting morphological size (tarsus length), body condition (scaled mass index and muscle score), physiological stress (heterophil–lymphocyte (H-L) ratio) and inflammation status (erythrocyte sedimentation rate (ESR)) using a partial cross-fostering experiment by swapping nestlings between smaller and larger nesting forest patches. We focused on nesting forest patch size because previous studies have shown that decreased patch size is associated with increased physiological stress and lowered body condition in Treecreeper nestlings in the study population (Suorsa *et al.* 2003b, 2004). Based on the available literature, we predicted that smaller nesting forest patch area is related to smaller tarsus size, poorer physiological condition, higher stress and increased infections among Treecreeper nestlings. This is, to the best of our knowledge, the first time that genetic and parental effects have been directly controlled for in a study examining habitat fragmentation.

METHODS

Study species

The Eurasian Treecreeper is a small arboreal passerine that specializes in searching for invertebrates on tree trunks (Suhonen & Kuitunen 1991) and shows a clear preference for old-growth forests (Suorsa *et al.* 2005). Treecreepers construct natural nests under a flap of loose bark or in crevices in tree trunks (Cramp & Perrins 1993) but also readily accept specially designed nestboxes. Males defend a breeding territory that reaches at least 70 m from the nest (Cramp & Perrins 1993), and feeding trips within the home-range can extend three times this distance (H. Hakkarainen unpubl. data).

Data collection

We conducted the experiment in summer 2002 in a study area covering 1150 km^2 in central Finland (centred on 62°37′N, 26°20′E) subjected to intensive commercial forestry. Our study site contained 218 nestbox sites in habitats with differing degrees of habitat loss and fragmentation. The partial cross-fostering experiment was performed by swapping half of the offspring from each brood between different-sized nesting forest patches. This enabled us to estimate the effect of forest patch size while controlling for genetic and parental effects (Merilä & Sheldon 2001). Nesting forest patch size was determined from classified Landsat

TM 5 images (from 1995 to 1997) of the study area produced by the National Land Survey of Finland (Vuorela 1997). The forested area around nestboxes was determined by summing the total area of pixels classified as containing over 50 m^3/ha of wood. A threshold of 50 m^3/ha of wood was used since breeding Treecreepers are rarely observed in forests below this level of timber volume (Suorsa *et al.* 2005).

The size of forest patches occupied in 2002 ranged from 1.5 to 12.75 ha with a mean of 7.85 ha ($n = 79$ occupied out of 218 patches). Each forest patch was occupied by only one breeding pair of Treecreepers. From these, 22 synchronously hatched pairs of nests having similar brood size (± 1 chick, one duplicate had a difference of two chicks), later referred to as duplicates, were selected. The hatching dates of nestlings included in the experiment were similar to the population mean, although they were slightly more concentrated towards the beginning of the breeding season when conditions are more severe: 1 May = 1, experiment: mean ± sd = 10.77 ± 4.38 days, range: 4–19 days, population: 11.74 ± 5.8 days, range: 3–27 days. We used the mean size of occupied forest patches as a reference point to split the 44 nesting patches used in this study into those smaller than 7.85 ha (mean: 5.25 ha, range: 1.50–7.63 ha) and those larger than 7.85 ha (mean: 9.88 ha, range: 8.00–12.44 ha). This split was used to create two classes of patches between which to swap nestlings. The forest patch sizes used here are very similar to those of Suorsa *et al.* (2004), where an effect of nesting forest patch size on physiological stress of Treecreeper nestlings was found (mean: 7.38 ha, range: 0.5–12.75 ha).

Within duplicates, half of the offspring were randomly selected from smaller patches and swapped with an equal number of randomly selected offspring from larger patches, and vice versa. Half of the offspring in each nest were not moved and served as controls. The swapping of chicks was performed 2 days after hatching, keeping the brood size constant in all but one nest. At least two nestlings were swapped from each nest, except one nest where only one nestling was moved. The brood sizes of nests used in this experiment ranged between four and six chicks. Nestlings could not be swapped by sex, because determination of nestling sex at this stage requires molecular methods. The swapped nestlings were marked by clipping the downy feathers on their heads and by painting their claws. At day 5, we banded the nestlings with an aluminium ring and at day 11 took morphological measurements as well as blood samples from which the H-L ratio and ESR rate were determined. Parent birds were also trapped at day 11 with mist-nests and measured and aged as two calendar years or older based on plumage characteristics. Prior to fledging at day 14, we measured the morphological traits again.

Offspring traits

Tarsus length (day 11: $n = 207$, mean ± sd = 15.29 ± 0.55 mm, day 14: $n = 176$, 15.45 ± 0.41 mm) was used as a measure of the morphological size of nestlings. Body condition was estimated by calculating the scaled mass index (SMI) (day 11: $n = 207$, mean ± sd = 8.57 ± 0.82, day 14: $n = 176$, 8.12 ± 0.83), which has recently been shown to be a superior body condition index compared with, for example, the residual body mass commonly used in ecological studies (Peig & Green 2010). SMI was calculated using the method outlined in Peig and Green (2010), which uses the scaling relationship, obtained through standardized major axis regression (Warton *et al.* 2006), between mass and tarsus length to standardize body mass.

Muscle score (day 11: $n = 207$, mean ± sd = 2.25 ± 0.66, day 14: $n = 176$, 2.21 ± 0.75) was used to estimate body condition, as developed pectoral muscles are important for flight performance and protein storage (Lind & Jakobsson 2001). Therefore, quantifying the overall condition of the pectoral muscles in a bird gives a relatively stable measure of the condition of an individual. Muscle score was measured by visually inspecting the pectoral muscles of each individual nestling and then quantifying the amount of muscle on a scale of zero to four (modified from Gosler 1991).

The H-L ratio ($n = 139$, mean ± sd = 0.80 ± 0.83) was used as a measure of physiological stress, as it increases in moderately stressful conditions, making it suitable for detecting the presence of physiological stress for most stressors (Maxwell & Robertson 1998). The H-L ratio for each individual was determined with a microscope from a drop of blood that had been smeared and fixed onto a microscope slide (Ilmonen *et al.* 2003).

The ESR ($n = 189$, mean ± sd = 0.03 ± 0.01 mm/h) was used to estimate the inflammation status of nestlings. ESR is a measure of the rate at which red blood cells settle down a column in a preset amount of time, and is a non-specific measure of inflammation (Saino & Møller 1996). Individuals in poorer condition are likely to be subject to more infections by pathogens and parasites, resulting in increased ESR values. Blood samples were taken in heparinized capillary tubes by pricking the brachial vein of the nestling. ESR was calculated by dividing the volume of plasma not occupied by red blood cells by the total volume of blood in the capillary and dividing this by the time required for sedimentation to yield the proportion of blood sedimented per hour (Saino & Møller 1996).

Statistical analysis

We used general linear mixed models (Littell *et al.* 2006) and restricted maximum-likelihood estimation (REML) to determine the relative contributions of environmental, genetic and parental components to offspring traits (Table 1). In these models, original and rearing nesting forest patch sizes were included as continuous fixed effects. Brood size was also included as a categorical fixed effect in our models because we knew from previous research that an increased brood size was associated with increased physiological stress in Treecreeper nestlings (Suorsa *et al.* 2004).

Nest of origin, nest of rearing and nest pair duplicates were included as random factors and estimated via variance components. Both nest of origin and nest of rearing were nested within the term 'nest pair duplicate'. Nest of origin includes all genetic effects as well as early parental effects. Nest of rearing includes post-swapping parental effects, and the term duplicate reflects variation of offspring traits related to differences between nest pairs, such as time of season (Norte *et al.* 2009). We did not analyse possible genotype by environment interactions, as these effects are easily overlooked if sample sizes are low (Merilä & Sheldon 2001). To control the type I error rate and preserve the power of statistical tests of fixed effects, potential negative variance components of random factors were estimated instead of setting them to zero (i.e. excluding them from the models) (Littell *et al.* 2006). In biological terms, however, this equates to assuming that the random factor had no effect on the response, because, for example, heritability cannot have a negative value.

The residuals of tarsus length, H-L ratio and ESR were slightly skewed, so a log-normal distribution with an identity link function was used to ensure normally distributed residuals. Likelihood ratio tests with mixture distributions and *F*-tests were used to determine the significance of random (when positive) and fixed effects, respectively (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). All analyses were performed with SAS statistical software version 9.2 (SAS Institute, Cary, NC, USA).

Table 1. Effects associated with different components of offspring traits in the partial cross-fostering experiment conducted in central Finland.

Effect	Measures
Original patch size	Pre-swapping environmental effects caused by habitat quality
Rearing patch size	Post-swapping environmental effects caused by habitat quality
Original × rearing patch size	Effect of differing patch size differences within nest-pair duplicates
Nest of origin	Pre-swapping genetic, maternal and brood effects
Nest of rearing	Post-swapping brood effects
Duplicate	Hatching date and other differences between nest-pair duplicates

RESULTS

We found no evidence for effects of either original or rearing nesting forest patch size on any of the traits examined (Tables 2 and 3). There was a significant effect of nest of origin for tarsus length, SMI, muscle score and H-L ratio (Tables 2 and 3). Nest of origin explained 7–35% of the variance in offspring traits (Fig. 1). There was a significant 'nest of rearing effect' for all measured offspring traits (Tables 2 and 3), which explained 13–48% of the variance in offspring traits (Fig. 1).

Initial size differences between chicks of different origins in the same nest could lead to a competitive advantage for the larger chicks through competition for food in the nest (Kruuk & Hadfield 2007) and thus affect the response variables measured here. Therefore, we assessed whether weight differences at the time of swapping were correlated

Table 2. Results of general linear mixed models used to partition morphological size (tarsus length) and body condition (scaled mass index and muscle score) of nestlings into environmental, genetic and parental components.

	Tarsus length (n = 207)				Scaled mass index (n = 207)				Muscle score (n = 207)			
Independent variable	$df_{num,den}$	Estimate ± se	F/χ^{2*}	P	$df_{num,den}$	Estimate ± se	F/χ^{2*}	P	$df_{num,den}$	Estimate ± se	F/χ^{2*}	P
Fixed effects												
Original patch size	1, 25.70	0.0008 ± 0.015	0	0.96	1, 32.88	−0.010 ± 0.028	0.13	0.72	1, 21.83	0.013 ± 0.016	0.69	0.42
Rearing patch size	1, 18.25	0.001 ± 0.015	0	0.95	1, 26.19	−0.022 ± 0.024	0.82	0.37	1, 23.11	−0.001 ± 0.021	0	0.96
Brood size	2, 31.04	†	1.69	0.20	2, 43.41	†	0.07	0.94	2, 37.21	†	0.66	0.52
Random effects												
Nest of origin (duplicate)	1	0.036 ± 0.018	12.27	0.0002	1	0.200 ± 0.082	29.29	<0.0001	1	0.041 ± 0.021	11.71	0.0003
Nest of rearing (duplicate)	1	0.023 ± 0.017	4.40	0.018	1	0.112 ± 0.056	12.52	0.0004	1	0.089 ± 0.037	29.05	<0.0001
Duplicate	1	0.024 ± 0.026	1.00	0.16	1	−0.034 ± 0.071	0	‡	1	0.057 ± 0.051	1.59	0.10
Residual		0.020 ± 0.002				0.392 ± 0.042				0.454 ± 0.048		

*F-values were used for significance tests of fixed effects, χ^2 values were used for random effects.

†Parameter estimates of brood size (categorical variable) have been omitted due to multiple levels.

‡Test statistics not given due to negative variance component (see Methods).

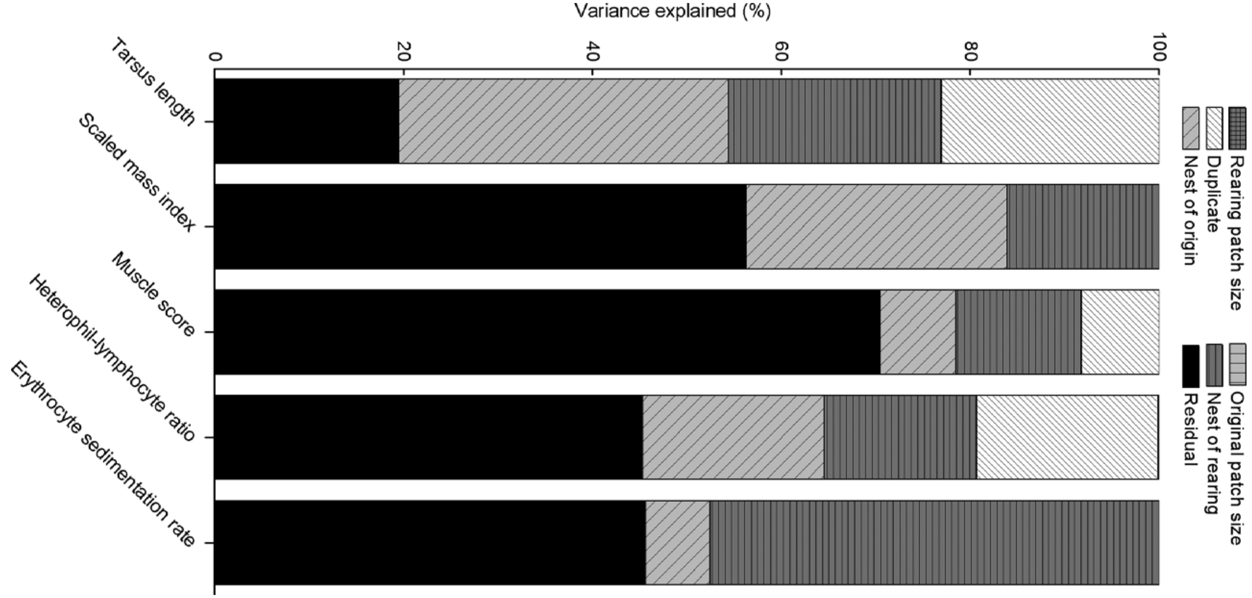

Figure 1. Relative contributions of the estimates of environmental, genetic and parental components of offspring traits measured from Eurasian Treecreepers sampled in this study.

with a higher variance of measured traits at the end of the breeding season (Norte *et al.* 2009). Muscle score (estimate ± se = 0.42 ± 0.17, $F_{1,18} = 6.47$, $P = 0.02$) and H-L ratio (estimate ± se = 0.31 ± 0.14, $F_{1,13} = 5.11$, $P = 0.04$) showed a significant association between initial size differences and variance at the end of the nestling period. This might have artificially increased the estimates of the nest

Table 3. Results of general linear mixed models used to partition physiological stress (heterophil–lymphocyte ratio) and inflammation status (erythrocyte sedimentation rate) into environmental, genetic and parental components.

	Heterophil–lymphocyte ratio (n = 139)				Erythrocyte sedimentation rate (n = 189)			
Independent variable	$df_{num,den}$	Estimate ± se	F/χ^{2*}	P	$df_{num,den}$	Estimate ± se	F/χ^{2*}	P
Fixed effects								
Original patch size	1, 20.55	−0.046 ± 0.029	2.52	0.13	1, 21.19	0.003 ± 0.011	0.09	0.77
Rearing patch size	1, 18.57	0.024 ± 0.030	0.64	0.43	1, 33.08	−0.006 ± 0.019	0.09	0.77
Brood size	2, 29.80	†	0.63	0.54	2, 37.83	†	0.37	0.69
Random effects								
Nest of origin (duplicate)	1	0.120 ± 0.074	6.20	0.006	1	0.014 ± 0.011	2.96	0.078
Nest of rearing (duplicate)	1	0.113 ± 0.076	4.87	0.014	1	0.095 ± 0.039	37.19	<0.0001
Duplicate	1	0.142 ± 0.116	1.88	0.085	1	−0.011 ± 0.028	0	‡
Residual		0.313 ± 0.049				0.086 ± 0.011		

*F-values were used for significance tests of fixed effects, chi-squared values were used for random effects.

†Parameter estimates of brood size (categorical variable) have been omitted due to multiple levels.

‡Test statistics not given due to negative variance component (see Methods).

of origin for these traits. None of the other traits showed a significant relationship ($P > 0.55$).

As the size difference between pairs of forest patches within duplicates was not constant (mean ± sd = 4.59 ± 1.91 ha, range: 1.63–8.25 ha), we also fitted the models including the interaction between original and rearing patch size. These interactions were not statistically significant ($F < 3.5$, $P > 0.06$) and their inclusion did not change the biological interpretation of genetic and parental effects in any of the models (results not shown). It should, however, be noted that the interpretation of this interaction might not be straightforward due to nestlings with a patch size difference of zero having the same nest of origin and rearing. We also assessed whether nesting forest patch size and clutch size were associated in order to examine the possibility that parents of higher reproductive success concentrated on larger forest patches. However, no significant relationship was found ($r_S = 0.12$, $P = 0.47$). Furthermore, we assessed whether older or larger birds, or those in better condition, occupied larger forest patches but did not find a significant relationship for age (males: $F_{1,35} = 0.79$, $P = 0.38$, females: $F_{1,37} = 2.03$, $P = 0.16$), tarsus length (males: $F_{1,35} = 0.35$, $P = 0.56$, females: $F_{1,37} = 0.15$, $P = 0.70$) or SMI (males: $F_{1,35} = 0.24$, $P = 0.63$, females: $F_{1,37} = 0.09$, $P = 0.77$).

DISCUSSION

Our partial cross-fostering experiment provided no evidence that the size of the nesting forest patch before or after swapping had an effect on offspring traits in the Eurasian Treecreeper population studied. Instead, morphological size, mass-derived body condition and physiological stress were mainly determined by genetic effects, whereas parental effects primarily determined muscle-derived body condition and inflammation status.

These results appear to contrast with previous correlative studies that have shown negative effects of decreased patch size on birds at the individual and population levels (Ribic *et al.* 2009, Bayard & Elphick 2010). However, many of these studies have been carried out in large continuously forested areas with much less disturbance than at our study site. Decreased patch size has been shown to be associated with reduced food supply in birds (Zanette *et al.* 2000) as well as altered microclimate (Vanwalleghem & Meentemeyer 2009), providing potential links to how the negative effects of reduced patch size can operate. In the case of the Treecreeper, larger forest patches should contain more food due to an increased number of tree trunks on which Treecreepers can feed (Kuitunen 1989). Furthermore, in our study area larger forest patches had a higher percentage of forest over 150 m^3/ha of wood ($r = 0.54$, $P = 0.0002$, $n = 43$), which is expected to lead to increased food supply, as invertebrate density and trunk circumference show a weak positive correlation ($r_s = 0.18$, $P = 0.066$, $n = 101$). Most importantly, decreasing forest patch size has been shown to be associated with reduced body condition and increased physiological stress in Treecreeper nestlings (Suorsa *et al.* 2003b, 2004). Although morphological size, muscle score and inflammation status might not have been sensitive enough to respond to decreased patch size, we would have expected body condition and physiological stress to do so.

We suggest three possible reasons that could have contributed to the lack of an effect of nesting forest patch size on the offspring traits measured: (1) Treecreeper nestling phenotype is mainly determined by genetic and parental effects, (2) favourable rearing conditions during the study year masked possible negative effects of small forest patch size or (3) the effects of forest patch size on offspring traits were sex-specific.

First, our results suggest that in Treecreeper offspring, genetic background is an important component of morphological size, body condition and physiological stress. Our results are therefore in line with previous studies that have found genetic effects for morphological size in Alpine Swifts *Apus melba* (Bize & Roulin 2009), body condition in Collared Flycatchers *Ficedula albicollis* (Merilä *et al.* 2001) and H-L ratio in domestic chickens *Gallus gallus* (Campo & Davila 2002). Muscle score and H-L ratio might have had artificially large genetic components due to pre-swapping parental effects (Kruuk & Hadfield 2007) (see Methods). Our results also showed that for muscle score and inflammation status, post-swapping parental effects were more important than genetic ones, and nearly equal in importance for physiological stress (Fig. 1). Previous cross-fostering studies have found a strong effect of post-swapping parental effects for tarsus length and body condition in Collared Flycatcher nestlings (Kruuk & Hadfield 2007) and for tarsus length in Great Tit *Parus major* nestlings (Norte *et al.* 2009). As we controlled for nesting forest patch size, it is likely that these effects were accounted for by effects arising from parental performance. Indeed, parental effort is known to play a major role in the condition of offspring in Siberian Jays *Perisoreus infaustus* (Ekman *et al.* 2000) and Blue Tits *Cyanistes caeruleus* (Tremblay *et al.* 2004), as well as increasing the first-year survival of offspring in Long-tailed Tits *Aegithalos caudatus* (MacColl & Hatchwell 2003).

Secondly, our experiment was conducted during only one breeding season, which means that we may have missed temporal variation in the environment (Harrison & Bruna 1999), such as year-to-year differences in climate. Previous research studying the effects of nesting forest patch size on the same Treecreeper population conducted in 2001 showed that a smaller forest patch was associated with increased physiological stress (Suorsa *et al.* 2004). We therefore compared the mean temperature of the nestling period (date of first hatching to date of last fledging) between 2001 and 2002 when the current study was conducted. This analysis showed that nestling period temperature was on average 3.5 °C higher in 2002 than in 2001 (ANOVA controlling for the heteroscedasticity of variances: $F_{1,61.7} = 18.3$, $P < 0.0001$, 2002: $n = 36$, mean ± se = 11.5 ± 0.67 °C, 2001: $n = 33$, 8.0 ± 0.47 °C). Moreover, there were a total of 22 rainless days during the nestling period in 2002 compared with 17 in 2001. The climatic conditions during the nestling period of 2002 could have been more beneficial for the development of the arboreal spiders (Huhta 1965) that Treecreeper nestlings mainly feed on. This would improve the foraging success of parent Treecreepers and lead to reduced nutritional stress in nestlings. Therefore, it is possible that favourable climatic conditions during the breeding season of 2002 lead to high food supply for Treecreeper nestlings. This is in line with the results of Suorsa *et al.* (2004), who found no effect of nesting patch size on the physiological stress of Treecreeper nestlings in broods with experimentally reduced number of nestlings.

Finally, previous studies have shown sex differences in the nestling responses to adverse environmental conditions, although the direction of such patterns may vary between the species due to, for example, nestling size difference between the sexes and clutch size (Råberg *et al.* 2005). We know from our population that males are more costly to raise and suffer higher mortality due to habitat degradation (Suorsa *et al.* 2003a). As we were not able to determine the sex of nestlings in this experiment, we cannot rule out the possibility that nestling sex confounded our results to some degree.

In conclusion, our results show that nesting forest patch size did not affect the offspring traits measured here in the Eurasian Treecreeper. Instead, our results highlighted the importance of genetic and parental effects in this species. Neither of these sources of variation has normally been considered when studying the effects of habitat loss and changes in habitat configuration on individuals. We thus suggest that future studies pay attention to such genetic and parental effects when investigating the effects of habitat loss on phenotypic variation. To further our understanding, we also suggest that similar studies using different indicators of habitat loss, fragmentation and quality be replicated over several years to address these questions.

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