Sympatric divergence and clinal variation in multiple coloration traits of *Ficedula* flycatchers

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Abstract

Geographic variation in phenotypes plays a key role in fundamental evolutionary processes such as local adaptation, population differentiation and speciation, but the selective forces behind it are rarely known. We found support for the hypothesis that geographic variation in plumage traits of the pied flycatcher *Ficedula hypoleuca* is explained by character displacement with the collared flycatcher *Ficedula albicollis* in the contact zone. The plumage traits of the pied flycatcher differed strongly from the more conspicuous collared flycatcher in a sympatric area but increased in conspicuousness with increasing distance to there. Phenotypic differentiation (P_{ST}) was higher than that in neutral genetic markers (F_{ST}), and the effect of geographic distance remained when statistically controlling for neutral genetic differentiation. This suggests that a cline created by character displacement and gene flow explains phenotypic variation across the distribution of this species. The different plumage traits of the pied flycatcher are strongly to moderately correlated, indicating that they evolve non-independently from each other. The flycatchers provide an example of plumage patterns diverg-

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ing in two species that differ in several aspects of appearance. The divergence in sympatry and convergence in allopatry in these birds provide a possibility to study the evolutionary mechanisms behind the highly divergent avian plumage patterns.

Introduction

Geographic variation in traits associated with fitness plays a key role in fundamental evolutionary processes such as local adaptation, population differentiation and ultimately, speciation (Mayr, 1942; Endler, 1977; Newton, 2003; Price, 2008). The degree of divergence between local populations depends on the interplay between local adaptive processes and gene flow between the populations (e.g. Hereford, 2009; Fraser *et al.*, 2011). However, random genetic drift may also create nonadaptive variation between populations, and the existence of variation as such does not prove selection or adaptation (Zink & Remsen, 1986; Newton, 2003). Moreover, although the comparison of variation in neutral genetic markers and phenotypes is useful for indicating that selection is acting on a trait (e.g. Lehtonen *et al.*, 2009a; Antoniazza *et al.*, 2010), the selective agents causing the variation are still rarely known (Mullen & Hoekstra, 2008). Knowing the selective agent causing divergence is, however, an essential component for understanding how selection may act in natural populations.

The dorsal coloration of *Ficedula* flycatcher males in Eurasia is a prominent example of extensive geographic variation in phenotype that has been related to selection and local adaptation. In particular, the coloration of the pied flycatcher *Ficedula hypoleuca* has been suggested to evolve through character displacement with the socially dominant collared flycatcher *Ficedula albicollis* in areas of sympatry (e.g. Røskaft *et al.*, 1986; Lundberg & Alatalo, 1992; Sætre *et al.*, 1997). The collared flycatcher is always black on the dorsal side, whereas the dorsal colour of pied flycatchers is typically predominantly black in allopatric populations but brown in the sympatric area (Røskaft *et al.*, 1986; Lundberg & Alatalo, 1992; Lehtonen *et al.*, 2009a). The females of both species are brown, but the brown pied flycatcher males resemble the females of the collared flycatcher more than the females of their own species (Calhim *et al.*, 2014). There is considerable evidence that this heterospecific female mimicry of the brown male colour type is beneficial for pied flycatchers in sympatric areas because it reduces harmful social/agonistic interactions (Slagsvold & Sætre, 1991; Alatalo *et al.*, 1994) and hybridization (Sætre *et al.*, 1997) between the species (reviewed by Qvarnström *et al.*, 2010; Sætre & Sæther, 2010). However, it is less clear whether these heterospecific interactions have an effect on the plumage coloration of

male pied flycatchers outside the sympatric area through gene flow. It is known that the male pied flycatchers are most commonly dark in allopatric populations (e.g. von Haartman, 1985; Røskaft *et al.*, 1986; Lundberg & Alatalo, 1992; Lehtonen *et al.*, 2009a), but it remains unknown whether male plumage coloration changes steeply or gradually outside the contact zone. A gradual change could be expected if there is selection to different directions in the areas but gene flow between them.

The interest in the geographic variation in the coloration of the pied flycatcher has almost exclusively focused on the black-brown coloration of males. However, the flycatchers also have other plumage traits that are equally conspicuous and variable as the dorsal coloration: white patches on the forehead, wing and tail that vary in size and shape, and ultraviolet reflectance (UV) (e.g. Lehtonen *et al.*, 2009b; Sirkiä & Laaksonen, 2009). Geographic variation in these traits across the breeding range remains mainly unknown. It is also poorly known how all these plumage traits relate to the same traits in the collared flycatcher. The genetic mechanisms behind the traits are not known, but it is known that black-brown dorsal coloration is highly heritable in all populations where studied ($h^2 = 0.60$–0.88; reviewed by Lehtonen *et al.*, 2009a), and there is also evidence that the forehead patch size is heritable (Potti & Canal, 2011). Understanding the variation and potential divergence in these other traits is important for understanding the evolution of the overall coloration patterns and the conflicting selection pressures on them. It is also important to know to what extent different traits are correlated, as the evolution of a trait depends on its correlation with other traits that may be subject to different selective forces than the focal trait (Merilä & Björklund, 2004).

We studied geographic variation in a number of pied flycatcher plumage traits throughout its breeding range, and in the same traits in two populations of the collared flycatcher. Our main aim was to gain a better understanding of the evolution of complex coloration patterns and geographic variation in them. Previous studies from single populations have indicated that different coloration traits are correlated in the pied flycatcher (e.g. Menchinsky, 2006; Ivankina *et al.*, 2007; Sirkiä & Laaksonen, 2009). We therefore first examined the intercorrelations between the traits, to investigate whether different traits evolve independent of each other or form a plumage trait complex indicative of phenotypic integration. Secondly, we

examined whether the plumage traits exhibit more phenotypic variation among populations (P_{ST}) than predicted by neutral genetic variation (F_{ST}), which could indicate that the variation is due to non-neutral evolutionary forces (e.g. Leinonen *et al.*, 2008; Brommer, 2011). A previous study showed that there is much more phenotypic variation in the dorsal black-brown coloration of pied flycatchers than predicted by neutral genetic variation (Lehtonen *et al.*, 2009a). This has not, however, been studied for any other plumage traits, and it is not even known what kind of geographic variation exists in the other traits. Finally, we tested the hypothesis that character displacement in the sympatric area, combined with gene flow, is one of the driving evolutionary forces behind geographic colour variation in the plumage traits of the pied flycatcher in allopatric areas. To our knowledge, this hypothesis has not been previously tested quantitatively in a geographic context. We predicted that if this hypothesis is an important explanation for the distribution-wide coloration pattern of pied flycatchers, they should differ from the collared flycatchers in the sympatric area but the difference should decrease with increasing distance from the sympatric area when controlling for genetic differentiation in neutral markers between the populations.

Methods

Study species

The pied and the collared flycatcher are closely related and very similar in their ecology (a general reference for the following species information is Lundberg & Alatalo, 1992). They winter in sub-Saharan Africa (collared more in the east) and breed in Europe. The breeding ranges of the species are, however, different: the pied flycatcher breeds in most of Europe, whereas the collared flycatcher is mainly restricted to central Eastern Europe and the Swedish islands of Gotland and Öland (Fig. 1). Where the two species live in sympatry, the collared flycatcher is socially dominant and the species can hybridize (reviewed by Qvarnström *et al.*, 2010). Both species breed exclusively in cavities and readily accept a nest box, which allows easy access to capturing them for measurements.

Study populations

Data were collected from 19 study populations across the breeding range of the pied flycatcher and from two populations of the collared flycatcher. The 19 populations form a considerable coverage over the breeding
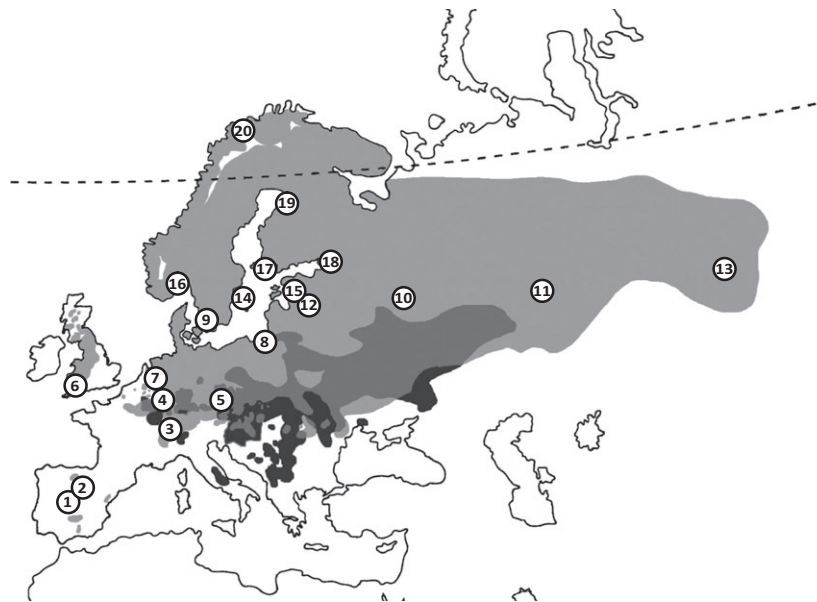

Fig. 1 Breeding ranges of the pied and collared flycatcher and the locations of the flycatcher populations studied. Light grey area illustrates the breeding range of the pied flycatcher in allopatry, the middle grey area illustrates the sympatric breeding area of both pied and collared flycatcher and the dark grey area illustrates the allopatric breeding area of the collared flycatcher. The map is modified from Birds of the Western Palearctic (Cramp & Simmons, 2006) and Flint *et al.* (1984) using local information of the authors. The white circles indicate the locations of the study populations: (1) Valsain-Lozoya, Spain; (2) La Hiruela, Spain; (3) Vaud, Switzerland; (4) Harthausen, Germany; (5) Moravia-Jeseníky*, Czech Republic; (6) East Dartmoor, United Kingdom; (7) Drenthe, the Netherlands; (8) Courish spit, Russia; (9) Vombs fure, Sweden; (10) Moscow region, Russia; (11) Middle Urals, Russia; (12) Kraslava, Latvia; (13) Tomsk, Russia; (14) Gotland**, Sweden; (15) Estonia; (16) Oslo, Norway; (17) Ruissalo, Finland; (18) Karelia, Russia; (19) Oulu, Finland; (20) Skibotn, Norway. *Samples from both the pied and the collared flycatcher. **Samples only from the collared flycatcher.

range of the species (Fig. 1). The data were collected between 2007 and 2010. The number of individuals measured per population and trait is presented in Table S1. The majority of the data were collected from males captured at the nest during nestling feeding. Therefore, our data represent the successful breeders in each population.

Collection and measurement of coloration data

The data were collected in a similar way in each population, following the same instructions on how to report male plumage traits, take photographs and collect feather samples. The feather samples and photographs were sent to PS who measured the UV reflectance and sizes of ornamental patches in the way that is described in the Supporting Information. Measurements from the feathers and pictures were taken in a similar way for both pied and collared flycatchers. The dorsal coloration of head and back of pied flycatcher males was estimated by Drost scores (Drost, 1936) in the field by the observer. Drost scores I and II represent males with black head and back, but in group I, the rump of a male is black, whereas in group II, it is more or less grey. In group VII, males are completely brown in their dorsal coloration (i.e. 0% of black) (for detailed description see Drost, 1936; Lundberg & Alatalo, 1992; Glutz von Blotzheim & Bauer, 1993). The colour scoring of the back of the males was performed only in pied flycatchers (as collared flycatchers are always black).

It is known that age affects plumage traits in flycatchers (Lundberg & Alatalo, 1992; Wiley *et al.*, 2005; Hegyi *et al.*, 2006). Ageing of the birds, however, requires specific experience, and it was not possible to get age data from several of the studied populations. Potential differences in age composition between the populations could therefore affect the differences between them. We, however, consider it unlikely that the differences in age composition would be very large. As it is also possible that correlations between traits could differ between age classes, the correlations between traits (across the breeding range) are presented separately for different age classes for the subset of data that included age information.

Neutral genetic and phenotypic differentiation among populations

Neutral genetic differentiation among pied flycatcher populations has been examined in previous studies using F_{ST} as a measure of genetic differentiation (Lehtonen *et al.*, 2009a). This data set was supplemented with new genetic data for pied flycatcher populations from Oulu, Finland and Courish Spit, Russia. Data for the new populations were carefully calibrated by re-analysing a small number of individuals from the original data set. The molecular genetic methods for the 17 microsatellite markers were analysed, and the methods for calculating F_{ST} are described in detail in Lehtonen *et al.* (2009a).

Adaptive divergence across populations is inferred when the quantitative genetic differentiation metric Q_{ST} exceeds the expected divergence due to genetic drift as estimated by F_{ST} (Spitze, 1993; reviewed in Leinonen *et al.*, 2008). There are, however, several important issues and caveats surrounding the appropriateness of $Q_{ST} - F_{ST}$ comparisons in inferring adaptive divergence (e.g. Chenoweth & Blows, 2008; Miller *et al.*, 2008; Whitlock, 2008). An important factor limiting its use in many organisms is that estimation of Q_{ST} requires rearing organisms in a common environment. In many organisms (especially birds), phenotypic differentiation (P_{ST}) has therefore been used as a proxy for Q_{ST} (Leinonen *et al.*, 2008).

We here estimated P_{ST} across 15 populations for all traits. We defined

$$P_{ST} = \frac{cV_B}{cV_B + 2h^2 V_W} = \frac{\left(\frac{c}{h^2}\right)V_B}{\left(\frac{c}{h^2}\right)V_B + 2V_W}, \quad (1)$$

where V_B is the phenotypic variance between populations and V_W the variance within populations. The scalars c and h^2 are the proportions of between-population and within-population variance caused by additive genetic effects, respectively. The proportion of within-population variance due to additive genetic effects is also referred to as the heritability (h^2) of a trait. These two parameters are explicitly included in eqn (1), because studies differ in the value they (implicitly or explicitly) assume for these parameters and hence differ in their interpretation of how P_{ST} is calculated (Brommer, 2011). The robustness of using P_{ST} as a proxy for Q_{ST} can be inferred on the basis of the lowest value of c/h^2 for which P_{ST} still exceeds F_{ST} (detailed in Brommer, 2011). If we would know the values for c and h^2, then our equation for P_{ST} would estimate Q_{ST}. However, we do not and likely cannot know the value for c. One can then define the critical c/h^2 value as the c/h^2 for which the upper 95% CI of F_{ST} equals to the lower 95% CI of P_{ST} (Brommer, 2011). In case $P_{ST} > F_{ST}$ also for low critical values of c/h^2, then one can be more confident that Q_{ST} will also exceed F_{ST} than if $P_{ST} > F_{ST}$ only for critical $c/h^2 \approx 1$.

Between-population and within-population variances V_B and V_W (eqn 1) were estimated using a linear mixed model implemented in a Bayesian Monte Carlo Markov Chain, using package 'MCMCglmm' (Hadfield, 2010) in R (R Core Team 2013). The models included 'year' as a factorial fixed effect and population as random effect. We included a burn-in of 10 000 iterations. Posteriors were sampled for 100 000 iterations with an interval of 100. Absence of autocorrelation in the posteriors was

confirmed for all traits. Following the specification of MCMCglmm (see Hadfield, 2010), priors for the random effect followed an inverse Wishart distribution. We defined a proper prior for the between-population variance with parameter 'V' (V.between) set at 10% of the phenotypic variance and parameter 'nu' set at 1. The prior for the residual (V.residual) was defined with 'V' at 90% of the phenotypic variance and 'nu' set at 1.

P_{ST} with its 95% credible interval was calculated on the basis of the posteriors and assuming $c = h^2$. As explained above, the robustness of P_{ST} as a proxy for Q_{ST} was evaluated for each trait as the critical c/h^2 value. This value was calculated by solving when the 95% lowest posterior density of P_{ST} (eqn 1) for each trait equalled the 95% upper confidence value for F_{ST}. Details of these calculations are provided by Brommer *et al.* (2014).

Distance to the sympatric area

We measured the geographic distance from each pied flycatcher population to the central and Eastern European breeding range of the collared flycatcher. This was carried out on the basis of the map in the Birds of the Western Palearctic (Cramp & Simmons, 2006), complemented with more local information from the authors. We neglected the populations of the collared flycatcher in the Swedish islands Gotland and Öland in this respect, as the recent expansion of collared flycatchers to these islands (ca. 150 years ago) is of limited area and yet without an apparent effect on pied flycatcher coloration even on the islands themselves (Qvarnström *et al.*, 2009, 2010). In addition, the frequency of the pied flycatchers on these islands has strongly decreased, which limits the potential gene flow from these areas to a very low level. We therefore assume that the occurrence of collared flycatchers, or gene flow from these rather small islands, has not yet affected selection on male pied flycatcher coloration in the surrounding areas.

Statistical analyses

Statistical analyses were conducted with SAS 9.2 and R 3.0.2 software (R Core Team, 2013). A principal component analysis was conducted to examine whether the variation in the five plumage traits of the pied flycatcher could be reduced to common axes of variation (see Results for details). The hypothesis that distance to the sympatric zone explains among-population differences in the phenotypic traits was examined with linear models, in which the trait values were response variables, and geographic distance and neutral genetic differentiation (F_{ST}) from the Czech sympatric zone explanatory variables. F_{ST} was included as a covariate in the model as it could be that a trait changes with distance to one population simply because of genetic

drift. Moran's I was used to examine for spatial autocorrelation in the residuals of the models (proc Variogram in SAS); no autocorrelation was observed. Methods described in Nakagawa & Cuthill (2007) were used to establish the strength of all reported relationships as the standardized effect size (ES) and its noncentral 95% confidence intervals (CI). We note that models run with either population means or with individual values (using population as a random effect in a mixed model) gave qualitatively the same results; thus, the results are presented for the population mean models only. We also rerun the analyses without the UV reflectance measurements from Oslo because the birds were captured earlier (at the mating stage) in this population, which could make a difference if UV reflectance changes due to feather wear. As there were no qualitative differences, we present the results where Oslo UV measurements are included.

Sample sizes

We report data collected from 19 pied flycatcher and two collared flycatcher populations (Table S1). However, the data that were collected varied between populations, and thus, also the samples sizes for the analyses vary between traits. The sizes of all ornamental patches are missing from three populations: Moscow region, Tomsk and Courish spit (Russia), as pictures for standardized measurements were not taken in these populations. Wing patch measurements are missing from Valsain-Lozoya (Spain). Genetic data were not available for the Tomsk population. Exploratory data analyses furthermore indicated that although the two Spanish study populations are physically separated by mountains, the trait values from the two locations were basically the same (Table S1) and the level of genetic differentiation was very low (Lehtonen *et al.*, 2009a). We therefore used only the data from the population with more samples and all traits measured (La Hiruela) in the statistical analyses, to derive conservative statistics for hypothesis testing. The number of populations with all traits measured and with genetic data available was therefore 15. The comparisons of F_{ST} and P_{ST}, and hypothesis testing for the principal component and patch sizes, were conducted on these 15 populations. The hypothesis testing for UV reflection and black-brown dorsal coloration was conducted on seventeen populations.

Results

Correlations between plumage traits in the pied flycatcher

The different plumage traits of the pied flycatcher were correlated (Table 1). In particular, the correlations between black-brown dorsal coloration and the other

Table 1 The relationships between different male plumage traits in the pied flycatcher *Ficedula hypoleuca*. Results are presented for all males pooled over age classes (see Table S2 for the same results in different male age classes). Standardized effect sizes (*r*) are given in the first row and their 95% confidence limits (following Nakagawa & Cuthill, 2007) in parentheses in the second row. Sample size is given in the third row. Low Drost score represents black males and high score brown males.

Variable	UV reflectance	Forehead patch size	Wing patch size	Tail patch size
Drost score	−0.62	−0.50	−0.44	0.45
	(−0.67, −0.57)	(−0.55, −0.45)	(−0.50, −0.39)	(0.40, 0.51)
	1648	1449	1276	1259
UV reflectance		0.32	0.42	−0.35
		(0.26, 0.38)	(0.36, 0.48)	(−0.41, −0.29)
		1165	1072	1034
Forehead patch size			0.36	−0.25
			(0.30, 0.41)	(−0.31, −0.20)
			1253	1236
Wing patch size				−0.11
				(−0.17, −0.050)
				1140

traits were moderate to strong (Table 1). Black pied flycatcher males have higher UV reflectance, larger forehead and wing patches, and smaller tail patches, than brown males (Table 1). The other correlations between the traits were also mainly towards increasing conspicuousness in both traits, with the exception that tail patch size mainly decreased with increasing conspicuousness in the other traits (Table 1). These results do not seem to be confounded by an effect of age, as the same relationships are apparent in both 1-year-old and older males (Table S2).

The moderate to strong relationships between the coloration traits suggested that the joined variation in them could be reduced to fewer variables representing common axes of variation. Principal component analysis (PCA) for all five plumage traits was possible for 879 individuals (from 15 populations). The first principal component (PC1) explained 48.9% of the variance (PC2 only 18.7%). Increasing PC1 represented darker coloration (smaller Drost score, eigenvector −0.54), higher UV reflectance (0.49), larger forehead (0.43) and wing patch (0.43), and smaller tail patch (−0.32). It can therefore be interpreted as increasing showiness/conspicuousness. However, as the first principal component only captured half of the variation in the plumage traits, and it was only possible to calculate it for less than half of the individuals, we also conducted the following analyses separately for each trait. This enabled a more direct and informative interpretation of the results.

Neutral genetic and phenotypic differentiation among populations

The global F_{ST} value was 0.009 (95% CI: 0.0067, 0.0123). The P_{ST} values for the phenotypic traits of the same 15 populations were all 10–40 times higher than F_{ST}: 0.40 (95% CI: 0.23, 0.59; critical $c/h^2 = 0.042$) for

PC1; 0.27 (0.15, 0.47; $c/h^2 = 0.071$) for Drost score; 0.15 (0.075, 0.28; critical $c/h^2 = 0.16$) for UV reflectance; 0.25 (0.15, 0.42; critical $c/h^2 = 0.070$) for forehead patch size; 0.094 (0.045, 0.21; critical $c/h^2 = 0.27$) for wing patch size; and 0.21 (0.097, 0.34; critical $c/h^2 = 0.12$) for tail patch size. This is clear evidence that the differentiation of traits between populations exceeds the neutral expectation.

Plumage coloration in relation to distance from sympatric zone

The pied flycatchers differed clearly from the collared flycatchers in all traits in the Czech sympatric zone (Fig. 2; all pairwise tests $P < 0.0001$). The overall conspicuousness of the average plumage coloration of pied flycatcher males (measured as PC1) increased with distance to the contact zone with the collared flycatcher (Fig. 2; Table 2). The same overall trend could be seen when different traits were examined separately (Fig. 2). The ES, however, varied across traits, and the confidence intervals included zero in other traits but forehead patch size and UV reflectance (Table 2). The pairwise neutral genetic differentiation (F_{ST}) from the Czech sympatric zone was included as a covariate in the above models because it was also significantly associated with differentiation in coloration for most traits (not shown); it was not, however, correlated with geographic distance from the sympatric zone ($r = 0.27$, $P = 0.28$, $N = 17$). We further examined whether latitude or longitude could be underlying the relationship between pied flycatcher coloration and distance to the sympatric zone. Longitude clearly had no association with coloration either alone or in combination with other variables ($P > 0.3$). Latitude had no association with PC1 alone, but when entered in the same model with distance to the sympatric zone, PC1 (conspicuousness) tended to increase towards north. How-
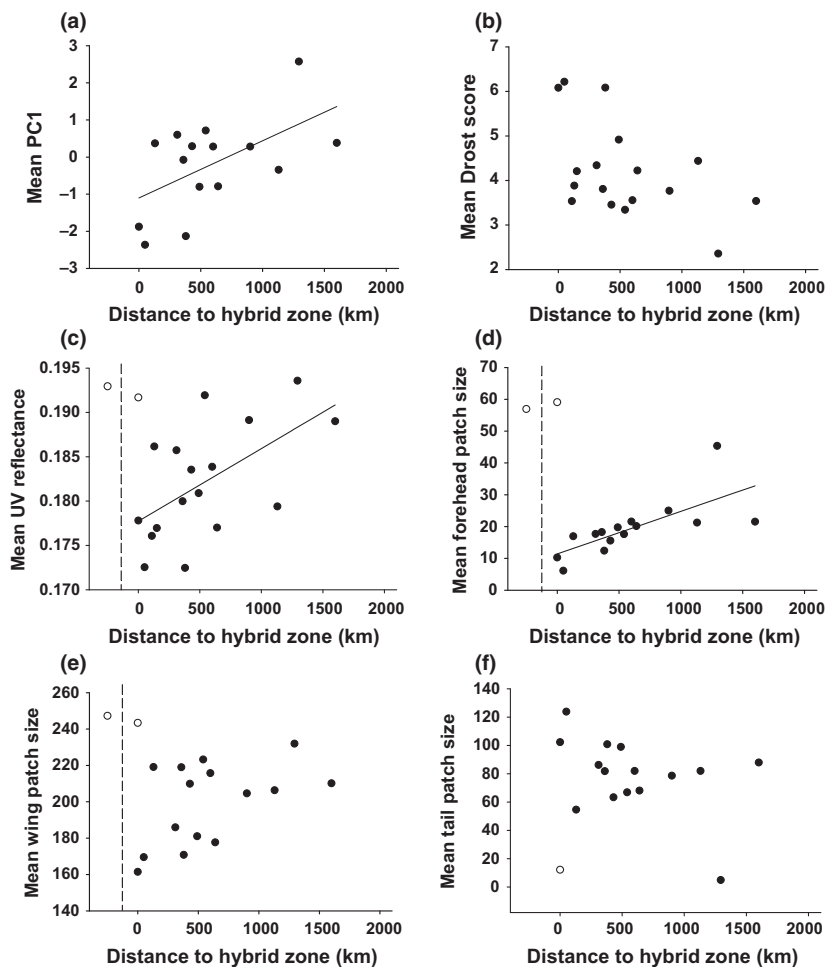
Fig. 2 Plumage traits of pied flycatcher males in relation to distance to the sympatric area with the collared flycatcher. Filled circles are pied flycatcher populations and white circles collared flycatcher populations for comparison. The collared flycatcher population at 0 km is the Czech (Moravia-Jeseníky) population that is situated within the sympatric zone; the Swedish (Gotland) collared flycatcher population is separated by the dashed line. (a) Principal component 1; (b) Drost score (increasing values indicate increasing proportion of brown in the plumage); (c) UV reflectance (proportion of reflectance in the UV wavelengths); (d) forehead patch size (mm^2); (e) wing patch size (mm^2); and (f) tail patch size (mm^2). Regression lines are added for the relationships where confidence intervals do not overlap with zero.

Table 2 The effect of distance to sympatric zone with collared flycatcher on different plumage traits of pied flycatcher males. The statistics are from linear regressions, in which the genetic distance (as measured by F_{ST}) to the sympatric zone population was held as a covariate. ES, standardized effect size (*r*) with 95% confidence intervals (following Nakagawa & Cuthill, 2007). *N*, number of populations; PC1, principal component 1. Low Drost score represents black males and high score brown males.

Response variable	Estimate ± SE	*t*	*P*	ES (95% CI)	*N*
PC1	0.0012 ± 0.00058	2.16	0.05	0.59 (0.02, 1.15)	15
Drost score	−0.00098 ± 0.000528	−1.86	0.08	−0.48 (−1.0, 0.05)	17
UV reflectance	0.0000070 ± 0.0000030	2.41	0.03	0.61 (0.08, 1.13)	17
Forehead patch size	0.011 ± 0.0027	3.95	0.002	0.98 (0.41, 1.54)	15
Wing patch size	0.020 ± 0.0113	1.74	0.11	0.48 (−0.08, 1.05)	15
Tail patch size	−0.015 ± 0.0102	−1.51	0.16	−0.42 (−0.99, 0.14)	15

ever, in this model, distance to sympatric zone was still significant, whereas the latitude effect was only marginally significant (Distance $F_{1,12} = 10.56$, $P = 0.007$; latitude $F_{1,12} = 3.76$, $P = 0.076$).

Most of the pied flycatcher populations differed clearly from the collared flycatchers in patch sizes, but the most conspicuous pied flycatcher populations (in particular Spanish) approached the level of col-

lared flycatchers (Fig. 2; Table S1). The UV reflectance of collared flycatchers was similar to that of pied flycatchers in the populations with highest UV reflectance (Fig. 2; Table S1). The two collared flycatcher populations did not differ from each other in any of the three traits that were measured from both populations (UV reflectance, forehead and wing patch size; Fig. 2; Table S1).

Discussion

Our results show that the several different but correlated plumage traits, and thus the overall conspicuousness of the nuptial plumage of pied flycatcher males, increase with distance to the collared flycatcher contact zone where the two species have diverged in all the traits. The phenotypic differentiation between the populations (P_{ST}) is much higher than that in neutral genetic variation (F_{ST}), and the effect of geographic distance to the sympatric zone remains when controlling for the neutral genetic differentiation. This indicates that evolutionary forces other than genetic drift have played a role in the differentiation. These results provide evidence that the pied flycatcher has diverged from the collared flycatcher in all these plumage traits in the area of secondary contact and that it is likely that the effect of gene flow from this area (Lehtonen *et al.*, 2009a) affects the coloration around the wide distribution of the species. Our results therefore support the hypothesis that character displacement is an important factor behind the variable colour patterns in flycatchers and that they provide compelling evidence of character displacement in the wild (Newton, 2003; Servedio & Noor, 2003). More generally, these results indicate that instead of adaptations to the specific local conditions along an environmental gradient, wide geographic variation in phenotypic traits may reflect a single selective force combined with gene flow. However, they could also indicate a combination of two spatially variable selection pressures if there is concurrent selection for black coloration in pied flycatchers in the areas of allopatry (Sætre *et al.*, 1997).

Neutral genetic vs. phenotypic differentiation

Concluding adaptive divergence based on $P_{ST} - F_{ST}$ comparisons alone has been criticized because environmental differences (and genotype–environment interactions) will inflate the phenotypic divergence across populations (P_{ST}) compared to the quantitative genetic divergence Q_{ST} (Pujol *et al.*, 2008). We found that $P_{ST} > F_{ST}$ even if we allowed for additive genetic effects to determine a much lower proportion of the between-population than within-population variance (i.e. $c < h^2$ in eqn 1). For example, the lower credible interval of phenotypic differentiation in melanin pigmentation (Drost score) and forehead patch still exceeded the upper confidence limit of F_{ST} when c was more than an order of magnitude smaller than h^2. Hence, even when most of the between-population variance would not be due to coding genes, the between-population differentiation in the traits considered here is still larger than expected on the basis of neutral genetic drift alone. In general, we find low (≤ 0.27) critical c/h^2 value for all traits considered, which is relatively low compared to other $P_{ST} - F_{ST}$ studies (Brommer, 2011). Although our evidence for P_{ST} exceeding F_{ST} is robust, it needs to be noted that the inference that local adaptation has caused the strong divergence of these traits remains an indirect assessment. Definitive concluding of adaptive divergence requires demonstrating that the genes coding for these traits have diverged more than expected by genetic drift alone (e.g. Vasemägi & Primmer, 2005). Our conclusion of local adaptation hence remains tentative until the (quantitative) genetic differences in these traits between these populations are resolved. The genetic mechanisms behind the coloration traits in the pied flycatcher have not been identified (Lehtonen *et al.*, 2012), but the high heritability of the dorsal melanin coloration ($h^2 = 0.60–0.88$; reviewed by Lehtonen *et al.*, 2009a), the knowledge on the genetic basis of melanin coloration in other species (Roulin & Ducrest, 2013) and the increasing knowledge on the genomic landscape of the flycatchers (Ellegren *et al.*, 2012) suggest that the mechanisms could soon be found.

Coloration cline with distance to sympatric area

The understanding of the geographic pattern of evolution in the coloration of pied and collared flycatchers has improved based on several intensive ecological and genetic studies in the last two decades (reviewed by Sætre & Sæther, 2010; Qvarnström *et al.*, 2010). The species have diverged during the Pleistocene glaciations (probably less than a million years ago; Backström *et al.*, 2013) in separate refugia in Spain (pied flycatcher) and the Balkans (collared flycatcher). After the last glaciation period that ended ca. 14 000 years ago, the species have spread northwards from their refugia. They came into secondary contact in Central Europe where strong selection for character displacement took place because of asymmetric interspecific competition and reinforcement via maladaptive hybridization. The evidence for these events at the contact zone can be considered substantial (Qvarnström *et al.*, 2010; Sætre & Sæther, 2010). Thereafter, the pied flycatcher has spread its range to the north and east, and now breeds in the northernmost parts of continental Europe and far in the East. The more restricted range of the collared flycatcher may be due to the higher sensitivity of the species to harsh environmental conditions (Qvarnström *et al.*, 2009).

The most recent range expansions of the pied flycatcher, covering more than 1000 km, have apparently occurred during the last two centuries in Scandinavia and even more recently in Siberia (the Tomsk population) (Lundberg & Alatalo, 1992). We suggest that the recent nature of these most distant expansions can still be seen in the coloration of the birds. In particular, the birds in the Tomsk population 2000 km away from the sympatric area are very dull (Table S1), which could be due to a founder effect. It could also be due to differential dispersal, as dispersal propensity has been shown to

be associated with melanin-based coloration in some other species (van den Brink *et al.*, 2012; Roulin, 2013; Saino *et al.*, 2014). A more detailed examination of the coloration pattern and genetic population history in the eastern part of the range would be very interesting. Genetic analyses so far show that although there is some differentiation between the western pied flycatcher populations, the populations in the eastern and northern parts of the breeding range do not exhibit any differentiation at neutral genetic markers (Lehtonen *et al.*, 2009a, 2012; Kuhn *et al.*, 2013). This area of no differentiation nevertheless includes populations that are mainly brown (Czech and eastern populations) or predominantly black (northern populations). Differentiation in phenotype has thus already taken place without differentiation at neutral genetic markers, which indicates that selection and not drift is likely to be the cause of differentiation (Lehtonen *et al.*, 2009a). In the most distinctly separated populations away from the sympatric area (in particular Spain), the pied flycatchers in fact almost resemble the collared flycatcher in some of the traits (as noted for some traits already by Curio, 1960).

It has previously been unclear whether the geographic pattern of variation in pied flycatcher coloration includes an abrupt change or a cline with gradual change in coloration (von Haartman, 1985; Røskaft *et al.*, 1986; Lundberg & Alatalo, 1992). Our results demonstrate the appearance of a gradual change, that is a cline, in the coloration traits. A cline is typically considered to be caused by local adaptation to an environmental gradient in combination with gene flow among the locally adapted populations (Slatkin, 1973; Kirkpatrick & Barton, 1997; Newton, 2003). In the light of the current evidence, the apparent clinal variation in the coloration of the pied flycatcher may in fact be caused by only a single selective force, namely the social environment in the sympatric area, which is affecting coloration also in the allopatric area through gene flow. However, it is also possible that the evolution of dark coloration in allopatric populations is driven by sexual selection processes towards conspicuous coloration (Sætre *et al.*, 1997). According to extensive studies on black-brown dorsal coloration, the evidence for such selection is overall very mixed (reviewed by Lundberg & Alatalo, 1992; Hill, 2006), but may be most apparent in Spain (e.g. Canal *et al.*, 2011; Moreno *et al.*, 2013). It is therefore unclear to what extent sexual selection or other selective forces are currently driving evolution towards darker coloration in allopatry, in particular as the dark phenotype appears to be the ancestral state and the brown phenotype a derived one (Sætre *et al.*, 2003). Therefore, the gradual increase in showiness with distance from sympatric area may also be explained by decreasing gene flow from the sympatric area and context-dependent fluctuating selection that favours different phenotypes in different conditions

(Sirkiä *et al.*, 2010, 2013). It also needs to be noted that there are other selective forces, for example temperature, humidity or predation that could contribute to the geographic variation in the coloration of pied flycatchers. Such ecogeographic patterns have been found particularly in melanin-based coloration in many other species (reviewed by Zink & Remsen, 1986; Newton, 2003; Roulin, 2004). It, however, seems unlikely that such environmental factors could explain the relationship between the colorations of the pied and the collared flycatcher. This is because it would require that the selective environmental factors should have completely opposite colour-associated effects on the two closely related species.

A geographic gradient in a trait does not necessarily mean genetic differences between populations, as phenotypic plasticity would allow the same genotypes to produce different phenotypes if the differences would be induced by the environment (e.g. Merilä *et al.*, 2001; Alho *et al.*, 2010). The flycatchers moult into their breeding plumage at their winter quarters in Africa, and thus, any environmentally induced coloration differences among individuals (and populations) should primarily take place there. We do not know much about the wintering ecology of these birds and even less about how populations may differ in their wintering ecology. However, we consider it highly unlikely for several reasons that the correlations between plumage traits and the remarkable among-population differences in coloration could be caused by environmental factors only. First, the black-brown dorsal coloration is highly heritable in all populations where studied ($h^2 = 0.60$–0.88; reviewed by Lehtonen *et al.*, 2009a). Also the forehead patch size has been shown to be heritable (Potti & Canal, 2011), and it is also highly repeatable across years when measured accurately from photographs ($r > 0.9$, P. Järvistö & T. Laaksonen unpublished). Although both heritability and repeatability could be caused by similar conditions experienced by fathers and sons, or by the same individual in consecutive years, it is unlikely that conditions at winter ranges in Africa would remain stable across years (e.g. Szép, 1995). Furthermore, isotope analyses of feathers moulted on the wintering grounds do not support colour-type segregation at the wintering grounds (T. Laaksonen, M. Klaassen & E. Lehikoinen, unpublished). On these bases, we consider it unlikely that environmentally induced phenotypic plasticity plays any major role in the geographic pattern of pied flycatcher colour variation and that it is more likely that the differences are genetic.

Conclusions

We have shown that several major plumage traits of the pied flycatcher have diverged from the collared flycatcher in the area of sympatry and that they increase

gradually in conspicuousness with distance to the sympatric zone. This suggests a cline maintained by gene flow. These traits are strongly to moderately correlated, suggesting that they may have evolved together. The pied and collared flycatchers are thus an exciting example of plumage patterns diverging into two species that differ in several major phenotypic traits. The divergence in sympatry and (potential) convergence in allopatry in these birds provides a possibility to study the evolution of highly divergent avian plumage patterns, which often involve rapidly evolved large differences between closely related species (Martin *et al.*, 2009). The study of patterns of variation and covariation between different traits in different populations might prove an insightful research avenue, indicating whether some traits are under stronger local selection than others (e.g. Rohwer *et al.*, 2001). They might also indicate which selective process may be in action (Dale, 2006). Finally, understanding the proximate physiological mechanisms behind the determination of the phenotypic traits would help in revealing the conflicting selection pressures, constraints and trade-offs involved in their development (Roulin & Ducrest, 2013).

Acknowledgments

References

Alatalo, R.V., Gustafsson, L. & Lundberg, A. 1994. Male coloration and species recognition in sympatric flycatchers. *Proc. R. Soc. Lond. Biol. Sci.* **256**: 113–118.

Alho, J., Herczeg, G., Söderman, F., Laurila, A., Jönsson, K.I. & Merilä, J. 2010. Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity. *BMC Evol. Biol.* **10**: 317.

Antoniazza, S., Burri, R., Fumagalli, L., Goudet, J. & Roulin, A. 2010. Local adaptation maintains clinal variation in mela-nin-based coloration of European barn owls (*Tyto alba*). *Evolution* **64**: 1944–1954.

Backström, N., Sætre, G.-P. & Ellegren, H. 2013. Inferring the demographic history of European *Ficedula* flycatcher populations. *BMC Evol. Biol.* **13**: 2.

van den Brink, V., Dreiss, A.N. & Roulin, A. 2012. Melanin-based coloration predicts natal dispersal in the barn owl, *Tyto alba*. *Anim. Behav.* **84**: 805–812.

Brommer, J.E. 2011. Whither P_{ST}? The approximation of Q_{ST} by P_{ST} in evolutionary and conservation biology. *J. Evol. Biol.* **24**: 1160–1168.

Brommer, J.E., Hanski, I.K., Kekkonen, J. & Väisänen, R.A. 2014. Size differentiation in Finnish house sparrows follows Bergmann's rule with evidence of local adaptation. *J. Evol. Biol.* **27**: 737–747.

Calhim, S., Adamik, P., Järvistö, P., Leskinen, P., Török, J., Wakamatsu, K. *et al.* 2014. Heterospecific female mimicry in *Ficedula* flycatchers. *J. Evol. Biol.* **27**: 660–666.

Canal, D., Potti, J. & Dávila, J.A. 2011. Male phenotype predicts extra-pair paternity in pied flycatchers. *Behaviour* **148**: 691–712.

Chenoweth, S.F. & Blows, M.W. 2008. Q_{ST} meets the G matrix: the dimensionality of adaptive divergence in multiple correlated quantitative traits. *Evolution* **62**: 1437–1449.

Cramp, S. & Simmons, K.E.L. 2006. *Birds of the Western Palearctic Interactive Version 2.0.1*. BirdGuides Ltd, Oxford University Press, Sheffield, Oxford.

Curio, E. 1960. Die systematische Stellung des spanischen Trauerschnäppers. *Vogelwelt* **81**: 113–121.

Dale, J. 2006. Intraspecific variation in coloration. In: *Bird Coloration: Function and Evolution* (G.E. Hill & K.J. McGraw, eds), pp. 36–86. Harvard University Press, Cambridge, MA.

Drost, R. 1936. Über das Brutkleid männlicher Trauerflie-genfänger, Muscicapa hypoleuca. *Vogelzug* **6**: 179–186.

Ellegren, H., Smeds, L., Burri, R., Olason, P.I., Backström, N., Kawakami, T. *et al.* 2012. The genomic landscape of species divergence in *Ficedula* flycatchers. *Nature* **491**: 756–760.

Endler, J.A. 1977. *Geographic Variation, Speciation, and Clines*. Princeton University Press, Princeton, NJ.

Flint, V.E., Boehme, R.L., Kostin, Y.V. & Kuznetsov, A.A. 1984. *A Field Guide to Birds of the USSR*. Princeton University Press, Princeton, NJ.

Fraser, D.J., Weir, L.K., Bernatchez, L., Hansen, M.M. & Taylor, E.B. 2011. Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity* **106**: 404–420.

Glutz von Blotzheim, U.N. & Bauer, K.M. 1993. *Handbuch der vögel Mitteleuropas*. Aula-Verlag, Wiesbaden.

von Haartman, L. 1985. The biological significance of the nuptial plumage of the male Pied Flycatcher. In: *Acta XVIII Congressus Internationalis Ornithologicus*, Moscow 1982 (V.M. Gavrilov & V.D. Ilyichev, eds), pp. 34–60. Nauka, Moscow.

Hadfield, J.D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**: 1–22.

Hegyi, G., Török, J., Toth, L., Garamszegi, L.Z. & Rosivall, B. 2006. Rapid temporal change in the expression and age-related information content of a sexually selected trait. *J. Evol. Biol.* **19**: 228–238.

Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *Am. Nat.* **173**: 579–588.

Hill, G.E. 2006. Female choice for ornamental coloration. In: *Bird Coloration: Function and Evolution* (G.E. Hill & K.J.

McGraw, eds), pp. 137–200. Harvard University Press, Massachusetts.

Ivankina, E.V., Kerimov, A.B., Grinkov, V.G. & Bushuev, A.V. 2007. Structural and functional aspects of variation of plumage ornamentation in *F. hypoleuca* males. *Zh. Obsch. Biol.* **68**: 278–295.

Kirkpatrick, M. & Barton, N.H. 1997. Evolution of a species' range. *Am. Nat.* **150**: 1–23.

Kuhn, K., Schwenk, K., Both, C., Canal, D., Johansson, U.S., van der Mije, S. *et al.* 2013. Differentiation in neutral genes and a candidate gene in the pied flycatcher: using biological archives to track global climate change. *Ecol. Evol.* **3**: 4799–4814.

Lehtonen, P.K., Laaksonen, T., Artemyev, A.V., Belskii, E., Both, C., Bureš, S. *et al.* 2009a. Geographic patterns of genetic differentiation and plumage colour variation are different in the pied flycatcher (*Ficedula hypoleuca*). *Mol. Ecol.* **18**: 4463–4476.

Lehtonen, P.K., Primmer, C.R. & Laaksonen, T. 2009b. Different traits affect gain of extra-pair paternity and loss of paternity in the pied flycatcher, *Ficedula hypoleuca*. *Anim. Behav.* **77**: 1103–1110.

Lehtonen, P.K., Laaksonen, T., Artemyev, A.V., Belskii, E., Berg, P.R., Both, C. *et al.* 2012. Candidate genes for colour and vision exhibit signals of selection across the pied flycatcher (*Ficedula hypoleuca*) breeding range. *Heredity* **108**: 431–440.

Leinonen, T., O'Hara, R.B., Cano, J.M. & Merilä, J. 2008. Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. *J. Evol. Biol.* **21**: 1–17.

Lundberg, A. & Alatalo, R.V. 1992. *The Pied Flycatcher*. T and AD Poyser, London.

Martin, P.R., Montgomerie, R. & Loughe, S.C., eds. 2009. Rapid sympatry explain greater color pattern divergence in high latitude birds. *Evolution* **64**: 336–347.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.

Menchinsky, K.M. 2006. Relationships between components of plumage coloration in the pied flycatcher (*Ficedula hypoleuca*). *Bull MOIP* **111**: 70–74.

Merilä, J. & Björklund, M. 2004. Phenotypic integration as a constraint and adaptation. In: *Phenotypic Integration – Studying the Ecology and Evolution of Complex Phenotypes* (M. Pigliucci & K. Preston, eds), pp. 107–129. Oxford University Press, London.

Merilä, J., Kruuk, L.E. & Sheldon, B.C. 2001. Cryptic evolution in a wild bird population. *Nature* **412**: 76–79.

Miller, J.R., Hamilton, M.B. & Wood, B.P. 2008. F_{ST} and Q_{ST} under neutrality. *Genetics* **180**: 1023–1037.

Moreno, J., Velando, A., Gonzalez-Braojos, S., Ruiz-de-Castaneda, R. & Cantarero, A. 2013. Females paired with more attractive males show reduced oxidative damage: possible direct benefits of mate choice in pied flycatchers. *Ethology* **119**: 727–737.

Mullen, L.M. & Hoekstra, H.E. 2008. Natural selection along an environmental gradient: a classic cline in mouse pigmentation. *Evolution* **62**: 1555–1569.

Nakagawa, S. & Cuthill, I.C. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev.* **82**: 591–605.

Newton, I. 2003. *The Speciation and Biogeography of Birds*. Academic Press, London.

Potti, J. & Canal, D. 2011. Heritability and genetic correlation between the sexes in a songbird sexual ornament. *Heredity* **106**: 945–954.

Price, T. 2008. *Speciation in Birds*. Roberts and Company Publishers, Greenwood Village, CO.

Pujol, B., Wilson, A.J., Ross, R.I.C. & Pannell, R. 2008. Are Q_{ST}-F_{ST} comparisons for natural populations meaningful? *Mol. Ecol.* **17**: 4782–4785.

Qvarnström, A., Wiley, C., Svedin, N. & Vallin, N. 2009. Life-history divergence facilitates regional coexistence of competing *Ficedula* flycatchers. *Ecology* **90**: 1948–1957.

Qvarnström, A., Rice, A.M. & Ellegren, H. 2010. Speciation in *Ficedula* flycatchers. *Philos. Trans. R. Soc. B Biol. Sci.* **365**: 1841–1852.

R Core Team. 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rohwer, S., Bermingham, E. & Wood, C. 2001. Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. *Evolution* **55**: 405–422.

Røskaft, E., Järvi, T., Nyholm, N.E.I., Virolainen, M., Winkel, W. & Zang, H. 1986. Geographic variation in secondary sexual plumage colour characteristics of the male pied flycatcher. *Ornis Scand.* **17**: 293–298.

Roulin, A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biol. Rev.* **79**: 815–848.

Roulin, A. 2013. Ring recoveries of dead birds confirm that darker pheomelanic Barn Owls disperse longer distances. *J. Ornithol.* **154**: 871–874.

Roulin, A. & Ducrest, A.-L. 2013. Genetics of colouration in birds. *Semin. Cell Dev. Biol.* **24**: 594–608.

Sætre, G.-P. & Sæther, S.-A. 2010. Ecology and genetics of speciation in *Ficedula* flycatchers. *Mol. Ecol.* **19**: 1091–1106.

Sætre, G.-P., Moum, T., Bureš, S., Král, M., Adamjan, M. & Moreno, J. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* **387**: 589–592.

Sætre, G.-P., Borge, T., Lindroos, K., Haavie, J., Sheldon, B.C., Primmer, C.R. *et al.* 2003. Sex chromosome evolution and speciation in *Ficedula* flycatchers. *Proc. R. Soc. Lond. B Biol. Sci.* **270**: 53–59.

Saino, N., Romani, M., Scandolara, C., Rubolini, D., Ambrosini, R., Caprioli, M. *et al.* 2014. Brownish, small and lousy barn swallows have greater dispersal propensity. *Anim. Behav.* **87**: 137–146.

Servedio, M.R. & Noor, M.A.F. 2003. The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Evol. Syst.* **34**: 339–364.

Sirkiä, P.M. & Laaksonen, T. 2009. Distinguishing between male and territory quality: females choose multiple traits in the pied flycatcher. *Anim. Behav.* **78**: 1051–1060.

Sirkiä, P.M., Virolainen, M. & Laaksonen, T. 2010. Melanin coloration has temperature-dependent effects on breeding performance that may maintain phenotypic variation in a passerine bird. *J. Evol. Biol.* **23**: 2385–2396.

Sirkiä, P.M., Virolainen, M., Lehikoinen, E. & Laaksonen, T. 2013. Fluctuating selection and immigration as determinants of the phenotypic composition of a population. *Oecologia* **173**: 305–317.

Slagsvold, T. & Sætre, G.-P. 1991. Evolution of plumage color in male pied flycatchers (*Ficedula hypoleuca*): evidence for female mimicry. *Evolution* **45**: 910–917.

Slatkin, M. 1973. Gene flow and selection in a cline. *Genetics* **75**: 733–756.

Spitze, K. 1993. Population structure in *Daphnia obtusa* – quantitative genetic and allozymic variation. *Genetics* **135**: 367–374.

Szép, T. 1995. Relationship between West-African rainfall and the survival of Central-European sand martins *Riparia riparia*. *Ibis* **137**: 162–168.

Vasemägi, A. & Primmer, C.R. 2005. Challenges for identifying functionally important genetic variation: the promise of combining complementary research strategies. *Mol. Ecol.* **14**: 3623–3642.

Whitlock, M.C. 2008. Evolutionary inference from Q_{ST}. *Mol. Ecol.* **17**: 1885–1889.

Wiley, C., Bengtson, J.M., Svedin, N. & Qvarnström, A. 2005. Hybridization cost of delayed maturation of secondary sexual traits in the collared flycatcher. *Evolution* **59**: 2711–2716.

Zink, R. & Remsen, J. Jr 1986. Evolutionary processes and patterns of geographic variation in birds. *Curr. Ornithol.* **4**: 1–69.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Details of colour measurements.

Table S1 Mean ± standard deviation of the five plumage traits and sample size in each study population.

Table S2 The relationships between different plumage traits in the pied flycatcher *Ficedula hypoleuca* in (a) 1-year-old, and (b) 2-year old or older males.