

Received Date : 20-Oct-2014

Revised Date : 23-Jan-2015

Accepted Date : 06-Feb-2015

Article type : Research Papers

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

Laaksonen, T^{1,2}, Sirkiä, PM¹, Calhim, S^{1,2}, Brommer, JE¹, Leskinen, PK¹, Primmer, CR¹, Adamík, P³, Artemyev, AV⁴, Belskii, E⁵, Both, C⁶, Bureš, S³, Burgess, M⁷, Doligez, B⁹, Forsman, JT¹⁰, Grinkov, V⁸, Hoffmann, U¹¹, Ivankina, E⁸, Král, M¹², Krams, I^{13,14}, Lampe, HM¹⁵, Moreno, J¹⁶, Mägi, M¹⁷, Nord, A¹⁸, Potti, J¹⁹, Ravussin, P-A²⁰, Sokolov, L²¹

¹ Department of Biology, University of Turku, Finland

² Finnish Museum of Natural History, University of Helsinki, Finland

³ Department of Zoology, Palacký University, Olomouc, Czech Republic

⁴ Institute of Biology, Karelian Research Centre, Russian Academy of Science, Petrozavodsk, Russia

⁵ Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Yekaterinburg, Russia

⁶ Centre for Ecological and Evolutionary Studies, University of Groningen, Haren, The Netherlands

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jeb.12604

This article is protected by copyright. All rights reserved.

Accepted Article

⁷ Centre for Research in Animal Behavior, College of Life and Environmental Sciences,
University of Exeter, UK

⁸ Department of Evolutionary Biology, Faculty of Biology, Moscow State University, Russia

⁹ Department of Biometry and Evolutionary Biology, University of Lyon, France

¹⁰ Department of Biology, University of Oulu, Finland

¹¹ Harthausen, D-67376, Germany

¹² Valšův Důl, CZ-783 86 Dlouhá Loučka, Czech Republic

¹³ Institute of Systematic Biology, University of Daugavpils, Latvia

¹⁴ Institute of Ecology and Earth Sciences, University of Tartu, Estonia

¹⁵ Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University
of Oslo, Norway

¹⁶ Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales-CSIC,
Madrid, Spain

¹⁷ Institute of Ecology and Earth Sciences, University of Tartu, Estonia

¹⁸ Department of Biology, Section of Evolutionary Ecology, Lund University, Sweden

¹⁹ Department of Evolutionary Ecology, Estación Biológica de Doñana-CSIC, Sevilla, Spain

²⁰ Rue du Theu 12, CH-1446 Baulmes, Switzerland

²¹ Biological Station of the Zoological Institute, Russian Academy of Science, Rybachy,
Russia

Corresponding author: Toni Laaksonen, Section of Ecology, Department of Biology, FI-
20014 University of Turku, Finland. Email: toni.laaksonen@utu.fi. Tel. +358 2 333 6357

Running title: Divergence and cline in *Ficedula* coloration

Keywords: hybridization, melanin, phenotypic integration, phenotypic variation, reinforcement, sexual selection, spatial variation, speciation

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 *F. albicollis* in the contact zone. The visual 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 together. The flycatchers provide an example of plumage patterns diverging in two species that differ in several aspects of appearance. The divergence in sympatry and convergence in allopatry in these birds provides 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 non-adaptive variation between populations, and the existence of variation as such does not prove selection or adaptation (Zink & Remsen, 1986; Newton, 2003). Moreover, while 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 *F. hypoleuca* has been suggested to evolve through character displacement with the socially dominant collared flycatcher *F. 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 color 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 more resemble the

Accepted Article

females of the collared flycatcher than the females of their own species (Calhim *et al.*, 2014). There is considerable evidence that this heterospecific female mimicry of the brown male color type is beneficial for pied flycatchers in sympatric areas because it reduces harmful social/agonistic interactions (Slagsvold & Sætre, 1991; Alatalo *et al.*, 1994) and hybridisation (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; but see Dale *et al.*, 1999). Understanding the variation and potential divergence in these other traits is important for understanding the evolution of the overall

Accepted Article

coloration patterns and the conflicting selection pressures on them. It is also important to know to what extent different traits are correlated, since 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 two distinctly separate 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 inter-correlations 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 geographical color 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 geographical context. We predicted that if this hypothesis is an important explanation for the distribution-wide coloration pattern of pied

Accepted Article

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

a) 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.

b) Study populations

Data was collected from nineteen study populations across the breeding range of the pied flycatcher and from two populations of the collared flycatcher. The nineteen populations form a considerable coverage over the breeding 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 supplementary table S1. The majority of the data were collected from

males captured at the nest during nestling feeding. Therefore, our data represents the successful breeders in each population.

c) 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 supplementary material.

Measurements from the feathers and pictures were done 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 color scoring of the back of the males was done 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). Aging 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. Since 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.

d) Neutral genetic and phenotypic differentiation among populations

Neutral genetic differentiation among pied flycatcher populations has been examined in previous studies by 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 analyzed, and 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

$$P_{ST} = \frac{cV_B}{cV_B + 2h^2V_W} = \frac{c/h^2 V_B}{c/h^2 V_B + 2V_W},$$

(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 eq. (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- and within-population variances V_B and V_W (eq. (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} (eq. (1)) for each trait equalled the 95% upper confidence value for F_{ST} . Details of these calculations are provided by Brommer *et al.* (2014).

e) 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 done 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, since 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 and 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.

f) 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 since 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 models residuals (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 standardised effect size (ES) and its non-central 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. Since there were no qualitative differences, we present the results where Oslo UV measurements are included.

g) *Sample sizes*

We report data collected from nineteen pied flycatcher and two collared flycatcher populations (supplementary table S1). However, the data that was 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 standardised measurements were not taken in these populations. Wing patch measurements are missing from Valsain-Lozoya (Spain). Genetic data was 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 (supplementary table 1) 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, in order to derive conservative statistics for hypothesis testing. The number of populations with all traits measured and with genetic data available was therefore fifteen. The comparisons of F_{ST} and P_{ST} , and hypothesis testing for the principal component (see below) and patch sizes, were conducted on these fifteen populations. The hypothesis testing for UV reflection and black-brown dorsal coloration was conducted on seventeen populations.

RESULTS

a) 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 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 one-year-old and older males (supplementary 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, since 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.

b) 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 fifteen 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.

c) 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 effect size 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 found relationship between pied flycatcher coloration and distance to the sympatric zone. Longitude clearly had no association with coloration either alone or in

Accepted Article

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. However, 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 collared flycatchers (Fig. 2; supplementary table S1). The UV reflectance of collared flycatchers was similar to that of pied flycatchers in the populations with highest UV reflectance (Fig. 2; supplementary 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; supplementary 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, increases 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 simultaneously 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 color 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- than within-population variance (i.e. $c < h^2$ in eq. (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/h² 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 other species (Roulin and 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. 14000 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 (Sætre & Sæther, 2010; Qvarnström *et al.*, 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 one thousand kilometers, 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 (supplementary table 1), 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 while 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 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

Accepted Article

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; Røskoft *et al.*, 1986; Lundberg & Alatalo, 1992). Our results demonstrate the appearance of a gradual change, i.e. 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 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 and 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 since 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 favors 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

Accepted Article

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 molt 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; but see Dale *et al.*, 1999) and it is also highly repeatable across years when measured accurately from photographs ($r > 0.9$, P. Järvisjö and T. Laaksonen unpublished data). While 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 molted on the wintering grounds do not support color-type

Accepted Article

segregation at the wintering grounds (Laaksonen, T, Klaassen, M and Lehtikoinen, E, unpublished data). On these bases, we consider it unlikely that environmentally induced phenotypic plasticity plays any major role in the geographic pattern of pied flycatcher color 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 co-variation 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).

ACKNOWLEDGEMENTS

We thank Andrey Bushuev, David Canal, Anders Herland, Dieter Hoffmann, Antero Järvinen, Anvar Kerimov, Tuija Koivisto, Mikhail Kozlov, Pipsa Lappalainen, Brigitte Planade, Kirsi Reponen, Johanne Skjerven and Helmut Sternberg, for help at various stages of the study. The study was funded by Emil Aaltonen Foundation (grant to TL), Academy of Finland (grants to TL, JTF and CRP), Ella and Georg Ehrnrooth Foundation and Turku University Foundation (grants to PS), the Spanish Government (grant CGL2009-10652 to JP), Russian Fund of Basic Research (grants 05-04-49173-a and 09-04-00162-a to V. G.), Program for support of Scientific Schools (NSh-2840.2014.4 to E. B.), the Russian Academy of Science (to A.A.), Estonian Science Foundation (grant # 8985), the Estonian Ministry of Education and Science (SF0180004s09) and European Union through the European Regional Development Fund (Center of Excellence FIBIR) (to M. M.).

REFERENCES

- Abramoff, M. D., P. J. Magelhaes, and S. J. Ram. 2004. Image processing with ImageJ. *Biophot. Int.* 11: 36–42.
- Alatalo, R. V., L. Gustafsson, and A. Lundberg. 1994. Male coloration and species recognition in sympatric flycatchers. *Proc. R. Soc. Lond. Biol. Sci.* 256: 113–118.
- Alho, J., G. Herczeg, F. Söderman, A. Laurila, K. I. Jönsson, and J. Merilä. 2010. Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity. *BMC Evol. Biol.* 10: 317.

- Antoniazza, S., R. Burri, L. Fumagalli, J. Goudet, and A. Roulin. 2010. Local adaptation maintains clinal variation in melanin-based coloration of European barn owls (*Tyto alba*). *Evolution* 64: 1944–1954.
- Backström, N., G.-P. Sætre, and H. Ellegren. 2013. Inferring the demographic history of European *Ficedula* flycatcher populations. *BMC Evol. Biol.* 13: 2.
- 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., I. K. Hanski, J. Kekkonen, and R. A. Väisänen. 2014. Size differentiation in Finnish house sparrows follows Bergmann’s rule with evidence of local adaptation. *J. Evol. Biol.* 27: 737–747.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference. A practical information-theoretic approach. Springer-Verlag, New York.
- Calhim, S., P. Adamik, P. Järvistö, P. Leskinen, J. Török, K. Wakamatsu, and T. Laaksonen. 2014. Heterospecific female mimicry in *Ficedula* flycatchers. *J. Evol. Biol.* 27: 660–666.
- Canal, D., J. Potti, and J. A. Dávila. 2011. Male phenotype predicts extra-pair paternity in pied flycatchers. *Behaviour* 148: 691–712.
- Chenoweth, S.F., and M.W. Blows. 2008. Q_{ST} meets the G matrix: The dimensionality of adaptive divergence in multiple correlated quantitative traits. *Evolution* 62: 1437–1449.
- Cramp, S., and K. E. L. Simmons. 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, S., T. Slagsvold, H. M. Lampe, and G.-P. Sætre. 1999. Population divergence in sexual ornaments: The white forehead patch of Norwegian pied flycatchers is small and unsexy. *Evolution* 53: 1235–1246.
- Dale, J. 2006. Intraspecific variation in coloration. Pp. 36–86 in G. E. Hill and K. J. McGraw, eds. *Bird coloration: function and evolution*. Harvard University Press, Massachusetts.
- Drost, R. 1936. Über das Brutkleid männlicher Trauerfliegenfänger, *Muscicapa hypoleuca*. *Vogelzug* 6: 179–186.
- Eaton, M. D., and S. M. Lanyon. 2003. The ubiquity of avian ultraviolet plumage reflectance. *Proc. R. Soc. Lond. Biol. Sci.* 270: 1721–1726.
- Ellegren, H., L. Smeds, R. Burri, P. I. Olason, N. Backström, T. Kawakami, A. Kunstner, H. Mäkinen, K. Nadachowska-Brzyska, A. Qvarnström, S. Uebbing, and J. B. Wolf. 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, New Jersey.
- Flint, V. E., R. L. Boehme, Y. V. Kostin, and A. A. Kuznetsov. 1984. *A field guide to birds of the USSR*, Princeton University Press, Princeton.
- Fraser, D. J., L. K. Weir, L. Bernatchez, M. M. Hansen, and E. B. Taylor. 2011. Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity* 106: 404–420
- Galván, I., and J. Moreno. 2009. Variation in effects of male plumage ornaments: the case of Iberian Pied Flycatchers. *Ibis* 151: 541–546.
- Glutz von Blotzheim, U. N., and K. M. Bauer. 1993. *Handbuch der vögel Mitteleuropas*, Aula-Verlag, Wiesbaden.

- Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Soft.* 33: 1–22.
- Hegyí, G., J., Török, L., Toth, L.Z. Garamszegi, and 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. Pp. 137–200 in G. E. Hill, and K. J. McGraw, eds. *Bird coloration: function and evolution*. Harvard University Press, Massachusetts.
- Ivankina, E. V., A. B. Kerimov, V. G. Grinkov, A. V. Bushuev. 2007. Structural and functional aspects of variation of plumage ornamentation in *F. hypoleuca* males. *Zh. Obsch. Biol.* 68: 278–295.
- Kirkpatrick, M., and N. H. Barton. 1997. Evolution of a species' range. *Am. Nat.* 150: 1–23.
- Kuhn, K., K., Schwenk, C. Both, D. Canal, U. S. Johansson, S. van der Mije, T. Topfer, and M. Päckert. 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., T. Laaksonen, A. V. Artemyev, E. Belskii, P. R. Berg, C. Both, L. Buggiotti, S. Bureš, M. D. Burgess, A. V. Bushuev, I. Krams, J. Moreno, M. Mägi, A. Nord, J. Potti, P.-A. Ravussin, P. M. Sirkkiä, G.-P. Sætre, W. Winkel, C. R. Primmer. 2012. Candidate genes for colour and vision exhibit signals of selection across the pied flycatcher (*Ficedula hypoleuca*) breeding range. *Heredity* 108: 431–440.
- Lehtonen, P. K., T. Laaksonen, A. V. Artemyev, E. Belskii, C. Both, S. Bureš, A. V. Bushuev, I. Krams, J. Moreno, M. Mägi, A. Nord, J. Potti, P. A. Ravussin, P. M.

- Accepted Article
- Sirkkä, G.-P. Sætre, and C. R. Primmer. 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., C. R. Primmer, and T. Laaksonen. 2009b. Different traits affect gain of extra-pair paternity and loss of paternity in the pied flycatcher, *Ficedula hypoleuca*. *Anim. Behav.* 77: 1103–1110.
- Leinonen, T., R. B. O’Hara, J. M. Cano, and J. Merilä. 2008. Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. *J. Evol. Biol.* 21: 1–17.
- Lundberg, A. and R. V. Alatalo. 1992. The pied flycatcher. T and AD Poyser, London.
- Mayr, E. (1942) Systematics and the origin of species, Columbia University Press, New York.
- Martin, P. R., R. Montgomerie, and S. C. Loughheed. 2009. Rapid sympatry explain greater color pattern divergence in high latitude birds. *Evolution* 64: 336–347.
- Menchinsky, K. M. 2006. Relationships between components of plumage coloration in the pied flycatcher (*Ficedula hypoleuca*). *Bull MOIP* 111: 70–74.
- Merilä, J., L. E. Kruuk, and B. C. Sheldon. 2001. Cryptic evolution in a wild bird population. *Nature* 412: 76–79.
- Merilä, J., and M. Björklund. 2004. Phenotypic integration as a constraint and adaptation. Pp. 107–129 in M. Pigliucci, and K. Preston, eds. Phenotypic integration – studying the ecology and evolution of complex phenotypes. Oxford University Press, London.
- Miller, J.R., M. B. Hamilton, and B. P. Wood. 2008. F_{ST} and Q_{ST} under neutrality. *Genetics* 180: 1023–1037.

- Moreno, J., A. Velando, S. Gonzalez-Braojos, R. Ruiz-de-Castaneda, and A. Cantanero. 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., and H. E. Hoekstra. 2008. Natural selection along an environmental gradient: A classic cline in mouse pigmentation. *Evolution* 62: 1555–1569.
- Nakagawa, S., and I. C. Cuthill. 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., and D. Canal. 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, Colorado.
- Pujol, B., A. J. Wilson, R. I. C. Ross, and R. Pannell. 2008. Are Q_{ST} - F_{ST} comparisons for natural populations meaningful? *Mol. Ecol.* 17: 4782–4785.
- Qvarnström, A., A. M. Rice, and H. Ellegren. 2010. Speciation in *Ficedula* flycatchers. *Philos. Trans. R. Soc. B Biol. Sci.* 365: 1841–1852.
- Qvarnström, A., C. Wiley, N. Svedin, and N. Vallin. 2009. Life-history divergence facilitates regional coexistence of competing *Ficedula* flycatchers. *Ecology* 90: 1948–1957.
- Røskaft, E., T. Järvi, N. E. I. Nyholm, M. Virolainen, W. Winkel, and H. Zang. 1986. Geographic variation in secondary sexual plumage colour characteristics of the male pied flycatcher. *Ornis Scand.* 17: 293–298.
- Rohwer, S., E. Bermingham, and C. Wood. 2001. Plumage and mitochondrial DNA haplotype variation across a moving hybrid zone. *Evolution* 55: 405–422.
- 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., and A.-L. Ducrest. 2013. Genetics of colouration in birds. *Semin. Cell Dev. Biol.* 24: 594–608.
- Saino, N., M. Romani, C. Scandolaro, D. Rubolini, R. Ambrosini, M. Caprioli, A. Costanzo, and A. Romano. 2014. Brownish, small and lousy barn swallows have greater dispersal propensity. *Anim. Behav.* 87: 137-146.
- Sætre, G.-P., T. Moum, S. Bureš, M. Král, M. Adamjan, and J. Moreno. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* 387: 589–592.
- Sætre, G.-P., E. Post, and M. Král. 1999. Can environmental fluctuation prevent competitive exclusion in sympatric flycatchers? *Proc. R. Soc. Lond. Biol. Sci.* 266: 1247–1251.
- Sætre, G.-P., T. Borge, T. Moum, C. R. Primmer, B. C. Sheldon, J. Haavie, A. Johnsen, and H. Ellegren. 2001. Speciation, introgressive hybridization and nonlinear rate of molecular evolution in flycatchers. *Mol. Ecol.* 10: 737–749.
- Sætre, G.-P., T. Borge, K. Lindroos, J. Haavie, B. C. Sheldon, C. R. Primmer and A. C. Syvänen. 2003. Sex chromosome evolution and speciation in *Ficedula* flycatchers. *Proc. R. Soc. Lond. B Biol. Sci.* 270: 53–59.
- Sætre, G.-P., and S.-A. Sæther. 2010. Ecology and genetics of speciation in *Ficedula* flycatchers. *Mol. Ecol.* 19: 1091–1106.
- Servedio, M. R., and M. A. F. Noor. 2003. The role of reinforcement in speciation: Theory and data. *Ann. Rev. Ecol. Evol. Syst.* 34: 339–364.
- Sirkkiä, P. M., and T. Laaksonen. 2009. Distinguishing between male and territory quality: females choose multiple traits in the pied flycatcher. *Anim. Behav.* 78: 1051–1060.

- Sirkiä, P. M., M. Virolainen, and T. Laaksonen. 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., M. Virolainen, E. Lehikoinen, and T. Laaksonen. 2013. Fluctuating selection and immigration as determinants of the phenotypic composition of a population. *Oecologia* 173: 305–317
- Slagsvold, T., and G.-P. Sætre. 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.
- van den Brink, V., A. N. Dreiss, and A. Roulin. 2012. Melanin-based coloration predicts natal dispersal in the barn owl, *Tyto alba*. *Anim. Behav.* 84: 805–812.
- von Haartman, L. 1985. The biological significance of the nuptial plumage of the male Pied Flycatcher. Pp. 34–60 in *Acta XVIII Congressus Internationalis Ornithologicus, Moscow 1982, Moscow*.
- Vasemägi, A., and C. R. Primmer. 2005. Challenges for identifying functionally important genetic variation: the promise of combining complementary research strategies. *Mol. Ecol.* 14: 3623–3642.
- Wiley, C., J. M. Bengtson, N. Svedin, and A. Qvarnström. 2005. Hybridization cost of delayed maturation of secondary sexual traits in the collared flycatcher. *Evolution* 59: 2711–2716.
- Whitlock, M.C. 2008. Evolutionary inference from Q_{ST} . *Mol. Ecol.* 17: 1885–1889.

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

FIGURE LEGENDS

Figure 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 and 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.

Figure 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 (mm²); e) wing patch size (mm²); and f) tail patch size (mm²). Regression lines are added for the relationships where confidence intervals do not overlap with zero.

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 supplementary table 2 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 and 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 (-0.67, -0.57) 1648	-0.50 (-0.55, -0.45) 1449	-0.44 (-0.50, -0.39) 1276	0.45 (0.40, 0.51) 1259
UV reflectance		0.32 (0.26, 0.38) 1165	0.42 (0.36, 0.48) 1072	-0.35 (-0.41, -0.29) 1034
Forehead patch size			0.36 (0.30, 0.41) 1253	-0.25 (-0.31, -0.20) 1236
Wing patch size				-0.11 (-0.17, -0.050) 1140

Table 2. The effect of distance to sympatric area 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 and Cuthill 2007). N = Number of populations. PC1 = principal component 1. Low Drost score represents black males and high score brown males.

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

Figure 1

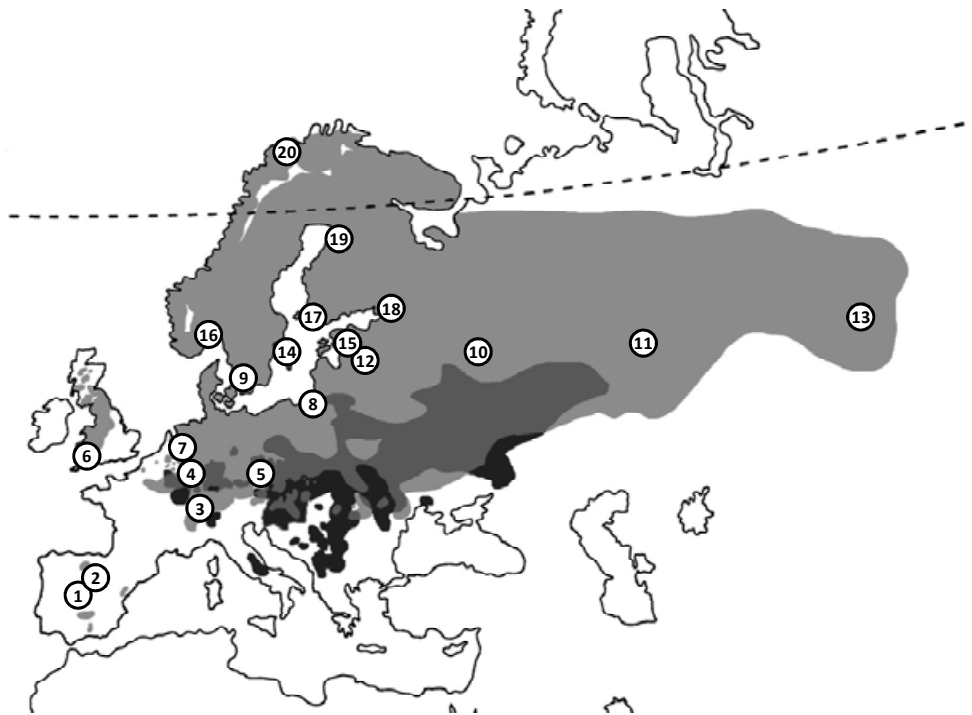


Figure 2

