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Low population genetic structuring of two cryptic bat species suggests their migratory behaviour in continental Europe

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Although two cryptic pipistrelle bat species, *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*, belong among the most common bat species in Europe, it is still unclear whether they can migrate over long distances between summer and winter roosts. Long-distance migratory species may be expected to show low levels of genetic structuring in large areas due to regular mixing of the gene pool by mating that occurs during migration and/or hibernation. Conversely, the dispersal of gametes in sedentary species is spatially restricted, populations are more genetically structured, and isolation by relatively short distance is visible. By analysing diversity of highly variable microsatellites within and among summer colonies of both studied species in central Europe, we found that differentiation between populations is very weak. Both classical F_{ST} and Bayesian clustering approach failed to detect genetic structure among colonies and there was no significant isolation-by-distance pattern. The analyses of relatedness, however, revealed that individuals within colonies are more related than random suggesting philopatry of at least one sex. The results were very similar for the two species. The high level of gene flow among central European populations, even on large geographic distances, is discussed in relation with migrations, dispersal, and mating behaviour. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **96**, 103–114.

ADDITIONAL KEYWORDS: colony structure – dispersal – gene flow – microsatellites – relatedness.

INTRODUCTION

Methods of population genetics have become a very important tool for studying the biology of animal populations, especially of those species that are difficult to follow by direct observations. Bats are one of the groups where the use of genetic markers has led to the description and understanding of the peculiarities of their social life (e.g. mating systems, roosting biology and sex-biased dispersal; McCracken, Lumsden & Kunz, 2006). Population genetic studies in bats have further revealed that geographic genetic differentiation can be affected by a variety of other factors, such as seasonal migrations, geographical barriers, and past processes (Burland & Worthington-Wilmer, 2001).

Genetic structure in migratory animals is assumed to be weak across the wide geographical range due to very intense gene flow over long distances and this is especially true in flying species such as birds (Buerkle, 1999; Davis *et al.*, 2006; Hellgren *et al.*, 2008) and bats (McCracken, McCracken & Vawter, 1994; Webb & Tidemann, 1996; Petit & Mayer, 1999, 2000; Russell, Medellín & McCracken, 2005). In temperate bats, the mating usually occurs after the end of reproductive period (i.e. during or after

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the movements between summer and winter roosts; Rachwald, 1992; Russ *et al.*, 2000; Sendor, Kugelschafter & Simon, 2000; Sendor & Simon, 2003). In species that move long distances (i.e. 'migratory'), an intensive mixing of the gene pool on relatively large areas therefore occurs. Conversely, the gene flow in non-migratory ('sedentary') species is more restricted and populations are genetically more structured (Burland *et al.*, 1999; Rossiter *et al.*, 2000; Rivers, Butlin & Altringham, 2005). Many examined sedentary bat species show female philopatry and strong substructure is visible on maternally inherited mitochondrial (mt)DNA. This differentiation generally disappears when biparentally inherited markers (such as autosomal microsatellites) are used, owing to male-mediated gene flow (Castella, Ruedi & Excoffier, 2001; Kerth, Mayer & Petit, 2002a). However, even in those cases, the intensity of gene flow decreases with increasing distance and isolation-by-distance pattern of genetic variation is visible (Burland *et al.*, 1999; Kerth *et al.*, 2002a; Rossiter *et al.*, 2007).

The common pipistrelle (*Pipistrellus pipistrellus s.l.*) has been considered a very common and widespread bat species in Europe (Jones, 1999). On the basis of two phonic types (45 and 55 kHz) and differences in the mtDNA sequences, two distinct species *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* (Ahlén, 1981; Zingg, 1990; Barratt *et al.*, 1995, 1997) are now recognized in most of Europe which differ in ecological requirements (Barlow & Jones, 1999; Bartonička & Řehák, 2004; Davidson-Watts & Jones 2006; Nicholls & Racey, 2006a, b; Sattler *et al.*, 2007). It is curious that, although both species belong among the most numerous bat species in Europe, the question of whether they can migrate over long distances between summer and winter roosts remains unanswered. Based on the recapture data of banded bats, *P. pipistrellus* in the broad sense has been considered as regional migrant (Hutterer *et al.*, 2005), although some very long-distance records are known in central and eastern Europe (Kepka, 1981; Sachteleben, 1991; Gaisler *et al.*, 2003). However, it is not clear to which of the two recently recognized species these records belong (Kaňuch *et al.*, 2007b).

The only study focussed (partially) on movements of species-identified pipistrelles is a very recent genetic study from Great Britain, where Racey *et al.* (2007) showed that the intensity of the gene flow is negatively correlated with distance and isolation-by-distance pattern occurs in both species. However, the biology of British populations can be affected by their insular position and, even if the evidence exists that pipistrelles can cross sea channels (Ahlén, 1997), the sea can be important barrier for long distance migrations (Castella *et al.*, 2000). Moreover, some other important regional differences between island and continental populations of bats were described (e.g. roosting behaviour of *Nyctalus leisleri*: Ruczyński & Bogdanowicz, 2005 versus Shiel & Fairley, 1999; flying behaviour and roost switching of *Pipistrellus pygmaeus*: Bartonička & Řehák, 2004 versus Davidson-Watts, Walls & Jones, 2006), suggesting that populations originating from various parts of the distribution area can behave differently. Partial migratory behaviour is well known in birds (Buerkle, 1999; Davis *et al.*, 2006), but also in some bats. The best known example is *Tadarida brasiliensis*, where one geographical part of populations is migratory and other populations are sedentary (Cockrum, 1969). Geographical variation in migratory tendency was expected also in European bat species *Vespertilio murinus*, *Nyctalus noctula*, and *Pipistrellus nathusii* (Strelkov, 1969; Rydell & Baagøe, 1994; E. Petit, pers. comm.) but rarely demonstrated by the field data (for a rare exception, see Rodrigues & Palmeirim, 2008).

The main question in the present study is whether genetic data can provide evidence of some limits in gene flow in continental populations of two cryptic *Pipistrellus* bat species. We used microsatellite markers to analyse their population genetic structure in the area of central Europe. First, we specifically analysed the distribution of genetic variation within and among summer colonies of the two species. Second, because the ecological and behavioural variations are often the most visible features of morphologically very similar species, we therefore focussed on the comparison of the population genetic structure between two cryptic species, especially whether putative differences in mating and dispersal behaviour are detectable by genetic markers.

MATERIAL AND METHODS

Samples

A total of 274 individuals of *P. pipistrellus* from 11 nursery colonies (with distances from each other in the range 22–651 km) and 233 individuals of *P. pygmaeus* from ten nursery colonies (with distances from each other in the range 13–761 km) were analysed (Fig. 1). The nursery colonies (here called populations) consisted only of adult females and their young occupying a common roost. If possible, samples (wing punches) were taken from adult females captured at the entrance of the roosts to avoid sampling first-order relatives. However, at three localities (Boskovštejn, Bratislava, Białowieża), it was not possible to obtain enough adult females and approximately one-third of the samples consisted of young born in these colonies. However, analyses of relatedness (see below) revealed that the proportion of close relatives (i.e.

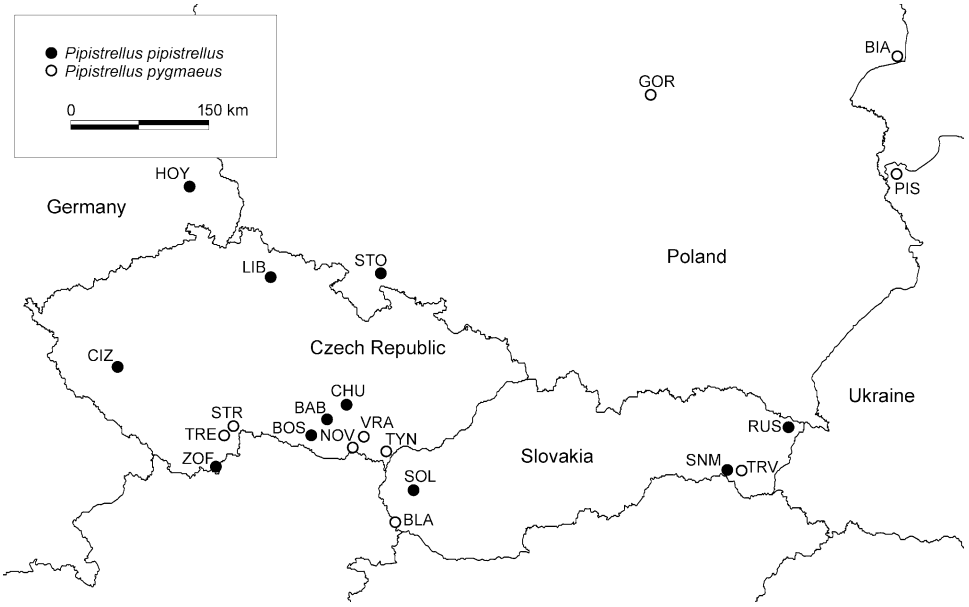

Figure 1. Geographical position of studied summer colonies of two cryptic pipistrelle species. For population abbreviations, see Table 2.

parent–offspring pairs) in these samples was comparable with samples composed only of adult females. All individuals were identified to species by the analysis of ultrasound detectors (recorded and analysed in time-expansion mode) and/or by a simple species identification polymerase chain reaction (PCR) test (Kaňuch *et al.*, 2007b). Geographical distances among populations (Fig. 1) were calculated from latitudinal and longitudinal coordinates (obtained from global positioning system data or maps) in GenAlEx, version 6.0 (Peakall & Smouse, 2006).

Genotyping

DNA was extracted by DNeasy Blood and Tissue Kit (Qiagen) and all individuals were genotyped on 13 microsatellite loci by using Multiplex PCR Kit (Qiagen) according to manufacturer's instructions. We used primers developed for other vespertilionid bat genera (Kaňuch *et al.*, 2007a) as well as primers designed directly for *Pipistrellus* bats (Racey *et al.*, 2007). Because some of the loci were not sufficiently amplified in both species or it was complicated to amplify them in multiplex kits, final analyses were restricted to eleven loci EF1, EF4, EF6, Paur05, NN18, NnP217, NnP219 (Kaňuch *et al.*, 2007a), Ppip01, Ppip02, Ppip04, and Ppip06 (Racey *et al.*, 2007). Fluorescently-labelled PCR products were separated by capillary electrophoresis on ABI 3130 Genetic Analyser (Applied Biosystems) and electrophoretograms were edited in GeneMapper, version 3.7 (Applied Biosystems). Every individual, which was successfully genotyped at some loci but not on the others in multiplex PCRs, was re-amplified by single PCRs to avoid primer competition (i.e. to confirm the presence of null allele homozygotes).

Intrapopulation genetic variability and relatedness

Deviations from Hardy–Weinberg equilibrium (HWE) were tested for each locus and population using the Markov chain method in the software GENEPOP, version 3.4 (Raymond & Rousset, 1995). Corrections for multiple tests were performed using the false discovery rate approach (Benjamini & Hochberg, 1995) and the QVALUE software (Storey, 2002). Number of alleles (A), observed (H_O) and expected (H_E; nonbiased estimate according to Nei, 1978) heterozygosities were calculated in GENETIX, version 4.05.2 (Belkhir *et al.*, 2001). Allelic richness (AR) corrected by the rarefaction method for the sample size (i.e. estimated for a minimum sample size of 12 diploid individuals in both species) were calculated for each population in FSTAT, version 2.9.3.2 (Goudet, 2001). Null alleles can lead to underestimation of measures of genetic variation within populations and to deviation from HWE. We therefore estimated the proportion of null alleles (NA) at each locus and population in the software FREENA (Chapuis & Estoup, 2007). The differences in AR between the two pipistrelle species were tested by using two-sided permutation test (1000 permutations) implemented in FSTAT.

Estimates of relatedness can be also biased by the presence of null alleles. A new approach has been proposed for estimating pairwise relatedness in the presence of null alleles, which was implemented in the software ML-RELATE (Kalinowski, Wagner & Taper, 2006), and this method was shown to be better than alternative methods of discarding the loci with null alleles or using uncorrected data (Wagner, Creel & Kalinowski, 2006). Maximum-likelihood estimates of pairwise relatedness (allowing values in the range 0 to 1) between all individuals of the same species were estimated from the complete data set and the variation of within- and between-colony relatedness was analysed by standard statistical procedures in Statistica 7.0 for Windows (StatSoft, Inc.). Subsequently, the proportion of related individuals (parent-offspring, full-sibs, half-sibs; estimated in ML-RELATE) within and between colonies was compared. Furthermore, to test for the existence of family groups in the populations, the degree of relatedness in terms of *rxy* (Queller & Goodnight, 1989) was estimated in each population using a permutation method implemented in IDENTIX (Belkhir, Castric & Bonhomme, 2002). The resampling (1000 permutations) was carried out at the genotypic level to estimate whether individuals within a population sample were genetically more related than expected in a randomly mating population. Null hypothesis of no relatedness is rejected with a significance level of 5% if the observed value of the relatedness is above the 95% level of resampled statistics (Belkhir *et al.*, 2002).

Differences between populations

Interpopulation genetic structure was estimated by two approaches. First, population differentiation for all population pairs and for all loci was tested by the exact *G*-test (Goudet *et al.*, 1996) implemented in FSTAT, and the overall loci test (not assuming HWE) was performed according to Petit, Balloux & Goudet (2001) in the same program (all tests by 1000 permutations). Second, the genetic differentiation between sampling sites was quantified by calculating estimators of F_{ST}, as described by Weir & Cockerham (1984). However, there are two possible sources of bias in comparisons of genetic differentiation by using F_{ST} approach. First, null alleles are known to overestimate the genetic differentiation between populations. We corrected for this effect, using the so-called ENA method implemented in FREENA software for estimating F_{ST}^{ENA}. This method efficiently corrects F_{ST} estimates for the positive bias introduced by the presence of null alleles (Chapuis & Estoup, 2007). Second, it is often difficult to interpret genetic differentiation values because of their dependence on the level of genetic variation at particular loci. We thus used a standardized measure of genetic differentiation, as proposed by Hedrick (2005), bringing values into the same range (0–1) for all levels of genetic variation. The standardized estimator $F'_{ST}{}^{ENA}$ was calculated by dividing the estimated value F_{ST}^{ENA} by the maximum value $F_{ST(max)}{}^{ENA}$ obtained using RecodeData, version 0.1 (Meirmans, 2006; available at: http://www.bentleydrummer.nl/software. Isolation by distance was analysed, for each F_{ST} estimator (i.e. F_{ST}^{ENA} and $F'_{ST}{}^{ENA}$), by regressing pairwise estimates of $F_{ST}/(1 - F_{ST})$ against ln-distance between sampling sites (Rousset, 1997). Mantel tests were used to test the correlation between matrices of genetic differentiation and Euclidean distances between sampling sites, by 5000 permutations in GENEPOP.

Bayesian clustering

A Bayesian clustering procedure, implemented in STRUCTURE, version 2.2 (Pritchard, Stephens & Donnelly, 2000) was used to infer the number of distinct genetic populations represented in the sample and the assignments of individuals to these genetic clusters. The current version of the programme allows also the analysis of loci with null alleles (Falush, Stephens & Pritchard, 2007). The Bayesian model assumes K (unknown) populations that have different allele frequencies at a set of independent loci. The programme was run with five independent simulations for each of K from 1 to 11, each one of 1 000 000 iterations, following a burn-in period of 100 000 iterations. In all simulations, admixture ancestry model and independent allele frequency models (with $\lambda = 1$) were used in the first steps. If the population structure is subtle, the model of correlated allele frequencies is often more effective to detect this structuring (Falush *et al.*, 2003). In the next step, we therefore analysed the data by using the correlated allele frequencies model. The likelihood of K [i.e. Ln $P_r(X|K)$], was used to infer the number of real populations in the datasets.

RESULTS

Detection of null alleles and intrapopulation genetic variation

We successfully genotyped 274 *P. pipistrellus* (20–45 individuals per population) and 233 *P. pygmaeus* (12–30) on 11 microsatellite loci. All loci were highly variable with both high number of alleles (11–38 in *P. pipistrellus* and 11–28 in *P. pygmaeus*) and high expected heterozygosity (0.75–0.90 in *P. pipistrellus* and 0.78–0.89 in *P. pygmaeus*) (Table 1). Lower observed than expected heterozygosities and consistent deviations from HWE on some loci were probably

Table 1. Microsatellite loci used in the present study

	Pipistrellus pipistrellus					*Pipistrellus pygmaeus*				
Locus	*A*	H_E	H_O	HW	*NA*	*A*	H_E	H_O	*HW*	*NA*
Ppip01	19	0.90	0.84	0.09	0.022	28	0.89	0.77	0.18	0.052*
Ppip02	11	0.75	0.80	0	0.016	10	0.78	0.78	0	0.010
Ppip04	18	0.90	0.61	0.73	0.140*	13	0.88	0.41	0.82	0.246*
Ppip06	38	0.90	0.89	0	0.001	22	0.87	0.60	0.73	0.129*
EF1	11	0.82	0.83	0	0.004	12	0.81	0.75	0.18	0.047*
EF4	17	0.86	0.75	0.09	0.045*	24	0.85	0.50	0.73	0.183*
EF6	19	0.89	0.89	0	0.008	12	0.86	0.84	0	0.027
Paur05	12	0.83	0.55	0.55	0.144*	11	0.82	0.82	0	0.011
NN18	15	0.86	0.86	0.09	0.008	17	0.83	0.48	0.64	0.181*
NnP217	20	0.84	0.82	0	0.004	21	0.81	0.81	0	< 0.001
NnP219	23	0.80	0.80	0	0.004	18	0.84	0.84	0	0.005

For each locus, the number of alleles (*A*), mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), proportion of populations with significant departure from Hardy–Weinberg (*HW*) equilibrium ($P < 0.05$ after false discovery rate correction), mean proportion of null alleles (*NA*) estimated in FreeNA are given. (*) Loci that were marked as 'loci with null alleles' in ML-Relate.

caused by the presence of null alleles, whose mean frequencies in populations were estimated up to 14.4% in *P. pipistrellus* (locus Paur05) and up to 24.6% in *P. pygmaeus* (locus Ppip04) (Table 1). We therefore carefully analysed the data taking into account the presence of null alleles at some loci.

Intrapopulation genetic variation was higher in *P. pipistrellus* than in *P. pygmaeus*. Allelic richness estimated by the rarefaction method for the smallest sample size ($N = 12$ diploid individuals) was 8.07–8.91 in the former and 7.20–8.32 in the latter species (Table 2) and this difference was significant (two-sided permutation test in FSTAT, $P < 0.001$). This result is not likely to be influenced by null alleles because simulations showed that estimates of AR are little affected by mean null allele frequencies below 0.15 (Brouat *et al.*, 2007). This was the case of our data where mean null allele frequency overall loci and populations was 0.036 for *P. pipistrellus* and 0.081 for *P. pygmaeus*.

Relatedness within and between colonies

Mean intra-colony relatedness (±SD) varied in *P. pipistrellus* between 0.044 ± 0.070 (population SNM) and 0.095 ± 0.102 (population STO), whereas, for two randomly chosen individuals from different colonies, the mean relatedness was 0.040 ± 0.063. In *P. pygmaeus*, intra-colony relatedness ranged from 0.046 ± 0.068 (population VRA) to 0.069 ± 0.089 (population STR), whereas mean inter-colony relatedness was 0.047 ± 0.071. In both species, intra-colony relatedness was significantly higher than inter-colony relatedness (Mann–Whitney *U* test; *P. pipistrellus*, $Z = 19.8$, $P < 0.001$; *P. pygmaeus*, $Z = 4.6$, $P < 0.001$). Higher proportion of relatives within colonies than between them is visible also from the relationship analysis. The most frequent relationship within colonies were half-sibs (8.70–30.83% in *P. pipistrellus*; 8.70–30.33% in *P. pygmaeus*), whereas parent–offspring and full-sibs pairs were observed only exceptionally (Table 2). Even though the percentage of related individuals within samples from the same colonies is very variable, a higher frequency of related individuals occurs inside any population sample than between two samples, with one exception in *P. pipistrellus* and two in *P. pygmaeus* (Table 2). The permutation tests in IDENTIX, however, indicated the presence of family groups only in two *P. pipistrellus* populations (RUS, HOY) (Table 2).

Population differentiation

Very weak differentiation between populations was observed (Table 3). No *G*-test (from 605 possible 'locus-population pair' tests) was significant after Bonferroni correction in *P. pipistrellus*, and only nine from 495 possible tests were significant in *P. pygmaeus* (eight significant tests were on loci with increased frequency of null alleles). Pairwise F_{ST}^{ENA} estimates were very low in both species. In *P. pipistrellus*, the mean ± SD pairwise $F_{ST}^{ENA} = 0.005 \pm 0.004$ (maximum $F_{ST}^{ENA} = 0.015$) and, in *P. pygmaeus*, the mean pairwise $F_{ST}^{ENA} = 0.006 \pm 0.005$ (maximum $F_{ST}^{ENA} = 0.017$). However, the populations are not totally panmictic as demonstrated by the global *G*-tests of population differentiation ($P < 0.001$ in both species) and global F_{ST}^{ENA} whose 95% confidence inter-

Table 2. Sample size (*N*), allelic richness (*AR*), and relatedness in studied populations as percentage of parent–offspring (*PO%*), full-sibs (*FS%*), half-sibs (*HS%*), and the total percentage of related individuals (*Sum%*)

	N	*AR*	*PO%*	*FS%*	*HS%*	*Sum%*	IDENTIX
Pipistrellus pipistrellus							
Chudčice, CZ (CHU)	25	8.07	0.67	0.67	18.33	19.67	0.097
Solirov, SK (SOL)	45	8.46	0.10	0	11.72	11.82	0.795
Žofín, CZ (ZOF)	21	8.78	0.95	0.48	11.90	13.33	0.678
Boskovštejn, CZ (BOS)	28	8.40	0.26	0.26	14.81	15.34	0.538
Ruské, SK (RUS)	24	8.68	0.72	0	14.86	15.58	0.018*
Slovenské Nové Mesto, SK (SNM)	23	8.91	0.40	0.40	8.70	9.49	0.476
Babylon-Kramolín, CZ (BAB)	20	8.77	0	1.05	11.05	12.11	0.129
Libštát, CZ (LIB)	20	8.85	0	0	10.53	10.53	0.581
Hoyerswerda, DE (HOY)	20	8.81	0	0	15.79	15.79	0.025*
Čižice, CZ (CIZ)	25	8.75	0	2.00	30.33	32.33	0.462
Stolec, PL (STO)	23	8.20	0.79	1.98	30.83	33.60	0.129
Between localities	–	–	0.03	0.14	9.62	9.79	–
Pipistrellus pygmaeus							
Týnec, CZ (TYN)	30	7.78	0.67	0.67	18.33	19.67	0.663
Novosedly, CZ (NOV)	22	7.77	0.10	0	11.72	11.82	0.146
Vranovice, CZ (VRA)	25	8.21	0.95	0.48	11.90	13.33	0.584
Třeboň, CZ (TRE)	22	7.20	0.26	0.26	14.81	15.34	0.254
Stráž nad Nežárkou, CZ (STR)	18	7.69	0.72	0	14.86	15.58	0.078
Bratislava, SK (BLA)	23	7.98	0.40	0.40	8.70	9.49	0.978
Trebišov, SK (TRV)	12	7.46	0	1.05	11.05	12.11	0.933
Pisocne jaziro – Sacki, UA (PIS)	27	8.32	0	0	10.53	10.53	0.973
Gorki, PL (GOR)	25	8.29	0	0	15.79	15.79	0.455
Białowieża, SK (BIA)	29	8.23	0	2.00	30.33	32.33	0.357
Between localities	–	–	0.03	0.36	11.21	11.60	–

Relatedness within populations and between them was calculated on the basis of pairwise maximum likelihood estimates of relatedness in the program ML-RELATE. AR (mean of all loci) was calculated for the smallest sample size ($N = 12$ diploid individuals). In the column IDENTIX, the results of permutation tests in IDENTIX are presented.
*Populations where the null hypothesis of no relatedness was rejected at $P < 0.05$.

vals (CI) are still higher than zero (*P. pipistrellus*, $F_{ST}^{ENA} = 0.0051$, 95% CI = 0.0031–0.0074; *P. pygmaeus*, $F_{ST}^{ENA} = 0.0053$, 95% CI = 0.0030–0.0085).

No genetic structure was found by simulations in the programme STRUCTURE. The highest Ln $P_r(X|K)$ was observed for $K = 1$ and decreased significantly with increasing number of putative populations in both species. This result was consistent for both correlated and independent allele frequencies models. Furthermore, the values of α greatly varied during the course of the runs and the proportions of the samples assigned to each population were approximately symmetric in the runs for $K > 1$. This is another evidence of the absence of population genetic structure in the data.

Spatial genetic structure

No significant isolation-by-distance pattern was found for any species. Although the highest pairwise F_{ST}^{ENA} estimates were usually obtained between the most distant populations in both species (Fig. 2), Mantel tests did not confirm correlation between geographic and genetic distances (*P. pipistrellus*, one-tailed test of isolation-by-distance, $P = 0.160$; *P. pygmaeus*, $P = 0.251$). Very similar results were obtained when using genetic distances corrected for variable level of genetic variation [i.e. $F'_{ST}{}^{ENA}/(1 - F'_{ST}{}^{ENA})$; *P. pipistrellus*, $P = 0.144$; *P. pygmaeus*, $P = 0.202$].

DISCUSSION

Differences between 'island' and 'mainland' populations

In the first detailed population genetic study of two cryptic pipistrelle bats in continental Europe, we found unexpectedly low level of genetic differentiation among nursery colonies in distances of up to almost 800 km from each other, therefore suggesting very intense gene flow between them. A recent population genetic study by Racey *et al.* (2007) described

Table 3. Pairwise F_{ST}^{ENA} values (below the diagonal) calculated for all loci, and number of significant ($P < 0.05$ after Bonferroni correction) *G*-tests of population differentiation (under the diagonal) for *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*

Pipistrellus pipistrellus	CHU	SOL	ZOF	BOS	RUS	SNM	BAB	LIB	HOY	CIZ	STO
CHU		–	–	–	–	–	–	–	–	–	–
SOL	0.006		–	–	–	–	–	–	–	–	–
ZOF	0.010	0.007		–	–	–	–	–	–	–	–
BOS	0.004	0.006	0.001		–	–	–	–	–	–	–
RUS	0.012	0.004	0.004	0.007		–	–	–	–	–	–
SNM	0.003	–0.002	–0.002	–0.001	0.002		–	–	–	–	–
BAB	0.008	0.005	0.001	0.005	0.006	0.007		–	–	–	–
LIB	0.000	–0.001	0.007	0.000	0.006	–0.003	0.001		–	–	–
HOY	0.006	0.006	0.011	0.015	0.008	0.013	0.012	0.004		–	–
CIZ	0.006	0.005	0.005	0.011	0.012	0.003	0.008	0.004	0.010		–
STO	0.002	0.004	0.005	0.006	0.014	0.003	0.011	–0.003	0.003	0.003	

Pipistrellus pygmaeus	TYN	NOV	VRA	TRE	STR	BLA	TRV	PIS	GOR	BIA
TYN		–	–	–	–	–	–	–	–	1
NOV	0.003		–	–	1	–	–	1	–	–
VRA	0.007	0.004		–	–	–	–	–	–	–
TRE	0.006	0.001	0.004		–	–	–	2	–	–
STR	0.013	0.010	0.008	0.010		–	1	1	1	–
BLA	0.004	–0.002	0.000	0.003	0.001		–	–	–	–
TRV	0.007	0.012	0.013	0.008	0.017	0.004		1	–	–
PIS	0.004	0.015	0.013	0.012	0.013	0.006	0.003		–	–
GOR	–0.002	0.005	–0.001	0.002	0.010	0.001	0.004	0.005		–
BIA	0.007	0.004	0.005	0.004	0.002	0.000	0.009	0.004	0.003	

For population abbreviations, see Table 2.

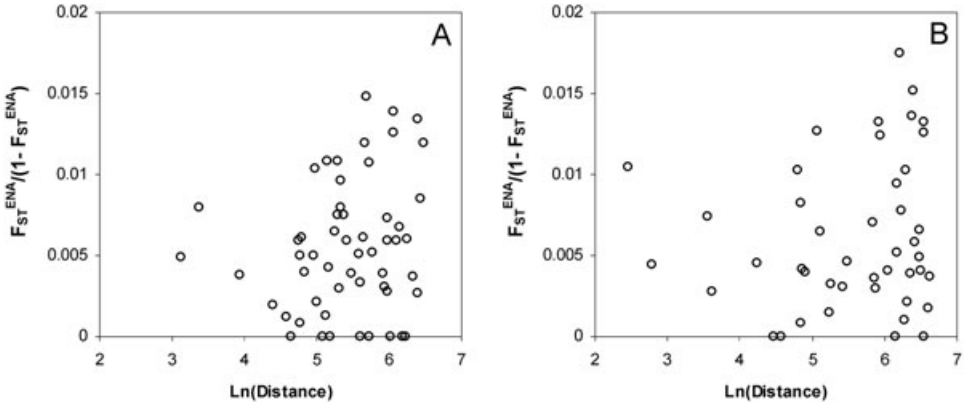

Figure 2. Correlation between genetic $F_{ST}^{ENA}/(1 - F_{ST}^{ENA})$ and geographical (ln scale) distance for two *Pipistrellus* species. The both relationships are not significant when tested by the Mantel tests. A, *Pipistrellus pipistrellus*; B, *Pipistrellus pygmaeus*.

microsatellite variation of the same species mainly in Great Britain. Even if we used similarly variable markers in the present study (some of them were identical in both studies) and the geographical scale was approximately the same, the nursery colonies in Great Britain were much more differentiated each other based on the F_{ST} approach. The mean pairwise F_{ST} was 0.029 for *P. pipistrellus* and 0.023 for *P. pygmaeus* (Racey *et al.*, 2007), making the genetic structure of both species comparable with sedentary bats such as *Plecotus auritus* (Burland *et al.*, 1999) or *Myotis bechsteinii* (Kerth *et al.*, 2002a). Furthermore the isolation-by-distance was significant, suggesting spatially limited gene flow in British pipistrelles. In the present study, the mean pairwise F_{ST} was 0.005 for *P. pipistrellus* and 0.006 for *P. pygmaeus* and no significant signs of isolation-by-distance were observed, which is similar to the migratory *N. noctula* (overall $F_{ST} = 0.006$, no isolation-by-distance; Petit & Mayer, 1999). The idea of regionally different intensity of gene flow between reproductive colonies is supported by the maximum recorded movement distance (Great Britain = 69 km, Avery, 1991; continental Europe = 1123 km, Benda *et al.*, 2003) and some differences in timing of mating (i.e. formation of mating groups in late summer before presumable migrations in Great Britain; Park, Altringham & Jones, 1996) versus mating during or after the seasonal movements in central Europe (Sachteleben & von Helversen, 2006).

Differences between populations of pipistrelles from the edge of their distribution area (e.g. Great Britain) and from the middle part of their distribution (e.g. central Europe) were also found in roost switching, colony size, and habitat use (Feyerabend & Simon, 2000; Bartonička & Řehák, 2004; Davidson-Watts *et al.*, 2006; Nicholls & Racey, 2006a, b; Sattler *et al.*, 2007). Geographical variation in behaviour is also known in other bat species; for example *Nyctalus leisleri* in continental Europe roosts exclusively in tree-hollows (Ruczyński & Bogdanowicz, 2005), whereas, in Ireland, nursery colonies occupy roof attics (Shiel & Fairley, 1999).

Movements from nursery colonies to hibernating sites and mating behaviour

Very weak genetic differentiation of reproductive colonies in central Europe could be explained by (1) long-distance movements between the places of reproduction and hibernation connected with mating (e.g. *N. noctula*; Petit & Mayer, 1999; Petit *et al.*, 2001) and/or by (2) long-distance dispersal of at least one sex (e.g. *Myotis myotis*; Castella *et al.*, 2001). Both hypotheses provide clear predictions, but banding data failed to provide sufficient information for their testing.

The first hypothesis predicts that at least a part of the population spend winter period in hibernacula far from the nursery colonies. Unfortunately, accessible data from direct observations are only fragmentary. Based on summer records, both species are very common in central Europe (Gaisler *et al.*, 2002); however, during winter, there are only few localities where hibernating pipistrelles have been found (Anděra & Hanák, 2007). Moreover, winter roosts of *P. pygmaeus* are almost unknown (P. Kaňuch, A. Fornůsková, T. Bartonička, J. Bryja, Z. Řehák, unpubl. data). It can be a result of hibernation in crevices of buildings or in trees, but it is also possible that the pipistrelles leave the nursing and foraging areas after reproduction (as observed in at least *P. pygmaeus*; Bartonička & Řehák, 2004) and migrate long distances to hibernacula. Large hibernating aggrega-

tions of tens of thousands individuals are known from few European localities (e.g. in Germany, Czech Republic, Slovakia and Romania: Dumitru, 1995; Kretzschmar & Heinz, 1995; Sendor *et al.*, 2000; Matis, Uhrin & Pjenčák, 2002; Nagy & Szanto, 2003; Anděra & Hanák, 2007) thus supporting the idea of winter concentration of large number of individuals coming from far away.

Direct data about long-distance movements are very rare and material reviewed from banding recoveries of *P. pipistrellus* in the broad sense did not provide an unambiguous view on species behaviour. Bat-ringing studies (mainly in Germany) have shown that pipistrelles in central Europe live in stationary populations that do not migrate (10–20 km; Hutterer *et al.*, 2005) and are often centred around large winter roosts (von Helversen *et al.*, 1987). However, several much longer movements of hundreds of kilometres were recorded in *P. pipistrellus s.l.* (Kepka, 1981; Sachteleben, 1991; Gaisler *et al.*, 2003), including the longest known movement of 1123 km from Ukraine to Bulgaria (Benda *et al.*, 2003). Circumstantial evidence of migratory behaviour is provided from homing experiments (e.g. bats successfully returned from distances up to 295 km; Roer, 1989), wing aspect ratio (based on wing morphology, *P. pipistrellus s.l.* was clearly classified to long-migratory species; Norberg & Rayner, 1987), and phylogeny (best known European migratory species are *Pipistrellus nathusii*, and representatives of phylogenetically close genus *Nyctalus*; Hutterer *et al.*, 2005).

Instead of migration, the mating during the movements or at hibernation sites is necessary prerequisite of long-distance gene flow. The mating system of *P. pygmaeus* was described as resource defence polygyny (Gerell & Lundberg, 1985; Lundberg & Gerell, 1986). By contrast, males of *P. pipistrellus* after the reproductive period occupy courtship territories along regular flight routes (Sachteleben & von Helversen, 2006) and the mating system can be characterized as a lek (Höglund & Alatalo, 1995) rather than a polygyny. Nevertheless, these studies probably do not cover the real complexity of mating behaviour of pipistrelles. First, the territorial activity and songflight calls of males of *P. pygmaeus* in central Europe were registered near the nursery colonies even before weaning the young (Bartonička & Řehák, 2004). These observations and the disappearance of large proportion of the population from nursery regions after weaning of young (Bartonička & Řehák, 2004) could suggest that mating occurs at nursery sites as well as during/after the movements to hibernacula. Second, the only study of mating behaviour of *P. pipistrellus* (Sachteleben & von Helversen, 2006) concerns the urban population of this species. They are known from many European towns (Gaisler *et al.*, 1998) and their behaviour includes very typical traits such as autumn invasions (Smit-Viergutz & Simon, 2000) and hibernation near the nursery colonies in the same town (Sendor *et al.*, 2000; Sachteleben & von Helversen, 2006). However, there are still numerous populations of *P. pipistrellus* living outside the big towns. Whether these individuals migrate and where do they mate and hibernate is still unknown but the autumn swarming at the entrances of the mass hibernacula in mines, buildings or natural caves (Kretzschmar & Heinz, 1995; Sendor *et al.*, 2000) could indicate that mating between individuals coming from far away can take place there (Furmankiewicz & Altringham, 2007).

'No migration, but dispersal' hypothesis

The second hypothesis that could explain the absence of genetic structuring on nuclear markers predicts that there is no regular seasonal long-distance migration (associated with mating) but that the gene flow is caused by the dispersal of at least one sex far from the birth site. In many species of temperate bats, the nursery colonies are composed of philopatric females, whereas young males abandon the birth place and mate with females from more distant colonies (e.g. *M. bechsteinii*: Kerth, Safi & König, 2002b; Kerth & Petit, 2005; *M. myotis*: Castella *et al.*, 2001; *N. noctula*: Petit & Mayer, 1999). In the present study, we found that nursery colonies are not random associations of females and that the relatedness between individuals within a colony is higher than the between-colony relatedness, therefore suggesting the idea of typical social system of temperate bats.

At least two approaches could be used in future studies to analyse the sex-biased dispersal and to distinguish it from regular seasonal migrations. First, direct observations using bat bands can provide definite information about movements (either dispersal and/or migrations). However, for the pipistrelle bats, the level of banding return is very low (e.g. 7978 individuals banded so far in the Czech and Slovak Republics in 1948–2000, of which only 2.7% were recaptured; Gaisler *et al.*, 2003). The second approach includes the use of genetic markers with sex-specific inheritance. This approach has been already successfully used in many bat species (see references above); however, to our knowledge, no study comparing sex-specific markers (mtDNA, Y-chromosome markers) with the autosomal ones exists for the pipistrelle bats. Furthermore, if the females are philopatric, the comparison of mtDNA of individuals from mass hibernacula and nursery colonies could provide the answer to the question where these individuals came from.

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REFERENCES

Ahlén I. 1981. Identification of Scandinavian bats by their sounds. *Swedish University of Agricultural Sciences, Rapport* **6:** 1–56.

Ahlén I. 1997. Migratory behaviour of bats at south Swedish coasts. *Zeitschrift Für Saugetierkunde* **62:** 375–380.

Anděra M, Hanák V. 2007. *Atlas of the mammals of the Czech Republic – a provisional version. V. Bats (chiroptera) – part 3*. Prague: National Museum.

Avery MI. 1991. Pipistrelle. In: Corbet GB, Harris S, eds. *Handbook of British mammals*. Oxford: Blackwell Scientific Publications, 124–128.

Barlow KE, Jones G. 1999. Roosts, echolocation calls and wing morphology of two phonic types of *Pipistrellus pipistrellus*. *Zeitschrift für Säugetierkunde* **64:** 257–268.

Barratt EM, Bruford MW, Burland TM, Jones G, Racey PA, Wayne RK. 1995. Characterization of mitochondrial DNA variability within the microchiropteran genus *Pipistrellus*: approaches and applications. *Symposia of the Zoological Society of London* **67:** 377–386.

Barratt EM, Deaville R, Burland TM, Bruford MW, Jones G, Racey PA, Wayne RK. 1997. DNA answers the call of pipistrelle bat species. *Nature* **387:** 138–139.

Bartonička T, Řehák Z. 2004. Flight activity and habitat use of *Pipistrellus pygmaeus* in a floodplain forest. *Mammalia* **68:** 365–375.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 2001. *GENETIX 4.02, Logiciel sous windows TM pour la Génétique des Populations*. Montpellier: Laboratoire Génome, Populations, Interactions, Université de Montpellier II.

Belkhir K, Castric V, Bonhomme F. 2002. IDENTIX, a software to test for relatedness in a population using permutation methods. *Molecular Ecology Notes* **2:** 611–614.

Benda P, Ivanova T, Horáček I, Hanák V, Červený J, Gaisler J, Gueorguieva A, Petrov B, Vohralík V. 2003. Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 3. Review of bat distribution in Bulgaria. *Acta Societatis Zoologicae Bohemicae* **67:** 245–357.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B, Statistical Methology* **57:** 289–300.

Brouat C, Loiseau A, Kane A, Ba K, Duplantier JM. 2007. Population genetic structure of two ecologically distinct multimammate rats: the commensal *Mastomys natalensis* and the wild *Mastomys erythroleucus* in southeastern Senegal. *Molecular Ecology* **16:** 2985–2997.

Buerkle CA. 1999. The historical pattern of gene flow among migratory and nonmigratory populations of prairie warblers (Aves: Parulinae). *Evolution* **53:** 1915–1924.

Burland TM, Barrat EM, Beaumont MA, Racey PA. 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.

Burland TM, Worthington-Wilmer J. 2001. Seeing in the dark: molecular approaches to the study of bat populations. *Biological Reviews* **76:** 389–409.

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.

Castella V, Ruedi M, Excoffier L, Ibánez C, Arlettaz R, Hausser J. 2000. Is the Gibraltar Strait a barrier to gene flow for the bat *Myotis myotis* (Chiroptera: Vespertilionidae)? *Molecular Ecology* **9:** 1761–1772.

Chapuis MP, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* **24:** 621–631.

Cockrum EL. 1969. Migration in guano bat, *Tadarida brasiliensis*. *Miscellaneos Publicaion, University of Kansas, Museum of Natural History* **51:** 303–336.

Davidson-Watts I, Jones G. 2006. Differences in foraging behaviour between *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825). *Journal of Zoology, London* **268:** 55–62.

Davidson-Watts I, Walls S, Jones G. 2006. Differential habitat selection by *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* identifies distinct conservation needs for cryptic species of echolocating bats. *Biological Conservation* **133:** 118–127.

Davis LA, Roalson EH, Cornell KL, McClanahan KD, Webster MS. 2006. Genetic divergence and migration patterns in a North American passerine bird: implications for evolution and conservation. *Molecular Ecology* **15:** 2141–2152.

Dumitru M. 1995. Mammal species from Romania categories of conservation. *Travaux du Museum National d'Histoire Naturelle Grigore Antipa* **35:** 549–566.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164:** 1567–1587.

Falush D, Stephens M, Pritchard JK. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* **7:** 574–578.

Feyerabend F, Simon M. 2000. Use of roosts and roost switching in a summer colony of 45 kHz phonic type pipistrelle bats *Pipistrellus pipistrellus* Schreber, 1774. *Myotis, Bonn* **38:** 51–59.

Furmankiewicz J, Altringham J. 2007. Genetic structure in a swarming brown long-eared bat (*Plecotus auritus*) population: evidence for mating at swarming sites. *Conservation Genetics* **8:** 913–923.

Gaisler J, Hanák V, Hanzal V, Jarský V. 2003. Results of bat banding in the Czech Republic and Slovakia, 1948–2000. *Vespertilio* **7:** 3–61.

Gaisler J, Řehák Z, Bartonička T. 2002. Mammalia: chiroptera. In: Řehák Z, Gaisler J, Chytil J, eds. *Vertebrates of the Pálava Biosphere Reserve of UNESCO*. Brno: Masaryk University, 139–149.

Gaisler J, Zukal J, Řehák Z, Homolka M. 1998. Habitat preference and flight activity of bats in a city. *Journal of Zoology, London* **244:** 439–445.

Gerell R, Lundberg K. 1985. Social organization in the bat *Pipistrellus pipistrellus*. *Behavioral Ecology and Sociobiology* **16:** 177–184.

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

Goudet J, Raymond M, deMeeus T, Rousset F. 1996. Testing differentiation in diploid populations. *Genetics* **144:** 1933–1940.

Hedrick PW. 2005. A standardized genetic differentiation measure. *Evolution* **59:** 1633–1638.

Hellgren O, Bensch S, Hobson KA, Lindström A. 2008. Population structure and migratory directions of Scandinavian bluethroats *Luscinia svecica* – a molecular, morphological and stable isotope analysis. *Ecography* **31:** 95–103.

von Helversen O, Esche M, Kretzschmar F, Boschert M. 1987. Die Fledermäuse Südbadens. *Mitteilungen des badischen Landesvereins für Naturkunde und Naturschutz NF* **14:** 409–475.

Hutterer R, Ivanova T, Meyer-Cords Ch, Rodrigues L. 2005. *Bat migrations in Europe. A review of banding data and literature*. Bonn: Federal Agency for Nature Conservation.

Höglund J, Alatalo RV. 1995. *Leks*. Princeton, NJ: Princeton University Press.

Jones G. 1999. *Pipistrellus pipistrellus* (Schreber, 1774). In: Mitchell-Jones AJ, Amori G, Bogdanowicz W, Kryštufek B, Reijnders PJH, Spitzenberger F, Stubbe M, Thissen JBM, Vohralík V, Zima J, eds. *The atlas of european mammals*. London: Academic Press, 126–127.

Kalinowski ST, Wagner AP, Taper ML. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes* **6:** 576–579.

Kaňuch P, Fornůsková A, Bartonička T, Bryja J. 2007a. Multiplex panels of polymorphic microsatellite loci for two cryptic bat species of the genus *Pipistrellus*, developed by cross-species amplification within the family Vespertilionidae. *Molecular Ecology Notes* **7:** 871–873.

Kaňuch P, Hájková P, Řehák Z, Bryja J. 2007b. A rapid PCR-based test for species identification of two cryptic bats *Pipistrellus pipistrellus* and *P. pygmaeus* and its application on museum and dropping samples. *Acta Chiropterologica* **9:** 277–282.

Kepka O. 1981. Fledermäuse der Steiermark. *Myotis, Bonn* **18**–19: 168–179.

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

Kerth G, Safi K, König B. 2002b. Mean colony relatedness is a poor predictor of colony structure and female philopatry in the communally breeding Bechstein's bat (*Myotis bechsteinii*). *Behavioral Ecology and Sociobiology* **52:** 203–210.

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

Kretzschmar F, Heinz B. 1995. Social behaviour and hibernation of a large population of *Pipistrellus pipistrellus* (Schreber, 1774) (Chiroptera: Vespertilionidae) and some other bat species in the mining-system of a limestone quarry near Heidelberg (South West Germany). *Myotis, Bonn* **32**–33: 221–231.

Lundberg K, Gerell R. 1986. Territorial advertisement and mate attraction in the bat *Pipistrellus pipistrellus*. *Ethology* **71:** 115–124.

Matis Š, Uhrin M, Pjenčák P. 2002. Hibernation of bats in Erna cave. *Vespertilio* **6:** 235–236.

McCracken GF, Lumsden LF, Kunz TH. 2006. Roosting ecology and population biology. In: Zubaid A, McCracken GF, Kunz TH, eds. *Functional and evolutionary ecology of bats*. New York, NY: Oxford University Press, 179–184.

McCracken GF, McCracken MK, Vawter AT. 1994. Genetic structure in migratory populations of the bat *Tadarida brasiliensis mexicana*. *Journal of Mammalogy* **75:** 500–514.

Meirmans PG. 2006. Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution* **60:** 2399–2402.

Nagy ZL, Szanto L. 2003. The occurrence of hibernating *Pipistrellus pipistrellus* (Schreber, 1774) in caves of The Carpathian Basin. *Acta Chiropterologica* **5:** 155–160.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583–590.

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

Nicholls B, Racey PA. 2006b. Habitat selection as a mechanism of resource partitioning in two cryptic bat species *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*. *Ecography* **29:** 697–708.

Norberg UM, Rayner JM. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptions, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London Series B* **316:** 335–427.

Park KJ, Altringham JD, Jones G. 1996. Assortative roosting in the two phonic types of *Pipistrellus pipistrellus* during the mating season. *Proceedings of the Royal Society of London Series B, Biological Sciences* **263:** 1495–1499.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6:** 288–295.

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.

Petit E, Mayer F. 2000. A population genetic analysis of migration: the case of the noctule bat (*Nyctalus noctula*). *Molecular Ecology* **9:** 683–690.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155:** 945–959.

Queller DC, Goodnight KF. 1989. Estimating relatedness using genetic markers. *Evolution* **43:** 258–275.

Racey PA, Barratt EM, Burland TM, Deaville R, Gotelli D, Jones G, Piertney SB. 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.

Rachwald A. 1992. Social organization, recovery frequency and body weight of the bat *Pipistrellus nathusii* from northern Poland. *Myotis, Bonn* **30:** 109–118.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetic software for exact tests and eucumenism. *Journal of Heredity* **86:** 248–249.

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.

Rodrigues L, Palmeirim JM. 2008. Migratory behaviour of the Schreiber's bat: when, where and why do cave bats migrate in a Mediterranean region? *Journal of Zoology, London* **274:** 116–125.

Roer H. 1989. Field experiments about the homming behaviour of the common pipistrelle (*Pipistrellus pipistrellus*, Schreber). In: Hanák V, Horáček I, Gaisler J, eds. *European bat research 1987*. Prague: Charles University Press, 551–558.

Rossiter SJ, Benda P, Dietz C, Zhang S, Jones G. 2007. Rangewide phylogeography in the greater horsehoe bat inferred from microsatellites: implications for population history, taxonomy and conservation. *Molecular Ecology* **16:** 4699–4714.

Rossiter SJ, Jones G, Ransome RD, Barratt EM. 2000. Genetic variation and population structure in the endangered greater horsehoe bat *Rhinolophus ferrumequinum*. *Molecular Ecology* **9:** 1131–1135.

Rousset F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation-by distance. *Genetics* **145:** 1219–1228.

Ruczyński I, Bogdanowicz W. 2005. Roost cavity selection by *Nyctalus noctula* and *N. leisleri* (Vespertilionidae, Chiroptera) in Bialowieza Primeval Forest, Eastern Poland. *Journal of Mammalogy* **86:** 921–930.

Russ JM, Hutson AM, Montgomery WI, Racey PA, Speakman JR. 2000. The status of Nathusius pipistrelle (*Pipistrellus nathusii* Keyserling et Blasius, 1839) in the British Isles. *Journal of Zoology, London* **254:** 91–100.

Russell AL, Medellín RA, McCracken GF. 2005. Genetic variation and migration in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). *Molecular Ecology* **14:** 2207–2222.

Rydell J, Baagøe HJ. 1994. Vespertilio murinus. *Mammalian Species* **467:** 1–6.

Sachteleben J. 1991. Zum 'Invasions' – Verhalten der Zwergfledermaus (*Pipistrellus pipistrellus*). *Nyctalus (NF)* **4:** 51–66.

Sachteleben J, von Helversen O. 2006. Songflight behaviour and mating system of pipistrelle bat (*Pipistrellus pipistrellus*) in an urban habitat. *Acta Chiropterologica* **8:** 391–401.

Sattler T, Bontadina F, Hirzel AH, Arlettaz R. 2007. Ecological niche modelling of two cryptic bat species calls for a reassessment of their conservation status. *Journal of Applied Ecology* **44:** 1188–1199.

Sendor T, Kugelschafter K, Simon M. 2000. Seasonal variation of activity patterns at a pipistrelle (*Pipistrellus pipistrellus*) hibernaculum. *Myotis, Bonn* **38:** 91–109.

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.

Shiel CB, Fairley JS. 1999. Evening emergence of two nursery colonies of Leisler's bat (*Nyctalus leisleri*). *Ireland. Journal of Zoology, London* **247:** 439–447.

Smit-Viergutz J, Simon M. 2000. Eine vergleichende Analyse des sommerrlichen Schwärmverhaltens der Zwergfledermaus (45 kHz Ruftyp, *Pipistrellus pipistrellus* Schreber, 1774) an den Invasionsorten und am Winterquartier. *Myotis, Bonn* **38:** 69–89.

Storey JD. 2002. A direct approach to false discovery rates. *Journal of the Royal Statistical Society Series B, Statistical Methology* **64:** 479–498.

Strelkov PP. 1969. Migratory and stationary bats (Chiroptera) of the European part of the Soviet Union. *Acta Zoologica, Cracow* **14:** 393–440.

Wagner AP, Creel S, Kalinowski ST. 2006. Estimating relatedness and relationships using microsatellite loci with null alleles. *Heredity* **97:** 336–345.

Webb NJ, Tidemann CR. 1996. Mobility of Australian flying-foxes, *Pteropus* spp. (Megachiroptera): evidence from genetic variation. *Proceedings of the Royal Society of London Series B, Biological Sciences* **263:** 497–502.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38:** 1358–1370.

Zingg P. 1990. Akustische Artidentifikation von Fledermäusen (Mammalia: Chiroptera) in der Schweiz. *Revue suisse Zoologie* **97:** 263–294.