

Morphological evolution of some migratory *Ficedula* flycatchers

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Abstract

Long-distance migration imposes physiological and morphological selection pressures on birds. The genus *Ficedula*, a lineage of Old World flycatchers, consists of long- and short-distance migratory species, as well as sedentary species. Members of each of these groups are not reciprocally monophyletic, yet each of the behavioral groups is morphologically distinguishable even when accounting for phylogeny. Long-distance migratory species have more pointed wings than either short-distance migratory or sedentary species, and migratory behaviors and wing pointed-ness are phylogenetically correlated. This suggests that migratory *Ficedula* species have converged on a migratory phenotype, and that migration may be a selective agent that has shaped the independently-derived migratory *Ficedula* species in similar ways.

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Introduction

Many studies over the last century have demonstrated consistent patterns across avian taxa regarding the relationship between wing length and migration distance (Baldwin *et al.*, 2010; Förschler and Bairlein, 2011; Marchetti *et al.*, 1995; reviewed in Mönkkönen, 1995). Generally, long-distance migratory species have longer wings per unit body size than short-distance migratory and sedentary species (Winkler and Leisler, 1992). Closely related bird species are often phenotypically similar, but more distantly related species can converge

on a particular morphology if they are subject to similar selection pressures, as has been shown among species in the genus *Anthus* with regard to both migratory and mating-display behaviors (*e.g.* Voelker, 2001). Comparative methods (Felsenstein, 1985; Harvey and Nee, 1997) can be used for such questions about convergent evolution, as they are here, to investigate the evolution of migratory behavior and associated phenotypic change (see also Richman and Price, 1992; Richman, 1996).

Studies of the evolution of migration increasingly suggest that long-distance migration has independently evolved multiple times within many avian lineages (Outlaw *et al.*, 2003; Outlaw and Voelker, 2006a, 2008; Boyle and Conway, 2007; Kondo and Omland, 2007; Rheindt *et al.*, 2008; Voelker *et al.*, 2009). Migratory behavior imposes a suite of selection pressures on birds (see Bowlin and Wikelski, 2008) that fly long distances, and this leads to the prediction that migratory birds, both within and across lineages, would have wing shapes, and perhaps overall body shapes, that are optimized for efficient long-distance flight.

Previously, Outlaw and Voelker (2006b) presented a molecular phylogeny of *Ficedula*, and in a separate paper, reconstructed the evolution of migratory behaviors within the genus (Outlaw and Voelker, 2008). Behavioral diversity within *Ficedula* flycatchers provides a study system to test hypotheses about convergent morphological evolution among independently-derived long-distance migratory species.

Material and methods

Ficedula species can be classified according to degrees of migratory behavior (Cramp, 1992; Ali and Ripley, 1996; Coates and Bishop, 1997; Grimmett *et al.*, 1999; Robson, 2000). Each species was assigned to one of the following categories: long-distance migrant,

Table 1. *Ficedula* species, behavioral assignments, migration distances, and geographic ranges. LDM: long-distance migratory species; S: sedentary species; SDM: short-distance migratory species. Migration distance is reported in degrees to the nearest 10°. Geographic range includes both breeding and wintering ranges for LDM species. Species are listed as in Fig. 2.

<i>Ficedula</i> species	Behavior	Migration distance	Geographic range
<i>nigrorufa</i>	S	0°	India
<i>hodgsonii</i>	SDM	10°	South and Southeast Asia
<i>dumetoria</i>	S	0°	Southeast Asia
<i>narcissina</i>	LDM	45°	Eastern Palearctic, South and Southeast Asia
<i>zanthopygia</i>	LDM	45°	Eastern Palearctic, South and Southeast Asia
<i>mugimaki</i>	LDM	40°	Eastern Palearctic, South and Southeast Asia
<i>westermanni</i>	SDM	0°	South and Southeast Asia
<i>superciliaris</i>	SDM	15°	South Asia
<i>strophciata</i>	SDM	0°	South Asia
<i>hyperythra</i>	SDM	0°	South and Southeast Asia
<i>albicollis</i>	LDM	60°	Western Palearctic, Africa
<i>hypoleuca</i>	LDM	60°	Western Palearctic, Africa
<i>parva/albicilla</i>	LDM	35°	Eastern Palearctic, South and Southeast Asia

short-distance, altitudinal or partial migrant (collectively hereafter: short-distance migrant), and sedentary (Table 1).

Morphological measurements

Specimens were selected for measurement from collections at the American Museum of Natural History, the British Museum of Natural History, the Delaware Museum of Natural History, and the Museum of Comparative Zoology (Harvard University). Many *Ficedula* species have poor representation within museums, and this limited the number of species included within this study. For each of 13 *Ficedula* species (Table 1), between 12 and 16 adult male specimens were measured from one geographic location (within a species) to minimize additional error associated with intraspecific variation. Seven morphological characters were selected including longest primary (usually the eighth) and first secondary lengths, tail length, bill length, width and depth, and tarsus length (Table 2). All were measured with digital calipers to the nearest 0.01 mm using standard measurement protocols (Proctor and Lynch, 1993).

Phylogeny

The 13 species measured for morphological characters are a subset of the species forming a monophyletic *Ficedula* (26 species; Outlaw and Voelker, 2006b). I pruned the topology to include only the 13 species for which morphological data were available, and calcu-

lated maximum likelihood branch lengths using the appropriate model of nucleotide substitution (GTR+I+ Γ ; from Outlaw and Voelker, 2006b). This tree was used in phylogeny-based analyses in this study.

Analyses of morphology

Body size correction in closely-related birds can be problematic because body weight varies tremendously throughout the annual cycle of birds, often regardless of whether birds migrate or not. Moreover, wing and tail measurements are sometimes not indicative of size (Rising and Somers, 1989). Another problem here is that reliable body weight data are not available for many *Ficedula* species. In order to deal with these problems, I log-transformed all data prior to analysis, and interpreted ordination results while considering differences in body size between behavioral groups, *i.e.* most traits load positively on the first factors indicating size differences (Price, 1991).

Analyses of Phenotypes. Discriminant function analyses (DFA) determine whether groups (*e.g.* LDM, SDM, sedentary) are diagnosable, and were conducted with both species and behavior as the grouping variables. Individual characters were included in analyses of variance (ANOVA); significant ANOVAs were followed by post-hoc tests between all behavioral groups using Dunnett's T3 for non-homogeneity of variance. The first and second principal component scores (from a covariance-based PCA) were included in an ANOVA following the preceding procedure (by behavioral groups; see Voss and Marcus, 1992). All

Table 2. Log-transformed means (M), standard deviations (SD) by species for each character and wing pointed-ness (WP). Number of specimens measured is listed after each species name. Species are listed as in Fig. 2.

Species		Longest primary	First secondary	Bill length	Bill depth	Bill width	Tail length	Tarsus length	WP
<i>nigrorufa</i> (12)	M	3.639	3.253	1.965	1.204	1.421	3.893	2.941	1.47
	SD	0.085	0.061	0.048	0.068	0.058	0.043	0.064	
<i>hodgsonii</i> (15)	M	3.891	3.362	1.826	1.106	1.180	3.988	2.721	1.70
	SD	0.039	0.078	0.055	0.064	0.074	0.047	0.064	
<i>dumetoria</i> (13)	M	3.627	3.321	2.128	1.371	1.503	3.827	2.868	1.35
	SD	0.069	0.073	0.044	0.061	0.056	0.048	0.077	
<i>narcissina</i> (16)	M	3.976	3.403	2.040	1.307	1.424	3.922	2.788	1.78
	SD	0.023	0.040	0.045	0.059	0.066	0.037	0.026	
<i>zanthopygia</i> (16)	M	3.881	3.338	2.032	1.338	1.445	3.793	2.800	1.73
	SD	0.038	0.059	0.048	0.067	0.060	0.082	0.031	
<i>mugimaki</i> (15)	M	3.904	3.365	1.849	1.087	1.233	3.928	2.732	1.72
	SD	0.027	0.119	0.061	0.043	0.065	0.047	0.037	
<i>westermanni</i> (15)	M	3.621	3.193	1.874	1.071	1.284	3.704	2.730	1.54
	SD	0.035	0.051	0.039	0.060	0.069	0.075	0.037	
<i>superciliaris</i> (15)	M	3.741	3.240	1.931	1.080	1.312	3.759	2.708	1.66
	SD	0.024	0.059	0.044	0.053	0.072	0.068	0.034	
<i>strophata</i> (15)	M	3.852	3.379	1.863	1.107	1.242	4.070	2.922	1.61
	SD	0.039	0.065	0.042	0.063	0.069	0.062	0.037	
<i>hyperthya</i> (15)	M	3.704	3.283	1.895	1.107	1.278	3.811	2.870	1.53
	SD	0.053	0.081	0.073	0.058	0.060	0.093	0.032	
<i>albicollis</i> (15)	M	4.068	3.435	1.899	1.183	1.386	3.897	2.817	1.89
	SD	0.026	0.047	0.046	0.060	0.086	0.042	0.036	
<i>hypoleuca</i> (16)	M	4.007	3.385	1.899	1.190	1.384	3.956	2.811	1.87
	SD	0.035	0.112	0.047	0.078	0.066	0.039	0.058	
<i>parva/albicilla</i> (15)	M	3.865	3.301	1.932	1.112	1.303	3.925	2.822	1.76
	SD	0.048	0.076	0.042	0.051	0.074	0.036	0.041	
Total (193)	M	3.836	3.329	1.931	1.173	1.337	3.883	2.807	
	SD	0.148	0.099	0.095	0.115	0.113	0.113	0.084	

statistical analyses were conducted in SPSS15.

Phylogenetic component of morphological similarity. To assess whether closely-related species are morphologically similar to one another due to shared ancestry, I calculated morphological Euclidean and uncorrected genetic distances. I then performed a one-tailed Mantel test on these two dissimilarity matrices with 100,000 permutations (XLSTAT 2008).

Phylogenetic relationship between behavior and morphology. Maximum likelihood based analyses of character evolution provide a way to evaluate whether characters are phylogenetically correlated (Discrete Program; Pagel, 1999) using likelihood ratio tests. I included migratory behavior, coded as migratory or non-migratory (1, 0, respectively), and three characters (separately): Principal Component 1, coded as above or below the averaged PC1 value (-0.03) for all species (1, 0, respectively); Principal Component 2, coded as above or below the averaged PC2 value (0.0095) for all species (1, 0, respectively); and a wing-pointed-ness

index (longest primary length/first secondary length, hereafter referred to as WP), coded as above or below the mean index value (1.14) for all species (1, 0, respectively). I coded characters in this way because these analyses require discrete, binary traits. I then conducted (MCMC) simulations to assign probabilities to character evolution likelihood ratio tests (see Outlaw and Voelker, 2006a). Finally, I used parsimony to reconstruct the evolution of WP (Mesquite Program; Maddison and Maddison, 2010).

Results

Overall, jackknifed DFA (Fig. 1a) was able to correctly classify 84% of individuals to species. In these analyses, ninth primary length was the most important discriminating character, followed by tarsus length and tail length. With regard to behavioral groups, jackknifed DFA correctly classified 87% of individuals to

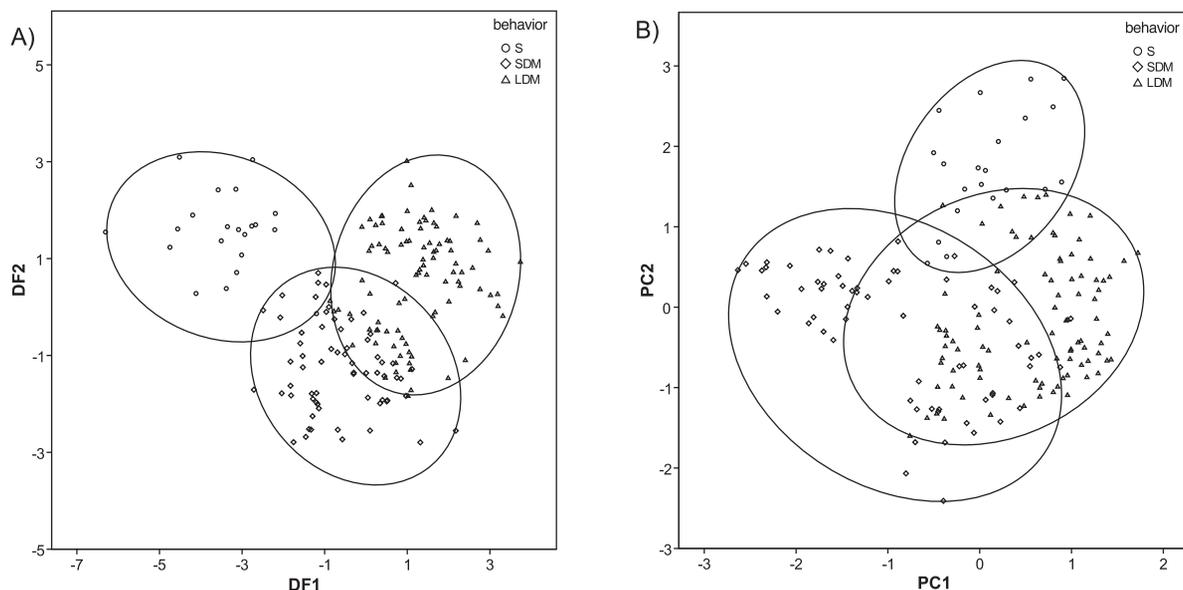


Fig. 1. Scatterplots of Discriminant Function (A) and Principal Component (B) scores for behavioral groups. S: sedentary, SDM: short-distance migratory, LDM: long-distance migratory.

group; incorrectly classified individuals fell between the SDM and LDM groups, but not between either of the latter and the S group; in these analyses, longest primary length was overwhelmingly the most important discriminating character.

ANOVAs on individual characters were significant across behavioral groups ($p < 0.001$ in all cases). Post-hoc tests revealed that behavioral groups were different in longest primary, and the three bill measurements ($p < 0.01$ in all cases). For first secondary and tail lengths, sedentary and LDM species are significantly different ($p < 0.01$), although sedentary and SDM species are not. In tarsus length, SDM and LDM groups are not significantly different, although sedentary species are different from both migrant groups ($p < 0.01$ in both cases).

In PCA (Fig. 1b), high positive loadings of wing and tail characters on the first component suggest that 37% of the variance between individuals is associated with differences in these characters (Table 3). The second component, 33% of the variance, has high positive loadings for the three bill characters. An ANOVA on Principal Component one (by behavior) indicated a linear increase from sedentary to short-distance to migratory species, which primarily reflects increasing size while Principal Component two, which suggests that wing and tail lengths are inversely related to bill traits, indicates the reverse trend (results not shown); the two components are significantly different ($p < 0.001$, overall and post-hoc) between groups.

A Mantel test of morphological and genetic distances suggests that the matrices are positively correlated

	Principal components		Character correlations	
	1	2	1	2
Longest primary	0.939	0.032	0.697	-0.562
First secondary	0.781	0.214	0.719	-0.341
Bill length	-0.169	0.869	0.367	0.780
Bill depth	0.127	0.896	0.611	0.616
Bill width	-0.085	0.876	0.410	0.729
Tail length	0.727	-0.161	0.539	-0.553
Tarsus length	-0.019	0.339	0.298	0.345
Variance explained	37.65%	33.41%		

Table 3. Rescaled principal component loadings for each character, correlations of each component with each character, and variance explained by each component. Principal components are based on covariance. All correlations are significant ($p < 0.01$).

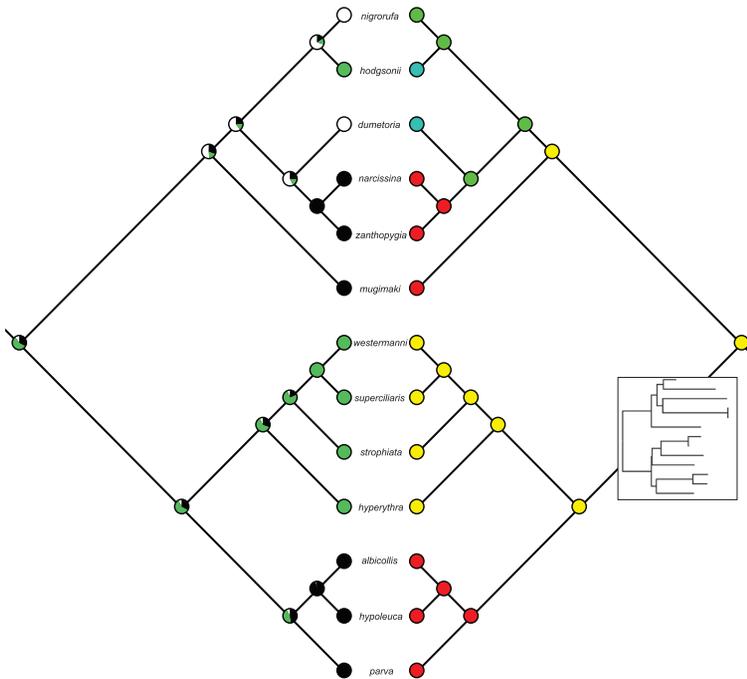


Fig. 2. Molecular phylogeny of *Ficedula* species included in this study (data from Outlaw and Voelker, 2006b). Circles at tips reflect character states of extant taxa and pie charts at nodes reflect ancestral character states. Left, migratory behaviors: white = S, black = LDM, green = SDM. Right, Wing pointed-ness from smallest to largest: green, blue, yellow, red. Note *F. parva* refers to *F. parva* and *F. albicilla*. Inset: phylogram with species ordered as in larger figure.

and that 36.5% of morphological variation can be explained by phylogenetic relatedness ($p < 0.001$).

Likelihood ratio tests of migratory behavior and morphology (above/below averaged PC1 mean; Table 3) as independent or dependent (phylogenetic correlation) indicate a significant difference: $\Delta\log\text{-likelihood} = 3.61, p < 0.001$ (simulation: 100 iterations). Likewise, there is a significant phylogenetic correlation for behavior and WP: $\Delta\log\text{-likelihood} = 5.47, p < 0.001$ (simulation: 100 iterations), but not for behavior and PC2: $\Delta\log\text{-likelihood} = 0.69$.

Discussion

Long-distance migratory species within the genus *Ficedula* exhibit morphological similarities, which suggest that migratory behaviors produce an “optimal” migratory phenotype. Although a proportion of variation among *Ficedula* species can be attributed to phylogenetic relatedness (36.5%), the results here suggest that sedentary, SDM and LDM *Ficedula* species form distinct morphological groups. One factor distinguishing these groups appears to be size; LDM species are larger than SDM, and SDM species are larger than sedentary species. However, WP accounts for size (being a ratio) and it is clear that LDM species have more pointed wings than SDM species, and SDM species more than sedentary species. The two species with the lowest

WP (*nigrorufa* and *dumetoria*) have the most restricted geographic ranges of species included here. Moreover, because some species within each behavioral group are independently-derived, part of the distinction between groups is due to non-phylogenetic causes, *i.e.* selection. The wing shape of LDM species can be construed as a reflection of their adaptation to increased flight efficiency. At the same time, SDM species primarily represent a middle-ground between LDM and sedentary species. Bill shape is highly variable between species, but so little is known about the diets and foraging habits of many Southeast Asian species, that I cannot speculate about selection on bill characters.

This study also shows that LDM behavior can predict morphology. When ancestral states shift from sedentary to migratory, the descendants are more likely to exhibit a particular phenotype (*i.e.*, are larger with more pointed wings; Fig. 2). The results are not unexpected because morphological differences have been and are consistently noted between sedentary and migratory lineages (at multiple taxonomic levels; see also Dawideit *et al.*, 2008), and a recent study highlights the biomechanical underpinnings to explain those differences (Bowlin and Wikelski, 2008).

A notable result from this study is that LDM *Ficedula* species, in very different breeding and wintering habitats, exhibit morphological similarities that have evolved repeatedly. This study suggests that convergent morphological evolution occurs in response to

the physical demands of migratory behaviors, and provides a framework for more detailed analyses within other lineages of birds.

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