

Flycatcher song in allopatry and sympatry – convergence, divergence and reinforcement

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Abstract

The theory of reinforcement predicts that natural selection against the production of unfit hybrids favours traits that increase assortative mating. Whether culturally inherited traits, such as bird song, can increase assortative mating by reinforcement is largely unknown. We compared songs of pied (*Ficedula hypoleuca*) and collared flycatchers (*F. albicollis*) from two hybrid zones of different ages with songs from allopatric populations. Previously, a character divergence in male plumage traits has been shown to reinforce premating isolation in sympatric flycatchers. In contrast, we find that the song of the pied flycatcher has converged towards that of the collared flycatcher (mixed singing). However, a corresponding divergence in the collared flycatcher shows that the species differences in song characters are maintained in sympatry. Genetic analyses suggest that mixed song is not caused by introgression from the collared flycatcher, but rather due to heterospecific copying. Circumstantial evidence suggests that mixed song may increase the rate of maladaptive hybridization. In the oldest hybrid zone where reinforcement on plumage traits is most pronounced, the frequency of mixed singing and hybridization is also lowest. Thus, we suggest that reinforcement has reduced the frequency of mixed singing in the pied flycatcher and caused a divergence in the song of the collared flycatcher. Whether a culturally inherited trait promotes or opposes speciation in sympatry may depend on its plasticity. The degree of plasticity may be genetically determined and accordingly under selection by reinforcement.

Introduction

When two populations come into secondary contact they may have diverged to such an extent that they do not interbreed (prezygotic barrier) or their hybrid offspring do not survive or reproduce (post-zygotic barrier). If pre- and post-zygotic barriers are incomplete, genes from each

population will mix (introgression) and over time the populations will blend. However, if the hybrids have low fitness, natural selection will favour traits that increase assortative mating, thereby reducing the frequency of maladaptive hybridization (Dobzhansky, 1937; Dobzhansky, 1940). This process, known as reinforcement, will cause signals used in mate recognition to diverge, thereby reinforcing prezygotic barriers (Sætre *et al.*, 1997b) eventually completing speciation in sympatry. Thus, depending on the fitness of the hybrid, prezygotic barriers will either increase or decrease (Parsons *et al.*, 1993; Sætre *et al.*, 1997b; de Kort *et al.*, 2002).

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Prezygotic barriers are generally assumed to be genetically determined (Dobzhansky, 1937). However, premating barriers may develop for nongenetical reasons (Grant & Grant, 1997a). Song in oscine birds is usually learned through an imprinting-like process (Kroodma, 1982; Catchpole & Slater, 1995). This property may cause song to change rapidly over time and vary geographically (for references see Catchpole & Slater, 1995). It has been suggested that such culturally inherited traits can increase assortative mating and possibly be the starting point for speciation in allopatry (Marler & Tamura, 1962; Baker & Mewaldt, 1978). However, when two closely related species come into secondary contact males may come to copy songs of the wrong model (Helb *et al.*, 1985). Consequently, heterospecific copying may lead to hybridization (Alatalo *et al.*, 1990; Eriksson, 1991; Grant & Grant, 1997c). Whether reinforcement acts on culturally inherited traits to increase assortative mating is largely unknown (Grant & Grant, 1997b). Thus, it is unclear whether culturally inherited traits promote or oppose speciation and the role of song in speciation remains controversial (Raikow, 1986; Baptista & Trail, 1992; Salomon & Hemim, 1992; Grant & Grant, 1997a; Irwin & Price, 1999; Irwin *et al.*, 2001; Slabbekoorn & Smith, 2002). By comparing traits of populations where their distribution overlaps (sympatry) with populations where they live separately (allopatry) we can examine the effects of coexistence on traits used in species recognition. In this way it may be possible to study whether culturally inherited traits promote or oppose speciation.

The pied flycatcher (*Ficedula hypoleuca*, Pall.) and the collared flycatcher (*F. albicollis*, Temm.) have overlapping breeding distributions in Central and Eastern Europe and on the Baltic islands of Gotland and Öland off the Swedish east coast (hereby referred to as the Swedish population) (Fig. 1). They are closely related species with genetic distance estimated at approximately 3% at mitochondrial DNA (Sætre *et al.*, 2001). Also, they are ecologically very similar as they compete over nest sites and territories in sympatry and affect each other's population dynamics (Sætre *et al.*, 1999b). The frequency of hybridization has been estimated at 2–7% in different sympatric populations (Alatalo *et al.*, 1982; Sætre *et al.*, 1999a; Veen *et al.*, 2001), and hybrids have greatly reduced fitness, consistent with Haldane's rule (Alatalo *et al.*, 1982; Sætre *et al.*, 1997b, 1999a, 2003). The pied and the collared flycatcher have a large overlapping breeding distribution (Fig. 1). However, hybridization occurs in a rather narrow zone corresponding to topography and habitat (Alerstam *et al.*, 1978; Sætre *et al.*, 1999a). Females of the two species are similar to the extent that males of both species are apparently unable to distinguish between them (Sætre *et al.*, 1997a). However, female mate choice is not random (Alatalo *et al.*, 1982; Sætre *et al.*, 1997b) and character displacement on male plumage colour has been found to reinforce premating

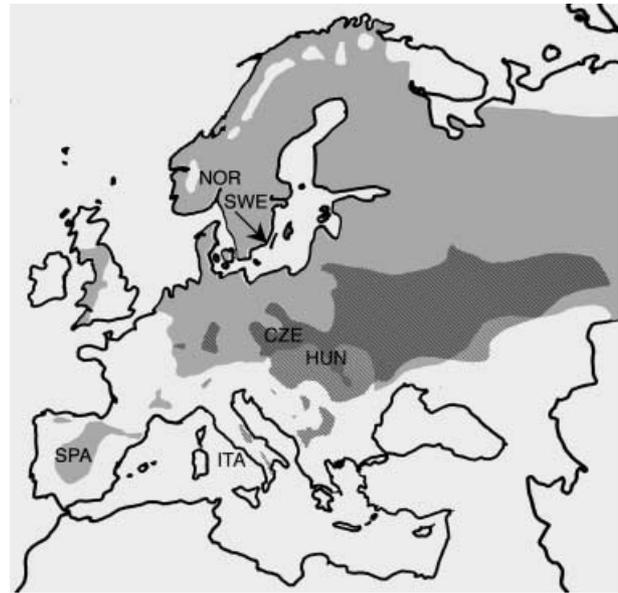


Fig. 1 The European distribution of the pied flycatcher (grey area) and collared flycatcher (hatched area). Song recordings are of allopatric pied flycatchers from Spain near Madrid (SPA) and Norway near Oslo (NOR), collared flycatchers from Italy in Abruzzo National Park (ITA) and Hungary near Budapest (HUN). Sympatric populations of pied and collared flycatchers were from the Baltic island of Öland, Sweden (SWE) (referred to as the Swedish populations) and the Czech Republic in the Jeseník mountains (CZE).

isolation in a Central European hybrid zone (Sætre *et al.*, 1997b). The hybrid zone in Central Europe is assumed to be of secondary contact after the Pleistocene glaciations (von Haartman, 1949; Sætre *et al.*, 2001) whereas the Swedish hybrid zone is apparently of more recent origin, possibly only about 150 years old (Alatalo *et al.*, 1990). Interestingly, the species differences in male plumage characters are more pronounced and the frequency of hybridization is lower in the Central European than in the Swedish hybrid zone (Alatalo *et al.*, 1994; Sætre *et al.*, 1999a, 2003). As plumage traits are used in species recognition, this suggests that the prezygotic barriers have evolved further in the Central European than in the Swedish hybrid zone (Sætre *et al.*, 1999a).

The songs of the pied and collared flycatcher are complex, repertoire sizes typically ranging from a few up to 100 unique song syllables (Lundberg & Alatalo, 1992), but easily distinguishable in the field mainly through difference in frequency and tempo (Gelster, 1987). In flycatchers, song has been shown to function in territory defence (Eriksson, 1991) and mate attraction (Eriksson, 1986). Females have been shown to base their mate choice on repertoire size (Eriksson, 1991; Lampe & Sætre, 1995) and song activity and complexity has been found to correlate with the health status and condition of the male (Garamszegi *et al.*, 2003; Lampe & Espmark, 2003).

Both character convergence and divergence have been suggested to occur in the songs of flycatchers. In sympatric populations some pied flycatchers sing a song resembling that of the collared flycatcher (Tischler, 1942; Löhrl, 1955; Gelter, 1987; Eriksson, 1991), a phenomenon known as mixed song (Eriksson, 1991). This has been suggested to be an example of character convergence in the pied flycatcher (Eriksson, 1991). However, Eriksson was not able to study character changes in the song of the collared flycatcher as no allopatric collared flycatcher population was used in the comparisons. By contrast Wallin (1985) argued for a divergent character displacement among sympatric collared flycatchers. However, the comparison was flawed. It was based on a very limited sample size; allopatric collared flycatchers represented with only one individual which has later been shown to belong to a distinct species – the semicollared flycatcher (*F. semitorquata*) (Sætre *et al.*, 1997b, 2001). To clarify the issues of possible character convergence and divergence in the song in the *Ficedula* flycatchers, we conducted a large-scale comparison of song characteristics using four allopatric and two sympatric populations. Also, we compared two hybrid zones with different population structures and assumed age of contact (Sætre *et al.*, 2003). Based on previous evidence on plumage colour evolution (see above) we predicted that adaptive changes in song are likely to be more pronounced in the Central European compared with the island hybrid zones. Finally, we applied genetic analysis to assess whether gene flow across the partial species barriers could affect the song characters in the sympatric populations.

Materials and methods

Song recordings were obtained during April–June 1999–2002. Allopatric pied flycatchers were from Spain (41°04'N, 3°27'W) and Norway (59°55'N, 10°46'E);

allopatric collared flycatchers were from Italy (41°49'N, 13°47'E) and Hungary (47°43'N, 19°01'E) (we classified the Hungarian population as allopatric, but note that the pied flycatcher is an occasional, rare breeding bird in this area). Sympatric populations were from Sweden (57°10'N, 16°58'E) and the Czech Republic (49°49'N, 17°10'E) (see Fig. 1 and Table 1 for further details). Species were identified by a combination of DNA markers, plumage characters and species-specific alarm calls (data not shown). Hybrids were excluded from the song analyses.

The songs were recorded with Sony DAT recorders (TCD-D7 and TCD-D10), a minidisk recorder (Kenwood DMC-J7R) or a Sony Tape recorder (TC-D5 PRO II). For recording we used a mono (Classic), a stereo (Science and PRO II) and a shotgun microphone mounted on a 55 cm parabolic dish (all products by Telinga, Tobo, Sweden). The songs were analysed in Avisoft-SASLab Pro (version 3.93, R. Specht) (Avisoft Bioacoustics, Berlin, Germany) using automatic parameter measurement with standard settings. To increase sensitivity, certain parameters were manually adjusted (automatic threshold relative to maximum set between –25 and –20 dB). Syllables were defined as continuous elements or groups of elements separated by noticeable time intervals on the sonograms (hold time set between 0.01 and 0.02 s). We separated songs by a minimum interval of 1 s. For each syllable in the song, start and end time, as well as maximum and minimum frequency were measured. From these basic measurements new variables were calculated. These were: song duration and duration of each syllable (to nearest 0.001 s), number of syllables per song, lowest frequency, highest frequency and frequency range (to nearest 1 Hz) and song tempo (calculated as the average number of syllables per second). Incomplete songs (consisting of less than three syllables) and enticement calls (songs with more than 20 syllables) were excluded from the analyses. A male flycatcher switches

Table 1 Mean values and standard deviations for the seven song variables of the pied and collared flycatcher in sympatric and allopatric populations. No. of songs refers to the average number of songs analysed per individual.

Species	Collared flycatcher								Pied flycatcher							
	Allopatry				Sympatry				Allopatry				Sympatry			
	Italy		Hungary		Sweden		Czech R		Norway		Spain		Sweden		Czech R	
Populations variable	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD
Song duration	2.75	0.65	3.18	0.46	3.00	0.63	2.96	0.73	1.86	0.29	1.88	0.38	1.98	0.32	2.14	0.34
No. of syllables	9.39	2.47	11.19	1.79	9.39	2.12	9.82	2.74	9.88	1.60	6.70	1.23	8.04	1.78	10.84	2.07
Low frequency	4101	260	4418	149	4962	191	4830	177	3525	157	3282	265	4212	392	3800	260
High frequency	5806	284	5800	148	6196	259	6105	202	5417	179	5488	170	5742	390	5383	305
Frequency difference	1705	348	1382	177	1235	175	1275	91	1892	152	2206	158	1530	261	1584	275
Syllable duration	0.17	0.03	0.15	0.01	0.20	0.02	0.17	0.01	0.11	0.01	0.18	0.01	0.16	0.02	0.12	0.01
Tempo	3.45	0.27	3.55	0.22	3.15	0.33	3.32	0.19	5.36	0.33	3.65	0.24	4.09	0.46	5.08	0.54
No. of songs	28.2	12.3	32.0	15.6	26.2	6.1	21.0	9.4	40.7	18.7	19.5	8.3	32.5	13.9	24.8	8.4
<i>n</i>	26		14		17		10		30		6		8		20	

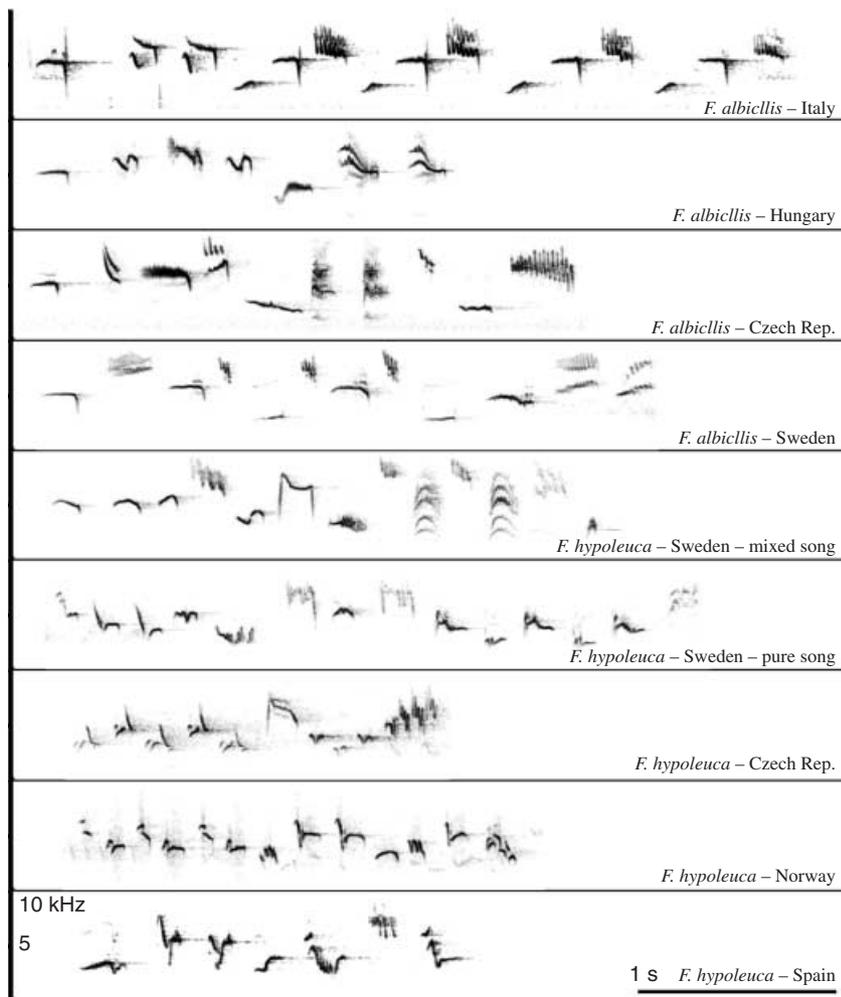


Fig. 2 Sonograms of pied and collared flycatchers from the study populations.

to enticement calls when a female visits his nest hole. This song type is therefore probably not directly comparable with normal territorial song. Mean values for each individual were calculated for all measurements. The choice of measurements in this study was based on

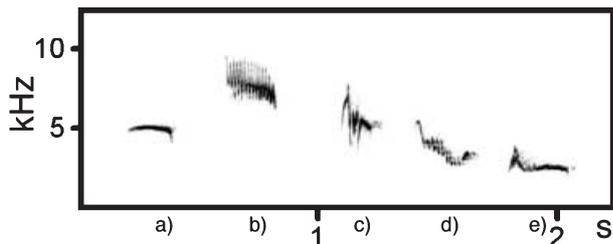


Fig. 3 The classification of syllables based on five categories. These categories were (a) note, (b) vibrato, (c) simple figure, (d) figure with a vibrato and (e) figure with a note. The two last categories defined syllables with elements that were produced either in sequence or simultaneously, independent of the order. The frequencies of the classified syllables are presented in Fig. 6.

earlier studies that found them useful in characterizing the species (Wallin, 1985; Gelter, 1987). To compare overall similarity between the species and the populations, the data were analysed in a principal component analysis and represented in scatterplots. One variable, the frequency range, was excluded from the principal component analysis because of low extraction commonality. Furthermore, the data were analysed in a discriminant function analysis. Convergence or divergence in the song characters was tested by ANOVAS. To compare population differences in song content, we made sonograms (see examples from the different populations in Fig. 2), and classified each syllable into one of five categories: note, vibrato, figure, figure/vibrato and figure/note (see Fig. 3 for details). The classifications were made blindly, that is, without knowing the species or population origin of the birds. The relative frequencies of syllables in each category were calculated to allow population comparisons.

Genetic data on birds from the sympatric population of Öland include genotypes at 21 genetic markers that have

been used to estimate the proportion of introgressed alleles in the genomes of the individual birds (Sætre *et al.*, 2003). For the present study, we compared these genetic results with the data on song, to investigate whether introgressive hybridization would affect the song of sympatric birds.

Results

The pied and the collared flycatcher were found to differ in several song characters in both sympatry and allopatry (Table 1). Qualitatively, the song of the collared flycatcher can be described as having longer songs, a lower tempo but a higher frequency (both minimum and maximum frequencies) than that of the pied flycatchers. To simplify the comparisons the data were analysed in a principal component analysis.

The two first principal components explained about 86% of the variation in the data and are presented in the scatterplots of allopatric and sympatric populations

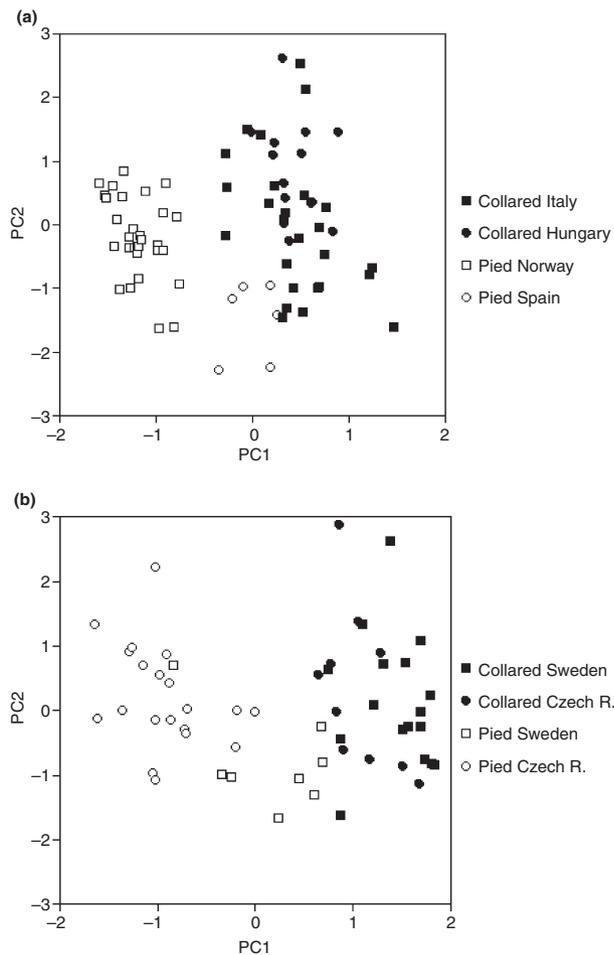


Fig. 4 Principal component scores of individuals from (a) allopatric and (b) sympatric pied and collared flycatcher populations.

Table 2 Correlation matrix of the variables and the first two components in the principal component analysis (PC1 and PC2) and the standardized canonical discriminant function coefficients (d_i).

Variable	PC1	PC2	d_i
Song duration	0.55	0.80	-0.44
No. of syllables	-0.23	0.97	0.09
Low frequency	0.86	0.20	-0.68
High frequency	0.85	0.11	-0.07
Syllable duration	0.91	-0.15	0.42
Tempo	-0.93	0.03	1.01

(Fig. 4). A high PC1 score is associated with longer syllables and song duration, lower tempo and higher minimum and maximum frequencies (Table 2). Further, a high PC2 score is associated with more syllables in the song and also longer songs.

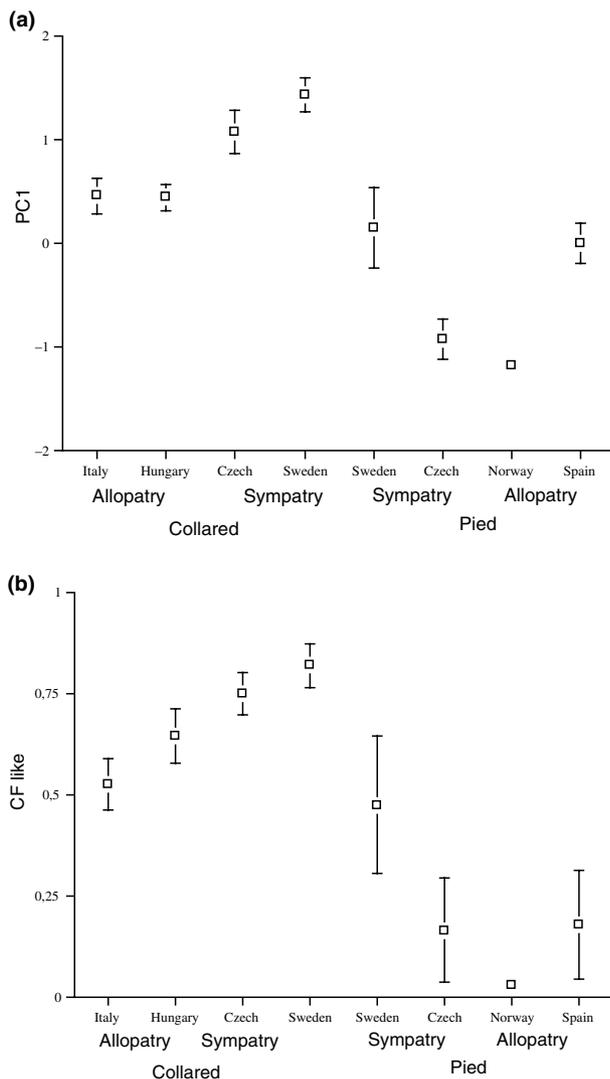
Collared flycatchers exhibited higher PC1 scores in sympatry than in allopatry (Figs 4 and 5a). Whereas the song variation largely coincides with allopatric vs. sympatric distributions in the collared flycatcher, it appears to be more complex in the pied flycatcher. In the pied flycatcher the allopatric Norwegian and the sympatric Czech populations both exhibited low PC1 scores. Also, the allopatric Spanish and the sympatric Swedish populations exhibited higher PC1 scores and were both quite similar to the allopatric collared flycatchers. For PC2 only minor and nonsignificant intraspecific variation was observed at the population level.

Some sympatric pied flycatchers got PC1 scores more similar to those of collared flycatchers than to allopatric pied flycatchers (Fig. 4). We performed a discriminant analysis to classify sympatric pied flycatchers as singing pied or collared flycatcher song. The relative contribution of the variables to the overall discrimination is presented in Table 2. Allopatric pied and collared flycatcher songs were used as templates for calculating the discriminant function. In the discriminant analysis, eight of 28 (29%) sympatric pied flycatchers were classified as singing collared flycatcher songs. These so-called mixed singers (Eriksson, 1991) were much more frequent in the Swedish (six of eight) than in the Czech samples (two of 20) ($\chi^2 = 8.859$, $n = 28$, $P < 0.05$). All sympatric collared flycatchers were classified to the right species. In sympatry, individuals classified as mixed singing pied flycatchers differed from pure singing pied flycatchers in five of the seven song characters (Table 3). Four of these five characters also differed between the allopatric and sympatric collared flycatchers.

About 88% of the song recordings of individual birds were of such quality that they allowed classification of the syllables in the song. Pied and collared flycatchers differed in the relative frequency of the five different classes of syllables (Fig. 6). Syllables classified as figure/notes were found almost exclusively in the collared flycatcher. Also, the vibratos and notes were much more

Table 3 Mean values and one-way ANOVAS of song variables comparing sympatric pied flycatcher scored as singing pure and mixed song in the discriminant analysis, and collared flycatcher from allopatry and sympatry.

Variable	Pied flycatcher					Collared flycatcher				
	Pure		Mixed			Allopatry		Sympatry		
	x	x	d.f.	F	P-value	x	x	d.f.	F	P-value
Song duration	2.12	2.03	1,26	0.41	0.527	2.90	2.98	1,65	0.28	0.601
No. of syllables	10.86	7.99	1,26	12.13	0.002	10.02	9.55	1,65	0.62	0.433
Low frequency	3749	4339	1,26	38.70	<0.001	4212	4913	1,65	132.97	<0.001
High frequency	5364	5789	1,26	10.52	0.003	5804	6162	1,65	35.51	<0.001
Frequency difference	1616	1450	1,26	2.31	0.141	1592	1250	1,65	24.67	<0.001
Syllable duration	0.12	0.16	1,26	31.13	<0.001	0.16	0.19	1,65	21.60	<0.001
Tempo	5.12	3.96	1,26	40.76	<0.001	3.48	3.21	1,65	15.65	<0.001

**Fig. 5** (a) Mean and 95% confidence intervals of the first principal component (PC1) and (b) the relative frequency of the three syllable types most typical for collared flycatchers (CF like) of pied and collared flycatcher populations.

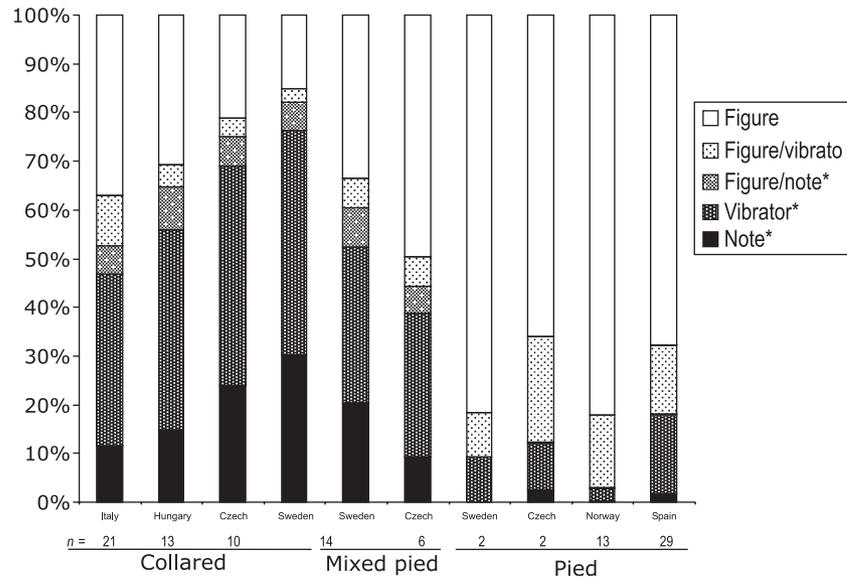
common in the collared than in the pied flycatcher song. These three classes of syllables comprised 53–82% in the collared flycatcher populations and between 3–48% in pied flycatchers (Fig. 5b). The results of the sonogram analysis were qualitatively similar to that of the principal component analysis of song characters (Fig. 5a and b). However, the Spanish pied flycatchers did not differ from Swedish pied flycatchers in the first principal component (Fig. 5a), but differed in the frequency of syllable types (Fig. 5b) being more similar to the Norwegian and Czech populations. Pied flycatchers classified as mixed singers in the discriminant analysis had 60% (the Swedish population) and 44% (the Czech population) of their syllables classified as typical of the collared flycatcher (Fig. 6). The relative frequency of these three syllable types were found to be significantly higher in sympatric than in allopatric collared flycatchers ($t = 6.24$, d.f. = 56, $P < 0.001$), whereas no difference was found between allopatric and pure singing sympatric pied flycatchers ($t = 1.46$, d.f. = 48, n.s.). Mixed singing pied flycatchers did not differ from allopatric collared flycatchers ($t = -0.14$, d.f. = 40, n.s.), but differed from sympatric collared flycatchers ($t = -5.44$, d.f. = 30, $P < 0.001$) and pure singing pied flycatchers ($t = 9.44$, d.f. = 55, $P < 0.001$).

Both genetic and song data was available for five of the pied flycatchers and nine of the collared flycatchers from the sympatric Swedish population. Four of the pied flycatchers were classified as mixed singers in the discriminant analysis. However, none of the pied flycatchers possessed any alleles introgressed from the collared flycatcher. We found no difference in song characteristics of five pure collared flycatchers compared with four collared flycatchers carrying at least one allele introgressed from the pied flycatcher (PC1: $t = 0.07$, d.f. = 7, n.s.).

Discussion

The theory of reinforcement suggests that natural selection against the production of unfit hybrids should

Fig. 6 The frequencies of syllable types of pied and collared flycatchers in allopatry and sympatry. Following the discriminant function analysis (see main text for further details), sympatric pied flycatchers have been grouped into mixed and pure singers. Syllable types that are typical in the collared flycatcher song have been marked with an asterisk (*). The frequencies of these syllables are presented in Fig. 5b).



favour traits that increase assortative mating. Given that hybridization is maladaptive and that song is a trait used in species recognition, the species differences in song characters should be larger in sympatry than in allopatry. The song analysis shows that there are quite clear species differences between pied and the collared flycatcher in allopatry. In sympatry some pied flycatchers resemble collared flycatchers in their song, known as mixed song (Eriksson, 1991). However, the species differences are maintained in sympatry because of a corresponding divergence in the collared flycatcher. Accordingly, the pattern of song variation can better be described as a character shift than a character displacement.

The pied flycatcher shows a complex pattern of geographic variation in song that warrants further explanation. First, the frequency of mixed song is higher in the Swedish than in the Czech sample of pied flycatchers. Secondly, in the principal component analysis the two allopatric pied flycatcher populations appeared to differ from each other. The Spanish population seems to be intermediate between the Norwegian population and the collared flycatcher (Figs 4a and 5a). In the classification of syllables, however, no differences between the allopatric pied flycatcher populations were evident (Fig. 5b). This implies that Spanish pied flycatchers are similar to the other pied flycatchers in song content, e.g. the relative frequency of the syllables in the songs, but resemble collared flycatchers in quantitative traits such as in syllable duration and tempo. Thus, the resemblance of the Spanish pied flycatcher to the collared flycatcher in the principal component analysis is not because of mixed singing.

Allopatric and sympatric populations may differ for many reasons, but it is likely that species interactions have affected pied and collared flycatcher in multiple

characters. Natural selection, such as adaptation to a different acoustical environment, sexual selection or random changes may cause population differences in song (West-Eberhard, 1983; Andersson, 1994; Butlin, 1995; Jones, 1997; Price, 1999). Hence, it does not necessarily follow that differences in signals between allopatric and sympatric populations are because of species interactions. In sympatric populations pied and collared flycatcher compete over resources (Sætre *et al.*, 1999b) and hybridize (Alatalo *et al.*, 1982; Sætre *et al.*, 1999a; Veen *et al.*, 2001). Hybrids have been found to have greatly reduced fitness (Alatalo *et al.*, 1982; Sætre *et al.*, 1997b, 1999a, 2003). A character displacement on male plumage traits has been found to reinforce species barriers (Sætre *et al.*, 1997b). The character displacement on plumage traits is a manifestation of the strength of species interaction suggesting that it could also affect other traits such as the song of the flycatchers.

Our DNA analyses suggest that social interspecific interaction rather than hybridization (*per se*) has affected the song of the flycatchers. Mixed singing has been described in a number of bird species (Helb *et al.*, 1985). However, only the recent application of DNA markers has made it possible to investigate whether the mixed singers are hybrids, backcrosses or individuals with pure parental genotype that learn heterospecific song (Bensch *et al.*, 2002). The comparison of genetics and song shows that none of the mixed singing pied flycatchers examined possessed any alleles introgressed from the collared flycatcher, thus they are not backcrosses. In fact, introgression has been found to occur nearly exclusively from pied to collared flycatchers and not vice versa (Sætre *et al.*, 2003). This implies that heterospecific learning is the dominant proximate factor explaining the occurrence of mixed song in the pied flycatcher. In accordance with

this, individual pied flycatchers have been observed singing pure song early in the season but changing to mixed song later in the season (Löhr, 1955). Thus, social interaction with the collared flycatcher is presumably the cause of mixed singing (Eriksson, 1991).

If song is affected by interspecific interactions it could be expected to differ in the two hybrid zones. The two hybrid zones differ in colonization history, population structure and age and this gives different predictions on the extent of adaptations to the coexistence in the pied and collared flycatchers. The hybrid zones on the Swedish Baltic islands are supposedly much younger than the one in Central Europe. Thus, any adaptations to coexistence, such as reinforcement of prezygotic barriers, would have had less time to evolve on the Swedish islands than in Central Europe (Lundberg & Alatalo, 1992). Moreover, the two classes of hybrid zones also differ in dynamics and structure (Sætre *et al.*, 1999a). The island populations consist of collared flycatchers evidently isolated from any continental population, whereas there would be extensive gene flow of allopatric pied flycatchers from the surrounding mainland. The pattern of gene flow is presumably more symmetrical in the continental hybrid zone (Sætre *et al.*, 1999a; S. Bures, M. Kral, G.-P. Sætre, unpublished data). Thus, any local adaptation in the pied flycatcher population on the Swedish islands is likely to be more strongly counteracted by gene flow from allopatry than those in Central Europe. Taken together, this renders reinforcement less likely on the islands than in the continental hybrid zones (Servedio & Kirkpatrick, 1997; Sætre *et al.*, 1997a). Furthermore, the Swedish collared flycatcher population is likely to have a Central European origin where it is sympatric with the pied flycatcher (Lundberg & Alatalo, 1992). Thus any adaptation in the Swedish population of the collared flycatchers may have its origin in the sympatric Central Europe populations. These geographic and demographic patterns suggest that adaptations to coexistence would have evolved further in the Czech than in the Swedish population of the pied flycatcher. Collared flycatchers, however, could be expected to show similar patterns in both hybrid zones because of common origin.

The variation in male plumage traits is consistent with the above mentioned predictions (Sætre *et al.*, 2003). Collared flycatchers are similarly black and white in the two hybrid zones, but Central European pied flycatchers are more brownish and have smaller patches of white compared with pied flycatchers found in the Swedish hybrid zone (i.e. they differ more from collared flycatchers). As the pied flycatcher is more similar to the collared flycatcher in the Swedish hybrid zone, the species differences are larger in the Czech than in the Swedish hybrid zone. If song plays a role in species recognition and speciation we could expect to find a similar pattern of variation.

The song analyses show that the collared flycatcher does show similar patterns of variation in both hybrid

zones. Also, mixed song in the pied flycatchers is more frequent in the Swedish than in the Czech population. Consequently, the species differences in song are larger in the Czech than in the Swedish hybrid zone; a pattern resembling that found in male plumage variation. Mixed song has been suggested to increase the probability of hybridization (Alatalo *et al.*, 1990; Eriksson, 1991). Accordingly, a plausible explanation for the difference between the hybrid zones in frequency of mixed song is that mixed singing is maladaptive and that the Central European population has developed better mechanisms to reduce the likelihood of heterospecific song learning. Also, mixed singing in the pied flycatcher may have caused a selection pressure for divergence of song characters in the collared flycatcher. Interestingly, our results show that the collared flycatchers in sympatry have diverged in the same characters that mixed singing pied flycatchers copy. If a reduction in the frequency of mixed singing and a divergence in the song of the collared flycatcher increases assortative mating and thus decreases the frequency of hybridization, this would be a form of reinforcement.

An alternative explanation could be that the probability of developing heterospecific song depends on the relative magnitude of exposure to con- and heterospecific song (Becker, 1982). When mixed singing occurs, it is usually most pronounced in the species with the lowest population density. The collared flycatcher constitutes 60–70 and 90% of the flycatcher population in the Swedish and the Czech hybrid zones respectively (Eriksson, 1991; Veen *et al.*, 2001). Thus, the density of the pied flycatcher relative to the collared flycatcher is higher in the Swedish than in the Czech population. Consequently, pied flycatchers in the Swedish hybrid zone should, if anything, be less biased in exposure to heterospecific song, contrary to our findings. Furthermore, if the frequency of mixed singing was only related to population densities we would expect to observe it also in the collared flycatcher, at least occasionally. However, mixed singing has never been reported in the collared flycatcher. Thus, different densities do not seem to be sufficient to explain this asymmetry between the species or between the hybrid zones. One explanation could be that pied flycatcher have rudimentary collared flycatcher structures in their song-learning predisposition (Eriksson, 1991). The collared flycatcher might already have diverged to such an extent that it is unable to learn the song of the pied flycatcher.

Song has been suggested to play an important role in the development of reproductive barriers in the early stage of speciation (Nottebohm, 1972; Grant & Grant, 1997a). In oscine passerines song is usually learned through an imprinting-like process (Kroodsma, 1982). Such culturally inherited traits may diverge faster than other genetically determined traits, thus increasing the rate of allopatric speciation (Grant & Grant, 1997a). It is unclear whether this property of song promotes or opposes speciation in sympatry (Raikow, 1986; Baptista

& Trail, 1992; Salomon & Hemim, 1992; Laland, 1994; Grant & Grant, 1997a; Irwin & Price, 1999; Irwin *et al.*, 2001). This plasticity has led researchers to suggest that song may not respond through reinforcement (Grant & Grant, 1997b). Indeed, there is little evidence to support the prediction of increased species differences in sympatry compared with allopatry in bird song (but see Salomon & Hemim, 1992). Rather, heterospecific copying seems to occur when two closely related species come to live in sympatry (Helb *et al.*, 1985). However, even culturally inherited traits, such as song in passerines, are under genetic constraints by an innate auditory song template (Kroodsma, 1982). Experiments have shown that the song template of birds is affected by both imprinting and genetics (Marler & Peters, 1977; Mundinger, 1995). The effect of imprinting has been found to vary even between closely related species (Slagsvold *et al.*, 2002). Thus, it seems likely that a genetic mechanism determining the plasticity of a trait may be under selection (Grant & Grant, 1997c; Irwin & Price, 1999). Thus, reinforcement may have caused a divergence of the innate auditory template of sympatric pied and collared flycatchers.

When two species come into secondary contact, species recognition mechanisms may be so similar that hybridization occurs. We suggest that at this early stage of speciation, traits used in species recognition that are culturally inherited may converge because of heterospecific copying (Fig. 7). Our data suggest that heterospecific copying in pied flycatchers caused the species differences in song to decrease. If hybrids have greatly reduced fitness, traits that increase assortative mating

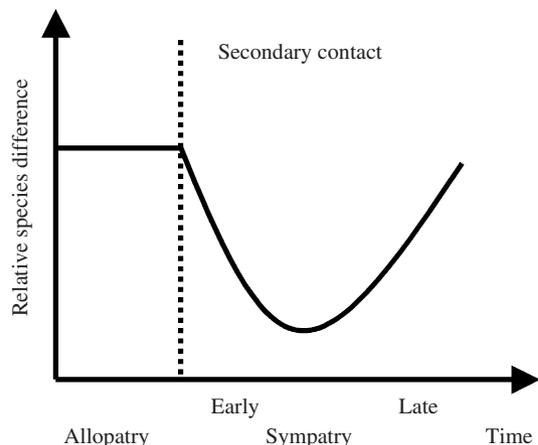


Fig. 7 A scenario of the species differences in song of the flycatchers. When the two species came into secondary contact heterospecific copying caused the species differences to decrease thereby leading to hybridization. Natural selection against maladaptive hybridization favoured traits that increased assortative mating e.g. by a divergence in an innate auditory template. Thus, over time the species differences increased and the frequency of hybridization decreased.

may evolve. These traits are most likely genetically determined, such as plumage traits. However, song may also have a genetic component affecting its plasticity and its expression. Accordingly, reinforcement may have caused innate auditory templates to diverge, such as in the collared flycatcher, and reduce its plasticity, such as the extent of heterospecific copying in the pied flycatcher. Thus, over time, the frequency of maladaptive hybridization will decrease, as the species differences in traits used in species recognition increase. Reproductive barriers seem to have evolved further in the Czech than in the Swedish hybrid zones. Thus, we suggest that the two different hybrid zones presented in this study may represent different stages in the speciation process. It could be speculated that if heterospecific copying increases the frequency of hybridization, it may increase the selective pressure for reinforcement of prezygotic barriers. Thus, culturally determined traits used in species recognition may, under certain circumstances, function as a catalyst in speciation.

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