The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2014, **113**, 1115–1125. With 2 figures

Strong matrilineal structure in common pipistrelle bats (*Pipistrellus pipistrellus*) is associated with variability in echolocation calls

ALENA FORNŮSKOVÁ[1,2,3,*], ERIC J. PETIT[3,4], TOMÁŠ BARTONIČKA[2], PETER KAŇUCH[5], ALAIN BUTET[3], ZDENĚK ŘEHÁK[2] and JOSEF BRYJA[1,2]

[1]*Institute of Vertebrate Biology, AS CR, Květná 8, 603 65 Brno, Czech Republic*
[2]*Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*
[3]*UMR CNRS 6553 ECOBIO, Université Rennes 1, Rennes, France*
[4]*Ecology and Ecosystem Health, UMR 985 Agrocampus Ouest–Institut National de la Recherche Agronomique, F-35042 Rennes, France*
[5]*Institute of Forest Ecology SAS, Zvolen, Slovakia*

Received 25 February 2014; revised 13 June 2014; accepted for publication 13 June 2014

The ontogeny and heritability of echolocation, an important sense in echolocating bats, is still not completely understood. Intraspecific variation in echolocation calls can be high, although the importance of possible explanatory variables (e.g. age, sex, social groups) remains largely unknown. Echolocation pulse features may vary among maternity roosts and this can theoretically be caused either by intercolony genetic differences or by vocal dialects learned during ontogeny within a roost (or a combination of both). In the present study, we analyzed intraspecific variation in echolocation parameters in relation to genetic structure at bi-parentally inherited microsatellites and maternally inherited mitochondrial (mt)DNA in maternal colonies of *Pipistrellus pipistrellus* in Central Europe. We found that individual colonies differ significantly in mtDNA, whereas the structure on nuclear markers is almost absent. This suggests a typical temperate bat social structure pattern, with strong sex-biased dispersal (i.e. philopatric females and dispersing males) (up to 92% of males leave their birth place according to our results). However, we show for the first time that genetic differentiation among mtDNA matrilines is associated with significant intercolony echolocation parameter differences. Because the genetic component of echolocation is not likely to be encoded by mtDNA, the results support the hypothesis of maternal echolocation dialect transmission to offspring, and the role of learning in this process is discussed. *Biological Journal of the Linnean Society*, 2014, **113**, 1115–1125.

ADDITIONAL KEYWORDS: dialects – maternal effects – philopatry – sex-biased dispersal – vocal learning.

INTRODUCTION

Sex-biased dispersal is common for many vertebrate species (Lawson Handley & Perrin, 2007). In birds, females usually disperse more frequently than males, whereas the opposite is generally true in mammals (Greenwood, 1980; Johnson, 1986; Kerth & Petit, 2005; Lawson Handley & Perrin, 2007). When the bias is strong, selective pressure can lead to environmental adaptive parental effects (Revardel, Franc & Petit, 2010). Male philopatry in birds, or female philopatry in mammals, can subsequently change the phenotype whereby offspring are significantly influenced by the philopatric parent (e.g. through genetic, behavioural or cultural inheritance) (Reinhold, 2002). For offspring, it is more advantageous to resemble the locally-adapted parent than the immigrant parent (Lawson Handley & Perrin, 2007; Wolf & Wade, 2009; Revardel *et al*., 2010). Examples of such

*Corresponding author. E-mail: fornuskova@ivb.cz

maternal effects (accompanied by male-biased dispersal) include adaptations to acidity described for the frog *Rana arvalis* (Räsänen, Laurila & Merilä, 2003) and vocal learning, frequently studied in whales such as *Orcinus orca* (Filatova, Burdin & Hoyt, 2010).

Temperate bat species evince the most common mammalian dispersal pattern (i.e. highly philopatric females and dispersing males) (Petit & Mayer, 1999; Castella, Ruedi & Excoffier, 2001; Petit, Balloux & Goudet, 2001; Kerth, Mayer & Petit, 2002), which provides an extraordinary opportunity to study the processes that lead to echolocation dialects. Female bats from temperate zones spend the nursing period in stable colonies made up of matrilines (e.g. *Myotis myotis*: Castella *et al*., 2001; *Myotis bechsteinii*: Kerth & Konig, 1999). Analysis of echolocation pulse features indicated variability among maternity roosts (Pearl & Fenton, 1996; Yoshino *et al*., 2008; Jiang *et al*., 2010a). Observed variability can be theoretically caused either by intercolony genetic differences caused by adaptations to local environmental conditions or by vocal dialects learned during ontogeny within a roost (or a combination of both: Gillam & McCracken, 2007; Yoshino *et al*., 2008; Chen, Jones & Rossiter, 2009). To our knowledge, these hypotheses have not been explicitly tested, although there are several studies suggesting an important role of learning in the postnatal modification of echolocation calls (Esser, 1994; Yoshino *et al*., 2008). Jones & Ransome (1993) showed that offspring of *Rhinolophus ferrumequinum* had a generic template that determined the basic structure of the call, although the final tuning of frequency parameters was partially learned from their mother.

It has recently been reported that bats use both known call types (social and echolocation calls) for communication between familiar and unfamiliar conspecifics (Voigt-Heucke, Taborsky & Dechmann, 2010). Variation in both communication signals at intraspecific level was already documented in several bat species (Kazial, Burnett & Masters, 2001; Pfalzer & Kusch, 2003; Gillam & McCracken, 2007; Carter *et al*., 2008; Yoshino *et al*., 2008; Chen *et al*., 2009). Although variation of social calls was connected with a broad spectrum of social interactions (Pfalzer & Kusch, 2003), intraspecific diversity in echolocation calls was connected with adaptations to a wide range of ecological niches, such as foraging habitat structure, foraging strategy, and body temperature (Schnitzler & Kalko, 2001; Gillam & McCracken, 2007; Jiang *et al*., 2010b). The possible influence of social variables (e.g. age, sex, social groups) on these calls remains largely unknown (Jones & Siemers, 2011); however, it was shown that echolocation calls of *Saccopteryx bilineata* encode information about individual and sex identity (Knornschild *et al*., 2012).

Populations of the common pipistrelle (*Pipistrellus pipistrellus*; Schreber, 1774), one of the most numerous bat species in Europe, have been expanding during Holocene in central and northern Europe (Sendor & Simon, 2003; Hulva *et al*., 2010). The *P. pipistrellus* population structure changes significantly throughout the year, with summer nursery colonies composed of (presumably) philopatric females and their offspring, whereas winter hibernacula are inhabited by both sexes (Bryja *et al*., 2009; Kanuch *et al*., 2010). Mating behaviour probably occurs during or after autumnal migrations. Evidence for intensive nuclear gene flow over long distances has been provided by three recent microsatellite-based genetic studies from continental Europe (Racey *et al*., 2007; Bryja *et al*., 2009; Sztencel-Jabłonka & Bogdanowicz, 2012).

In the present study, we used a large dataset to analyze relationships between intercolony genetic variation [assessed using both nuclear and mitochondrial (mt)DNA markers] and variation in echolocation calls. If echolocation calls are predominantly influenced by social environment in maternal colonies, divergence in echolocation parameters should be associated with mitochondrial (i.e. maternally inherited) population structure (Siemers & Ivanova, 2004; Yoshino *et al*., 2008). Male-biased dispersal and female philopatry predict strong population structure with respect to mtDNA. If the new maternal associations are created by a low number of female founders, they can be very distinct from neighbouring colonies not only with respect to mtDNA (as a result of strong genetic drift) but also echolocation pulse features (as a result of memetic drift; Burnell, 1998). Alternatively, if echolocation has a strong genetic basis (with corresponding genes carried by nuclear DNA), intercolony variation in echolocation parameters should be negligible, in accordance with lack of genetic structure and evidence for almost panmictic Central European population provided by nuclear genetic markers (Bryja *et al*., 2009).

MATERIAL AND METHODS

Genetic sampling and recording of echolocation calls

A small biopsy (2 mm^2) was taken from the wing membranes of 274 adult females from 11 Central European nursing colonies (20–45 individuals per colony; Fig. 1, Table 1) and subjected to genetic analysis. The wing punch sampling process and additional details about sampling are provided in Bryja *et al*. (2009). Echolocation calls of 20 adult females from each of 10 colonies were recorded prior to tissue sampling using a bat-detector with time-expansion

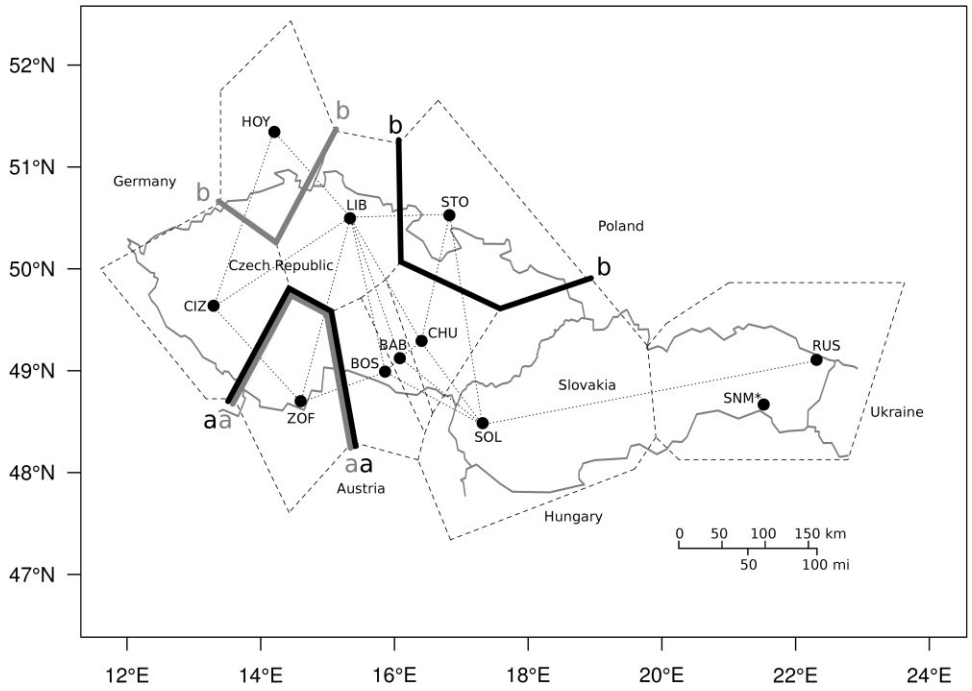

Figure 1. Area of Central European *Pipistrellus pipistrellus* colonies sampled, with barriers (zones of maximum dissimilarity) identified using Monmonier's algorithm. Barriers (tracing the edges of Voronoï polygons) reveal areas where populations from each side are more similar than populations from other sides. Results of $F_{ST\ mtDNA}$ matrix analysis are in black, whereas those from the echolocation call difference matrix are in grey. *a* and *b* indicate decreasing barrier importance. For colony name abbreviations, see Table 1. The colony with asterisk was removed from the analysis of Monmonier's algorithm because echolocation signals were not recorded there.

Table 1. Genetic variation between the colonies sampled

Name of locality	Code	N_{mic}	A_R	$H_{E\ mic}$	N_{mtDNA}	N_H	H_R	$H_{E\ mtDNA}$	π
Babylon–Kramolín (CZ)	BAB	20	8.77	0.836	17	6	5.946	0.831	2.99
Boskovštějn (CZ)	BOS	28	8.4	0.831	24	5	4.638	0.580	1.10
Čížice (CZ)	CIZ	25	8.75	0.825	22	9	8.589	0.887	2.23
Hoyerswerda (DE)	HOY	20	8.81	0.830	14	7	7.000	0.857	2.75
Chudčice (CZ)	CHU	25	8.07	0.828	23	7	6.667	0.802	2.62
Libštát (CZ)	LIB	20	8.85	0.823	17	6	5.973	0.853	3.21
Ruské (SK)	RUS	24	8.68	0.845	19	8	7.738	0.830	4.13
Slanské Nové Mesto (SK)	SNM	23	8.91	0.837	20	7	6.899	0.842	2.22
Solirov (SK)	SOL	45	8.46	0.844	30	7	6.348	0.809	1.89
Stolec (PL)	STO	23	8.2	0.813	16	4	3.976	0.442	1.56
Žofín (CZ)	ZOF	21	8.78	0.837	20	2	2.000	0.337	0.67
All populations		274	8.61	0.830	222	46	5.980	0.930	2.96

Code, colony name abbreviation; N_{mic}, microsatellite sample size; A_R, microsatellite allelic richness; $H_{E\ mic}$, microsatellite gene diversity; N_{mtDNA}, mtDNA sample size; N_H, number of haplotypes, H_R, mtDNA haplotype richness; $H_{E\ mtDNA}$, mtDNA gene diversity; π, nucleotide diversity, mean number of pairwise differences between haplotypes.

recording (D980; Pettersson Elektronik AB) in a 6 m × 3 m × 2 m experimental flight tent (Siemers & Ivanova, 2004; Siemers & Schnitzler, 2004). All animals were released after recording and tissue sampling.

Genotyping

Both nuclear and mitochondrial genetic markers were investigated to analyze genetic population structure. A detailed description of the DNA extraction

techniques used and methods for genotyping 11 nuclear microsatellites is provided in Bryja *et al*. (2009). The second hypervariable domain (HV-II) of the mitochondrial control region was analyzed by the polymerase chain reaction and sequenced as described in Castella *et al*. (2001). Species identification was based on the combined analysis of nuclear DNA and mtDNA and analysis of echolocation calls by ultrasound detectors (Bryja *et al*., 2009; A. Fornůsková, T. Bartonička, P. Kaňuch, Z. Řehák, unpublished results).

Analysis of genetic variation

Mitochondrial DNA

Sequence alignment was used to describe the number of haplotypes and their distribution between populations using ARLEQUIN, version 3.11 (Excoffier, Laval & Schneider, 2005). The median-joining network algorithm (Bandelt, Forster & Röhl, 1999), implemented in NETWORK, version 4.5.1.0 (Copyright Fluxus Technology Ltd, 1999–2008), was used to construct the haplotype network. For each population, standard genetic diversity indices, such as haplotype richness (H_R; the number of haplotypes corrected for sample size) and gene diversity ($H_{E\,mtDNA}$), were computed using the FSTAT, version 2.9.3 (Goudet, 2001). The mean number of pairwise differences (P_I) for all populations and genetic differentiation between colonies ($F_{ST\,mtDNA}$) was estimated using ARLEQUIN, version 3.11 (Excoffier *et al*., 2005).

Spatial analysis of molecular variance (SAMOVA, version 1.2) was calculated in ARLEQUIN, version 3.11 (Excoffier *et al*., 2005) to define genetically differentiated spatial groups. This procedure aims at maximizing the proportion of total genetic variance as a result of differences between groups of populations (Dupanloup, Schneider & Excoffier, 2002), equivalent to finding the partition that maximizes F_{CT}, or the genetic differentiation among groups.

Microsatellite analysis

Equivalent genetic diversity and inter-population microsatellite differentiation indices [allelic richness (A_R) and $F_{ST\,mic}$ (pairwise genetic differentiation corrected for the presence of null alleles; i.e. F_{ST}^{ENA})] were used *sensu* Bryja *et al*. (2009). Gene diversity ($H_{E\,mic}$) was calculated using ARLEQUIN, version 3.11. Because the Bayesian clustering procedure in STRUCTURE clearly revealed the best model for $K = 1$ (i.e. no evident population structure; Bryja *et al*., 2009), no other spatial analysis was performed.

Estimation of sex-biased dispersal

Sex-biased dispersal was estimated by comparing genetic structures assessed using markers with different modes of inheritance (control region versus microsatellites), in accordance with the method of Petit *et al*. (2001). First, we calculated female dispersal rate d_f from mitochondrial DNA ($F_{ST\,mt}$) using Equation 5.16 in Hartl & Clark (1997). We let N_f (effective number of females per population) vary within the range of known colony size variation. Based on personal knowledge of *P. pipistrellus* roosting habits, N_f varies from 64 to 500 when the expected maximum number of females per nursery colony is 500 (Nicholls & Racey, 2006; Racey *et al*., 2007). Then, we estimated d_m, the male dispersal rate, from microsatellite $F_{ST\,mic}$ using Wang's (1997) equation *sensu* Petit *et al*. (2001). Logically, N_m (the effective numbers of males) should be lower than N_f because almost all females reproduce each year, although it is suggested that the same rule does not apply for bat males (Heise, 1989). As a result, a number of equations were produced using a range of female and male effective size and sex ratios; with the constraint that sex ratio must be female-biased. We then used those equations that provided results of greatest contrast in terms of effective size and sex ratio: (1) a 1 : 1 effective sex ratio with lowest male and female effective size (there is no solution to the nuclear $F_{ST\,mic}$ equation below these numbers; in our case this is 64 females and 64 males) and random male dispersal; (2) a female-biased sex ratio with the highest biologically plausible female effective size (500 females and 36 males) and random male dispersal (there is no solution to the equation if the number of males is below 36); and (3) a 1 : 1 sex ratio with maximum females and maximum males (500 females and 500 males) and limited male dispersal. Finally, we quantified sex bias in dispersal as the percentage of male dispersers, calculated as $100 \times N_m d_m/(N_f d_f + N_m d_m)$.

Echolocation signal variability in relation to genetic structure

We recorded echolocation calls of all bats (20 in each colony) during flight in a tent toward a microphone located approximately 2–3 m straight ahead. Detector output was filtered by a 200-kHz, low-pass, anti-liasing filter and stored directly to a computer file. All pulses from three search-phase sequences were analyzed for each bat. After each set of three to six successive calls (see Supporting information, Table S1), the computer displayed call amplitudes allowing the set to be rejected if amplitude was too low or too high (Kazial *et al*., 2001). Only the first (fundamental) harmonic pulse was analyzed, as well as only sequences with a good signal-to-noise ratio (approximately 45 dB). Signal parameters were measured using AVISOFT, version 4.1 (SAS Lab-Pro software). Spectrograms and power spectra were con-

structed from 1024-point fast Fourier transforms (FFTs) using a sampling frequency of 22.05 kHz and the Hamming window function with an 87% overlap between consecutive FFTs, giving a frequency resolution of 280 Hz and time resolution of 0.60 ms. Only peak frequency (F_{peak}) was measured. This parameter was chosen over all the others because it allows simple comparison with previous studies (Boughman, 1998).

Associations between genetic differentiation (pairwise $F_{ST\,mic}$ or $F_{ST\,mtDNA}$ estimators), geographical distance, and intercolony differences in echolocation calls (matrices of absolute *t*-test values comparing F_{peak} among colonies; see Supporting information, Table S3c) were tested using the Mantel test in PASSAGE (Rosenberg & Anderson, 2011), with association significance being tested through 10 000 permutations. Patterns in genetic isolation caused by geographical distance were tested using $F_{ST\,mic}/(1-F_{ST\,mic})$ or $F_{ST\,mtDNA}/(1-F_{ST\,mtDNA})$ and the natural logarithm of geographical distance. We used a partial Mantel test to assess whether associations between genetic and echolocation differentiation were independent of geographical distance, with the matrix of geographical distance being held constant when genetic and echolocation distance matrix relationships were determined. Echolocation signals were not recorded at Slanské Nové Mesto and, therefore, this locality was removed from all Mantel tests. As an alternative, we used Monmonier's maximum difference algorithm, a computational geometry approach available in Barrier, version 2.2 (Monmonier, 1973; Manni, Guérard & Heyer, 2004) that enables the determination of maximum discontinuity zones in mitochondrial genetic structure (using a matrix of pairwise $F_{STmt\,DNA}$) and echolocation parameters (using the *t*-test statistics matrix from the F_{peak} pairwise comparisons). A Voronoï tessellation was obtained from the intersection of triangle medians defined by the Delaunay network connecting adjacent populations. A barrier, tracing the edge of the Voronoï polygons, revealed areas where populations from either side of the barrier were more similar than populations from other sides.

RESULTS

Intracolony genetic diversity

We obtained a 282-bp sequence of HV-II from 222 randomly selected specimens of *P. pipistrellus*. The sequence displayed 45 variable sites with 46 different mitochondrial haplotypes (see Supporting information, Table S2), the haplotype network having a clear star-like pattern (see Supporting information, Fig. S1). The most frequent haplotype (h4) was found in 21% of all specimens and nine out of 11 colonies. Other haplotypes differed by only one to four substitutions and 80% of them were specific to a particular colony (Table 2).

Gene diversity was high in all colonies using both types of genetic marker with exception of two colonies Žofín (ZOF) and Stolec (STO), where the variability of mtDNA was low (Table 1). All four measures of mitochondrial intracolony diversity were significantly correlated ($r = 0.67$–0.99; $P < 0.05$ for all pairs), with positive but nonsignificant correlations also found between microsatellite A_R and $H_{E\,mic}$ ($r = 0.33$, $P = 0.325$). No correlation was found between mitochondrial and nuclear diversity ($r = 0.13$–0.31; $P > 0.05$ for all pairs).

Intercolony genetic differentiation and isolation by distance

Global genetic differentiation among colonies, based on mtDNA, was significantly different from zero ($F_{ST\,mtDNA} = 0.226$, $P < 0.05$), with pairwise $F_{ST\,mtDNA}$ varying between 0.073 and 0.615 (see Supporting information, Table S3). SAMOVA analysis separated two populations (ZOF and STO) from each other, and from other populations ($F_{CT} = 29.4\%$; Table 3). ZOF had only two haplotypes, none of which were shared with other populations (h45 and h46; Table 2). The most similar to both of them was the haplotype h8 (differing by only one substitution), found amongst others in a geographically close population BOS (see Supporting information, Fig. S1). STO had only four haplotypes, all of which were shared with other populations. The most prevalent was h24, present also in the two geographically closest populations, Libštát (LIB) and Hoyerswerda (HOY). The two genetically distinct populations, STO and ZOF, also displayed very low haplotype richness and gene diversity ($H_R/_{ZOF} = 2$; $H_R/_{STO} = 3.976$; $H_{E\,mtDNA}/_{ZOF} = 0.337$; $H_{E\,mtDNA}/_{STO} = 0.442$; Table 1), suggesting a founder effect with a low number of female founders. By contrast, analyses based on microsatellites did not reveal any genetic structure (Bryja *et al.*, 2009).

Correlations between genetic and geographical distance were nonsignificant for mitochondrial markers (Mantel test, 10 000 permutations, $r = 0.063$, $P = 0.362$), and significant for nuclear microsatellites (Mantel test, 10 000 permutations, $r = 0.311$, $P = 0.038$) (see Supporting information, Fig. S2).

Sex-biased dispersal

Equations for d_f and d_m yielded variable results, dependent on female and male effective size (Table 4). Comparison of all three effective size and sex ratio

Table 2. Haplotype distribution for each colony

Haplotype	BAB	BOS	CIZ	HOY	CHU	LIB	RUS	SNM	SOL	STO	ZOF	Total	GenBank accession number
h1	1											1	HM106059
h2	5											5	HM106060
h3	2			1	1	3						7	HM106061
h4	4	15	3	1	9	3		3	8	1		47	HM106062
h5	4							2	6			12	HM106105
h6	1				4							5	JX912916
h7		5										5	JX912917
h8		2		4					1	2		9	HM106068
h9		1										1	JX912918
h10		1										1	JX912919
h11					2				9			11	HM106109
h12					2							2	JX912920
h13					1							1	JX912921
h14					4							4	JX912922
h15			6									6	HM106065
h16			3									3	HM106066
h17			2									2	JX912923
h18			2									2	JX912924
h19			1									1	JX912925
h20			3									3	JX912926
h21			1							1		2	HM106088
h22			1									1	JX912927
h23				4								4	JX912928
h24				2		5				12		19	HM106067
h25				1								1	JX912929
h26				1								1	JX912930
h27						2						2	JX912931
h28						1						1	JX912932
h29						3						3	JX912933
h30							1					1	HM106101
h31							1					1	HM106102
h32							4					4	HM106103
h33							2					2	HM106104
h34							1					1	JX912934
h35							7	2				9	JX912935
h36							2					2	JX912936
h37							1					1	JX912937
h38								3				3	HM106106
h39								7				7	HM106107
h40								1				1	HM106108
h41								2				2	JX912938
h42									3			3	HM106110
h43									2			2	HM106111
h44									1			1	JX912939
h45											4	4	HM106063
h46											16	16	HM106064

For colony name abbreviations, see Table 1.

Table 3. Results of spatial analysis of molecular variance, indicating F_{CT} (genetic differentiation between groups) and F_{SC} (genetic differentiation between populations within groups)

Number of groups	F_{CT}	F_{SC}	Composition of groups			
2	0.236	0.229	(BAB, BOS, CHU, CIZ, HOY, LIB, SNM, SOL, RUS, ZOF)	(STO)		
3	0.294	0.167	(BAB, BOS, CHU, CIZ, HOY, LIB, SNM, SOL, RUS)	(STO)	(ZOF)	
4	0.240	0.160	(BOS, CHU, CIZ, HOY, LIB, SNM, SOL, RUS)	(STO)	(BAB)	(ZOF)

For colony name abbreviations, see Table 1.

Table 4. Estimation of male-biased dispersal in *Pipistrellus pipistrellus* under three hypothetical scenarios

Setting	N_f/N_m	d_f/d_m	Bias (%)
1	64/64	0.044/1.000	96
2	500/36	0.006/0.982	92
3	500/500	0.006/0.096	94

N_f/N_m, effective number of females/males per population; d_f/d_m, female/male dispersal rates calculated on the basis of Wang's (1997) equation. Bias, sex bias in dispersal as the percentage of male dispersers.

settings, however, consistently revealed that dispersal was highly male-biased (more than 92% of dispersers were male).

Relationships between genetic and echolocation parameters

The highest values for F_{peak} were recorded at BOS and Ruské (RUS), whereas the lowest values were measured at STO, ZOF, and HOY. Localities with the lowest values of F_{peak} had the highest *t*-statistic values from pairwise *t*-tests (see Supporting information, Tables S1 and S3c). When controlled for geographical distance, F_{peak} differences between colonies (Fig. 2) were positively associated with genetic differentiation using mtDNA (partial Mantel test, 10 000 permutations, $r = 0.344$, $P = 0.034$). The positive association between mtDNA and echolocation distance remained significant even after genetically excluding the least similar population (ZOF), which was clearly founded by just a few genetically distinct females ($r = 0.517$, $P = 0.025$). The association between F_{peak} and differences at nuclear microsatellites (controlled for geographical distance) was not significant (partial Mantel test, 10 000 permutations, $r = 0.189$, $P = 0.143$).

Monmonier's algorithm, which was based on a matrix of differences in echolocation calls, separated the ZOF and HOY populations from each other and from all remaining populations (Fig. 1, barrier *b*). ZOF was also separated from other populations using the Φ_{ST} data matrix in the first rank of importance (barrier *a*). Hence, the geographical pattern for zones of maximum mtDNA dissimilarity was partly concordant with geographical differences in echolocation calls (Fig. 1).

DISCUSSION

Sex-biased dispersal

Female philopatry with male dispersal is the prevalent dispersal pattern in temperate bats, as has been shown for a number of species through detailed comparisons of genetic structure using nuclear and mitochondrial markers. Summer colonies usually consist of matrilines because colonies are probably founded by a few adult females and their daughters (Kerth, 2008). During the mating season, females meet and mate with unrelated males at so-called swarming sites (Rivers, Butlin & Altringham, 2005), resulting in an absence of genetic structure when analyzed using nuclear markers (e.g. microsatellites). This behaviour, however, is not expressed in the same way in all species. For example in *Plecotus auritus*, some males are philopatric (Burland *et al*., 1999), although the general pattern has been found in most species studied (e.g. *Nyctalus noctula*, Petit & Mayer, 1999; *M. myotis*, Castella *et al*., 2001; *M. bechsteinii*, Kerth, Mayer & König, 2000; Kerth *et al*., 2002; *R. ferrumequinum*, Rossiter *et al*., 2000; *Myotis lucifugus*, Dixon, 2011).

In the present study, we estimated for the first time sex-bias in dispersal in one of the most common of European bats, *P. pipistrellus*, with more than 92% of dispersing animals being male. Microsatellite variation between *P. pipistrellus* populations revealed very low differentiation at the nuclear DNA level and only weak (even if significant in the present study) isolation-by-distance pattern at studied scale, which can be explained by intensive gene flow during long seasonal migrations and through mating during or after such migrations at swarming or hibernating sites (Bryja *et al*., 2009). On the other hand, mtDNA provided evidence showing that summer colonies

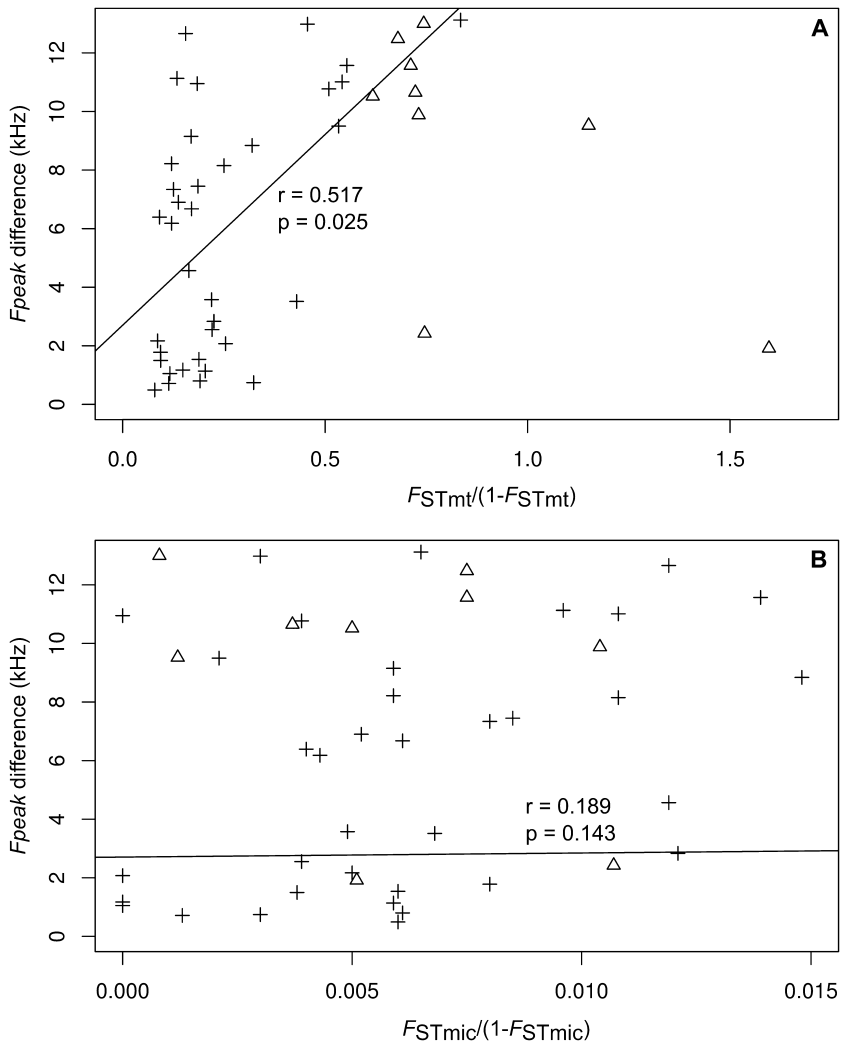

Figure 2. Association between pairwise genetic differentiation and differences in echolocation calls (*Fpeak_diff*). Pairwise estimates with the locality ZOF (i.e. the locality with two fixed private mtDNA haplotypes) are shown as triangles. A, mitochondrial genetic differentiation between colonies [i.e. $F_{ST\,mtDNA}/(1-F_{ST\,mtDNA})$]. B, nuclear genetic differentiation between colonies [i.e. $F_{ST\,mic}/(1-F_{ST\,mic})$]. The line represents the linear relationship between $F_{ST\,mtDNA}/(1-F_{ST\,mtDNA})$ and F_{peak} after excluding pairs with the locality ZOF.

were formed on the basis of a mother–daughter association, and that even two neighbouring colonies could differ significantly from each other at mtDNA. As shown at ZOF, the number of colony founders can be low, with just two haplotypes present and both private for this colony. According to our results, females live in closed societies with a very limited exchange of females among neighbouring colonies. New colonies are most likely formed from neighbouring nurseries, which is supported by the presence of similar haplotypes in nearby colonies (see Results; see also Supporting information, Table S2), although very strong genetic drift (founder effects) sufficiently masks the genetic isolation by geographical distance (see Supporting Information, Fig. S2).

Matrilineal structure and echolocation dialects

Observed associations between matrilineal genetic structure and the variability of echolocation calls provide new insight regarding the study of echolocation transmission between generations and the evolution of regional vocal dialects. Variability of echolocation calls was already documented between maternal colonies of *M. lucifugus* (Pearl & Fenton, 1996). Strong matrilineal structure and almost absolute female philopatry could lead to the evolution of local differences in maternally-transmitted cultural traits (e.g. echolocation call dialects) (Chen *et al.*, 2006, 2009).

We found significant differences in echolocation between colonies (i.e. local dialects), which were not related to geographical distance. Echolocation variation was better explained through mtDNA genetic structure (i.e. colonies that had similar mtDNA emitted similar peak frequency echolocation calls). This was particularly visible in the most genetically (but not geographically) distant colony (ZOF), which also differed most from other colonies in echolocation calls. No such association was found between echolocation and genetic differentiation at nuclear DNA.

The association between matrilineal structure and echolocation call pattern observed in the present study may suggest a potentially important role for postnatal learning of echolocation call structure. Regional differences in frequency parameters could be explained, therefore, by maternal transmission followed by cultural drift and selection during the isolation of matrilines (Jones & Ransome, 1993; Boughman, 1998; Yoshino *et al*., 2008; Chen *et al*., 2009).

Bats have a high capacity for vocal learning, as has previously been described for a number of species (Jones & Ransome, 1993; Esser, 1994). It was suggested that echolocation call parameters are partially inherited, although their final form is also significantly influenced by learning during the first 2 months after birth (Jones & Ransome, 1993). Learning of mothers vocalization signals was also documented in *Tadarida brasiliensis mexicana* pups (Gelfand & McCracken, 1986). Our results therefore provide indices leading to the maternal transmission hypothesis, whereby differences in acoustic signals between populations are maintained by mother–offspring transmission (Yoshino *et al*., 2008); note that parallels also exist between linguistic change and gene flow in humans (Cavalli-Sforza & Wang, 1986). These results now need to be confirmed through, for example, cross-fostering experiments or genetic studies investigating how much genes affect echolocation (Shimada, Shikano & Merilä, 2011). Also, detailed studies of parentage, where the call frequencies of mothers, fathers and offspring were all known could be highly informative.

ACKNOWLEDGEMENTS

We thank the Czech Science Foundation (206/06/0954) and the Long-Term Research Plan (MSM 0021622416) of Masaryk University for financial support. AF and JB worked also under institutional support grant no. RVO 68081766. Special thanks are extended to the French Government for the financial support of AF's Master study at the University of Rennes 1. We also thank D. Čížková, A. Konečný, and H. Patzenhauerová for their help in the Studenec laboratory. Finally, we thank three anonymous reviewers for their helpful comments.

REFERENCES

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16:** 37–48.

Boughman J. 1998. Vocal learning by greater spear-nosed bats. *Proceedings of the Royal Society of London Series B, Biological Sciences* **265:** 227–233.

Bryja J, Kaňuch P, Fornůsková A, Bartonička T, Rehák Z. 2009. Low population genetic structuring of two cryptic bat species suggests their migratory behaviour in continental Europe. *Biological Journal of the Linnean Society* **96:** 103–114.

Burland T, Barratt E, Beaumont M, Racey P. 1999. Population genetic structure and gene flow in a gleaning bat, *Plecotus auritus*. *Proceedings of the Royal Society of London Series B, Biological Sciences* **266:** 975–980.

Burnell K. 1998. Cultural variation in savannah sparrow, *Passerculus sandwichensis*, songs: an analysis using the meme concept. *Animal Behaviour* **56:** 995–1003.

Carter GG, Skowronski MD, Faure PA, Fenton B. 2008. Antiphonal calling allows individual discrimination in white-winged vampire bats. *Animal Behaviour* **76:** 1343–1355.

Castella V, Ruedi M, Excoffier L. 2001. Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. *Journal of Evolutionary Biology* **14:** 708–720.

Cavalli-Sforza LL, Wang WSY. 1986. Spatial distance and lexical replacement. *Language* **62:** 38–55.

Chen S, Jones G, Rossiter S. 2009. Determinants of echolocation call frequency variation in the Formosan lesser horseshoe bat (*Rhinolophus monoceros*). *Proceedings of the Royal Society of London Series B, Biological Sciences* **276:** 3901–3909.

Chen SF, Rossiter SJ, Faulkes CG, Jones G. 2006. Population genetic structure and demographic history of the endemic Formosan lesser horseshoe bat (*Rhinolophus monoceros*). *Molecular Ecology* **15:** 1643–1656.

Dixon MD. 2011. Population genetic structure and natal philopatry in the widespread North American bat *Myotis lucifugus*. *Journal of Mammalogy* **92:** 1343–1351.

Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* **11:** 2571–2581.

Esser KH. 1994. Audio-vocal learning in a non-human mammal: the lesser spear-nosed bat *Phyllostomus discolor*. *Neuroreport* **5:** 1718–1720.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* **1:** 47–50.

Filatova OA, Burdin AM, Hoyt E. 2010. Horizontal transmission of vocal traditions in killer whale (*Orcinus orca*) dialects. *Biology Bulletin* **37:** 965–971.

Gelfand DL, McCracken GF. 1986. Individual variation in the isolation calls of Mexican freetailed bat pups (*Tadarida brasiliensis mexicana*). *Animal Behaviour* **34:** 1078–1096.

Gillam EH, McCracken GF. 2007. Variability in the echolocation of *Tadarida brasiliensis*: effects of geography and local acoustic environment. *Animal Behaviour* **74:** 277–286.

Goudet J. 2001. *FSTAT, a program to estimate and test gene diversities and fixation indices*, Version 2.9.3). Available at: http://www2.unil.ch/popgen/softwares/fstat.htm

Greenwood P. 1980. Matings systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* **28:** 1140–1162.

Hartl DL, Clark AG. 1997. *Principles of population genetics*, 3rd edn. Sunderland, MA: Sinauer Associates Inc.

Heise G. 1989. Ergebnisse reproduktionsbiologisher Untersuchungen am Abendsegler (*Nyctalus noctula*) in der Umgebung von Prenzalu/Uckermark. *Nyctalus* **3:** 17–32.

Hulva P, Fornůsková A, Chudárková A, Evin A, Allegrini B, Benda P, Bryja J. 2010. Mechanisms of radiation in a bat group from the genus *Pipistrellus* inferred by phylogeography, demography and population genetics. *Molecular Ecology* **19:** 5417–5431.

Jiang T, Liu R, Metzner W, You Y, Li S, Liu S, Feng J. 2010a. Geographical and individual variation in echolocation calls of the intermediate leaf-nosed bat, *Hipposideros larvatus*. *Ethology* **116:** 691–703.

Jiang T, Metzner W, You Y, Liu S, Lu G, Li S, Wang L, Feng J. 2010b. Variation in the resting frequency of *Rhinolophus pusillus* in Mainland China: effect of climate and implications for conservation. *Journal of the Acoustical Society of America* **128:** 2204–2211.

Johnson C. 1986. Sex-biased philopatry and dispersal in mammals. *Oecologia* **69:** 626–627.

Jones G, Ransome R. 1993. Echolocation calls of bats are influenced by maternal effects and change over a lifetime. *Proceedings of the Royal Society of London Series B, Biological Sciences* **252:** 125–128.

Jones G, Siemers BM. 2011. The communicative potential of bat echolocation pulses. *Journal of Comparative Physiology A* **197:** 447–457.

Kanuch P, Fornuskova A, Bartonicka T, Bryja J, Rehak Z. 2010. Do two cryptic pipistrelle bat species differ in their autumn and winter roosting strategies within the range of sympatry? *Folia Zoologica* **59:** 102–107.

Kazial KA, Burnett SC, Masters WM. 2001. Individual and group variation in echolocation calls of big brown bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae). *Journal of Mammalogy* **82:** 339–351.

Kerth G. 2008. Animal sociality: bat colonies are founded by relatives. *Current Biology* **18:** R740–R742.

Kerth G, Konig B. 1999. Fission, fusion and nonrandom associations in female Bechstein's bats (*Myotis bechsteinii*). *Behaviour* **136:** 1187–1202.

Kerth G, Mayer F, König B. 2000. Mitochondrial DNA (mtDNA) reveals that female Bechstein's bats live in closed societies. *Molecular Ecology* **9:** 793–800.

Kerth G, Mayer F, Petit E. 2002. Extreme sex-biased dispersal in the communally breeding, nonmigratory Bechstein's bat (*Myotis bechsteinii*). *Molecular Ecology* **11:** 1491–1498.

Kerth G, Petit E. 2005. Colonization and dispersal in a social species, the Bechstein's bat (*Myotis bechsteinii*). *Molecular Ecology* **14:** 3943–3950.

Knornschild M, Jung K, Nagy M, Metz M, Kalko E. 2012. Bat echolocation calls facilitate social communication. *Proceedings of the Royal Society of London Series B, Biological Sciences* **279:** 4827–4835.

Lawson Handley LJ, Perrin N. 2007. Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* **16:** 1559–1578.

Manni F, Guérard E, Heyer E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology* **76:** 173–190.

Monmonier M. 1973. Maximum-difference barriers: an alternative numerical regionalization method. *Geographical Analysis* **3:** 245–261.

Nicholls B, Racey PA. 2006. Contrasting home-range size and spatial partitioning in cryptic and sympatric pipistrelle bats. *Behavioral Ecology and Sociobiology* **61:** 131–142.

Pearl DL, Fenton MB. 1996. Can echolocation calls provide information about group identity in the little brown bat (*Myotis lucifugus*)? *Canadian Journal of Zoology* **74:** 2184–2192.

Petit E, Balloux F, Goudet J. 2001. Sex-biased dispersal in a migratory bat: a characterization using sex-specific demographic parameters. *Evolution* **55:** 635–640.

Petit E, Mayer F. 1999. Male dispersal in the noctule bat (*Nyctalus noctula*): where are the limits? *Proceedings of the Royal Society of London Series B, Biological Sciences* **266:** 1717–1722.

Pfalzer G, Kusch J. 2003. Structure and variability of bat social calls: implications for specificity and individual recognition. *Journal of Zoology* **261:** 21–33.

Racey P, Barratt E, Burland T, Deaville R, Gotelli D, Jones G, Piertney S. 2007. Microsatellite DNA polymorphism confirms reproductive isolation and reveals differences in population genetic structure of cryptic pipistrelle bat species. *Biological Journal of the Linnean Society* **90:** 539–550.

Räsänen K, Laurila A, Merilä J. 2003. Geographic variation in acid stress tolerance of the moor frog, *Rana arvalis*. I. Local adaptation. *Evolution* **57:** 352–362.

Reinhold K. 2002. Maternal effects and the evolution of behavioral and morphological characters: a literature review indicates the importance of extended maternal care. *Journal of Heredity* **93:** 400–405.

Revardel E, Franc A, Petit R. 2010. Sex-biased dispersal promotes adaptive parental effects. *BMC Evolutionary Biology* **10:** 217.

Rivers NM, Butlin RK, Altringham JD. 2005. Genetic population structure of Natterer's bats explained by mating at swarming sites and philopatry. *Molecular Ecology* **14:** 4299–4312.

Rosenberg MS, Anderson CD. 2011. PASSaGE: pattern analysis, spatial statistics and geographic exegesis. Version 2. *Methods in Ecology and Evolution* **2:** 229–232.

Rossiter S, Jones G, Ransome R, Barrattt E. 2000. Genetic variation and population structure in the endangered greater horseshoe bat *Rhinolophus ferrumequinum*. *Molecular Ecology* **9:** 1131–1135.

Schnitzler HU, Kalko EKV. 2001. Echolocation by insect-eating bats. *Bioscience* **51:** 557–569.

Sendor T, Simon M. 2003. Population dynamics of the Pipistrelle bat: effects of sex, age and winter weather on seasonal survival. *Journal of Animal Ecology* **72:** 308–320.

Shimada Y, Shikano T, Merilä J. 2011. A high Incidence of selection on physiologically important genes in the three-spined stickleback, *Gasterosteus aculeatus*. *Molecular Biology and Evolution* **28:** 181–193.

Siemers B, Ivanova T. 2004. Ground gleaning in horseshoe bats: comparative evidence from *Rhinolophus blasii*, *R. euryale* and *R. mehelyi*. *Behavioral Ecology and Sociobiology* **56:** 464–471.

Siemers BM, Schnitzler HU. 2004. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature* **429:** 657–661.

Sztencel-Jabłonka A, Bogdanowicz W. 2012. Population genetics study of common (*Pipistrellus pipistrellus*) and soprano (*Pipistrellus pygmaeus*) pipistrelle bats from central Europe suggests interspecific hybridization. *Canadian Journal of Zoology* **90:** 1251–1260.

Voigt-Heucke SL, Taborsky M, Dechmann DKN. 2010. A dual function of echolocation: bats use echolocation calls to identify familiar and unfamiliar individuals. *Animal Behaviour* **80:** 59–67.

Wang J. 1997. Effective size and F-statistics of subdivided populations. II. Dioecious species. *Genetics* **146:** 1456–1474.

Wolf J, Wade M. 2009. What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **364:** 1107–1115.

Yoshino H, Armstrong K, Izawa M, Yokoyama J, Kawata M. 2008. Genetic and acoustic population structuring in the Okinawa least horseshoe bat: are intercolony acoustic differences maintained by vertical maternal transmission? *Molecular Ecology* **17:** 4978–4991.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Median-joining network indicating relationships between 46 D-loop sequence haplotypes. Haplotypes are denoted as circles, with size proportional to the number of individuals with a particular variant. Colours indicate the geographical origin of particular haplotypes. Small red circles represent hypothetical unsampled haplotypes that connect existing sequences within the network with maximum parsimony. The length of branches is proportional to the number of mutational steps.

Figure S2. Correlation between geographical distance (natural logarithm) and linearized estimates of genetic differentiation based on (a) mtDNA haplotype frequency $F_{ST\,mtDNA}/(1 - F_{ST\,mtDNA})$ and (b) microsatellite $F_{ST\,mic}/(1 - F_{ST\,mic})$.

Table S1. Mean, minimum, maximum, SD values for F_{peak} (peak frequency) for all *Pipistrellus pipistrellus* sequences; *n*, number of pulses analyzed; Calls, number of calls per female; Sequence, number of calls in one sequence (one flight toward a microphone).

Table S2. Polymorphic sites differentiating haplotypes h1–h46

Table S3. Pairwise difference matrices for genetic and echolocation parameters.