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Genetic structure in a swarming brown long-eared bat (*Plecotus auritus*) population: evidence for mating at swarming sites

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Abstract *Plecotus auritus*, a small, gleaning bat species, lives in small, isolated summer colonies in which both males and females show a high degree of natal philopatry. Despite this, colonies have high gene diversities and low inbreeding coefficients. It has been suggested that inbreeding is avoided because mating occurs during autumnal and spring swarming at hibernation sites. We tested this hypothesis by comparing microsatellite profiles, based on eight loci, of bats from six summer colonies and two swarming sites they were known to visit from radiotelemetry studies. We found high gene diversities (H_s = 0.77) at both swarming sites and summer colonies which were not statistically different. There was no detectable isolation by distance and F_{ST} was low (0.001). Together, these results suggest high gene flow between sites. Despite this, there was small but significant genetic differentiation amongst summer colonies and between summer colonies and the primary swarming site. We suggest that swarming is important for gene flow and for maintaining genetic diversity in this highly philopatric species and discuss possible reasons for the genetic differentiation observed. The identification and protection of swarming sites should be a major conservation priority for this and other temperate bat species.

Keywords Genetic structure · Mating · Swarming · *Plecotus* · Brown long-eared bat

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Introduction

Populations of highly mobile bat species are expected to exhibit less genetic structure and have higher rates of between-population gene flow and larger effective breeding populations than do sedentary species. In migratory bats dispersal distances may be long and genetic structure of populations is lower than in most other species (Petit and Mayer 1999; Burland and Worthington Wilmer 2001; Webster et al. 2002). Many bat populations are close to panmixia, but some degree of population structure is usually found due to physical barriers to gene flow, genetic drift and selection, life history traits such as mobility and dispersal patterns and mating systems (e.g. Petit and Mayer 1999; Castella et al. 2000; Lenormand 2002; Lowe et al. 2004). For example, in South Africa *Miniopterus schreibersii natalensis* consists of three distinct subpopulations which are correlated with habitat type (Miller-Butterworth et al. 2003) and in North America *Leptonycteris curasoae* populations align with their migration routes (Wilkinson and Fleming 1996).

Gene flow among populations of non migrating species appears more restricted than in migratory species (reviewed by Burland and Worthington Wilmer 2001). Many non-migratory species are highly philopatric and form small, closed societies of related individuals (Burland et al. 1998, 1999; Kerth et al. 2002b), which implies the existence of genetically subdivided populations. The tropical emballonurid *Saccopteryx bilineata* and the European species *Myotis myotis*, *M. bechsteinii* and *Plecotus auritus* exhibit microgeographical genetic differentiation among colonies (McCracken 1984; Petri et al. 1997; Burland et al. 1998; Kerth et al. 2000). These sedentary bat species are

potentially more prone to inbreeding depression than migratory species, but genetic studies of *P. auritus* and *M. bechsteinii* have shown that colony inbreeding coefficients are low and gene diversities are high (Burland et al. 1998; Kerth et al. 2003; Veith et al. 2004). This must result from a mating system that prevents inbreeding, which is perhaps facilitated by the mobility conferred by flight. Such a system may be especially important in *P. auritus*, since it forms small, mixed-sex summer colonies that may share the same roost from the end of March to the end of November (Entwistle et al. 2000, J. Furmankiewicz, unpublished). Furthermore, both sexes show natal philopatry and long-term association with a colony (Entwistle et al. 2000). It has been suggested that, in *P. auritus* and *M. bechsteinii*, inbreeding is avoided via extra-colony copulations (Burland et al. 1998, Kerth et al. 2003). There is some direct evidence for mating in colony roosts (Stebbings 1966, 1970) and in hibernacula in *P. auritus* (Moffat 1922).

A behaviour known as swarming occurs in the autumn, which is the primary mating period of European bat species. This activity involves vocalization, chasing and sporadically observed copulation of often large aggregations of bats in and around many hibernacula (e.g., Davis and Hitchckock 1965, Hall and Brenner 1968, Fenton 1969, Horáček and Zima 1978, Thomas et al. 1979, Bauerová and Zima 1988, Furmankiewicz 2002, Furmankiewicz and Górniak 2002, Parsons et al. 2003). Swarming bats frequently return to their summer (or transitional) roosts after swarming, particularly early in the swarming season (Parsons and Jones 2003; J. Furmankiewicz in prep.). Swarming males show signs of sexual activity, with distended cauda epididymides (Kerth et al. 2003; Parsons et al. 2003; Furmankiewicz 2004) and it is now widely believed that swarming in hibernacula has a mating function in many temperate zone bat species (Fenton 1969; Horáček 1975; Horáček and Zima 1978; Thomas et al. 1979; Furmankiewicz and Górniak 2002; Parsons et al. 2003; Rivers et al. 2005, 2006). Swarming populations are large: Rivers et al. (2006) estimated 4,000 Natterer's bats at one site (see also Bauerová and Zima 1988; J. Furmankiewicz in prep.) and are composed of bats from different summer roosts (Parsons and Jones 2003; Veith et al. 2004; Rivers et al. 2006; Furmankiewicz 2004, in prep.) suggesting that this behaviour significantly increases the effective population size.

Swarming sites used by *M. nattereri*, *M. daubentonii* and *M. lucifugus* attract bats from great distances (e.g. Davis and Hitchcock 1965; Parsons and Jones 2003; Rivers et al. 2006). Relative to these and most other species, *P. auritus* has low aspect ratio wings and low wing loading (Norberg and Rayner 1987), which facilitate slow and highly manoeuvrable flight in cluttered environments, but increase flight costs. Home ranges are small (Fuhrmann and Seitz 1992; Entwistle et al. 1996; J. Furmankiewicz, unpublished) and we might expect *P. auritus* to travel significantly shorter distances to swarming sites. However, swarming *P. auritus* were radiotracked to roosts up to 31 km from the swarming site (Furmankiewicz 2004, in prep.) and ringed bats have been recorded to move further (66 km in Masing (1989) and 88 km in Gaisler et al. (2003)). Furthermore, they frequently fly from distant roosts to a swarming site for just a few hours (Furmankiewicz 2004, in prep.). *Plecotus auritus* appears unusual in that it swarms twice a year, in autumn and in spring (Furmankiewicz 2004, in prep): most species appear only to swarm in the autumn (e.g. Parsons and Jones 2003; Rivers et al. 2006). Swarming must play an important role in this species' life cycle, given the high cost of these frequent and long flights.

Several functions have been attributed to swarming: mating (Fenton 1969; Thomas et al. 1979; Parsons et al. 2003; Parsons and Jones 2003; Kerth et al. 2003; Rivers et al. 2005), information transfer about suitable hibernacula (Fenton 1969; Horáček 1975; Veith et al. 2004) and seasonal migration (Hall and Brenner 1965, 1968; Horáček and Zima 1978). These functions are not mutually exclusive, but the best evidence suggests that mating is important: swarming appears to promote gene flow between bat colonies, increasing genetic diversity (Kerth et al. 2003; Veith et al. 2004; Rivers et al. 2005). In sedentary, non-migratory species such as *Plecotus auritus*, the seasonal, long-distance movements involved in swarming could be essential in prevent inbreeding (e.g. Petit and Mayer 2000; Burland and Worthington Wilmer 2001).

If many bats from different summer colonies meet at swarming sites and mate with individuals from colonies other than their own, this should result in high gene diversity and genetic similarity between the spatially isolated summer colonies. We studied the microgeographical genetic structure of *P. auritus* using microsatellite markers. We investigated two swarming sites and six summer colonies known to swarm at these sites. Our field data confirm the movement of bats between the sampled summer and swarming sites. Previous studies (Kerth et al. 2003; Veith et al. 2004) used only genetic data on a larger landscape scale and lacked behavioural support from field studies or concentrated only on summer colonies without reference to mating sites (Burland et al. 1999). Therefore our investigation provides strong, direct evidence for the importance of

swarming sites and their role in determining population structure.

If mating and significant gene flow occur at swarming sites, we predicted:

- High gene diversity and low inbreeding coefficients in summer colonies and swarming populations
- Little or no genetic differentiation and no isolation by distance among summer colonies
- Low mean within summer colony relatedness

These would confirm the importance of autumn swarming to *P. auritus* and would confirm the need for conservation of underground swarming sites.

Methods

The study was performed in SW Poland in the hilly and agricultural landscape of the Sudetic Foreland. Swarming bats were sampled primarily at one site, an abandoned mine in Skałki Stoleckie (Stoleckie Rocks, 50°34′ N, 16°52′ E), where high autumn and spring swarming activity of *P. auritus* has been observed regularly since 2000 (Furmankiewicz 2004) (Fig. 1). A second swarming site (Szklary, three small abandoned mines), located about 6 km NW of the first and with lower activity, was sampled at irregular intervals. All

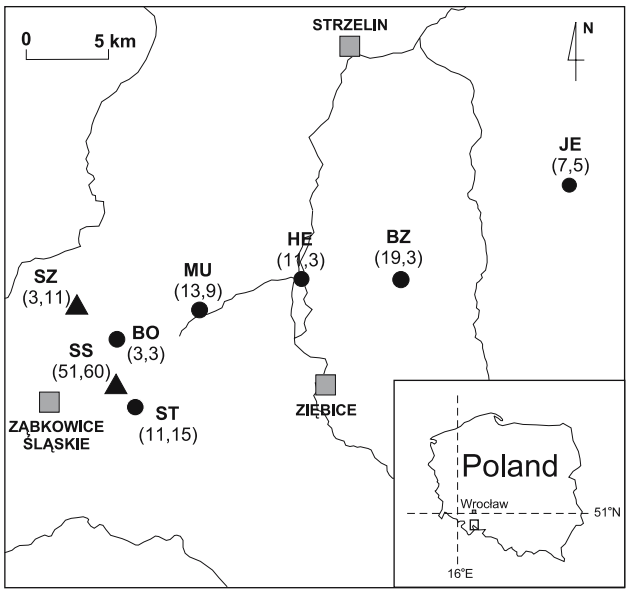

Fig. 1 Study area with the sample sites of swarming (SS—Skałki Stoleckie, SZ—Szklary) and summer populations (BO—Bobolice, BZ—Bożnowice, HE—Henryków, JE—Jeszkotle, MU—Muszkowice, ST—Stolec) of *Plecotus auritus*. The number of genotyped bats (female, male) are given in brackets. Individuals from all of the summer colonies are known to visit the swarming site at Skałki Stoleckie

sampled summer colonies were found by radiotracking swarming bats to their day roosts. All roosts within a single village or small forest area (about 2 km^2) were considered to be a single summer population. In some cases they may comprise a single colony, with free interchange between roosts. We sampled from one to four roosts from each population. From the tracking and ringing studies we know that at least 1 to 6 bats from each population visited the mine during the autumn and spring swarming seasons (Furmankiewicz 2004, in prep).

Bats were caught using mist nets or harp traps. All bats were sampled in 2003, swarming bats in March–April and August–October, bats at summer roosts throughout the summer, except mid-May–end of June to avoid disturbing nurseries. Wing membrane biopsies, 3 mm in diameter, were taken from both wings of each bat (Worthington Wilmer and Barratt, 1996). The total numbers of sampled bats at each site are shown in Fig. 1. Samples were stored in 99% ethanol. DNA isolation was done using a phenol-chloroform procedure (Sambrook et al. 1989). Eight dinucleotide microsatellite markers were used. Five were specifically developed for *P. auritus*: Paur01, Paur02, Paur04, Paur05, Paur06 (Burland et al. 1998) and three for other bat species: H29, D15, G30 (Castella and Ruedi 2000; Kerth et al. 2003). Samples were amplified using PCR with MJResearch DNA Engine Tetrad (Table 1 for conditions) and genotyped with an ABI3730 sequencer (Applied Biosystems) and GeneMapper 3.0 software. Each PCR solution contained 1.0–5.0 ng genomic DNA, 0.1–1.0 μM of each primer, 1.0–2.5 μl 50 mM $MgCl_2$ (Table 1), 1 μl 2 mM dNTPs, 1.0 μl 10 × *Taq* buffer and 0.5 U of *Taq* polymerase (ABgene). The optimized PCR profiles were as follows: 94°C for 3 min.; 35 cycles at 94°C for 30 s, 48.4–66°C (annealing temperature depending on primer, see Table 1) for 30 s and 72°C for 30 s; 72°C for 10 min. We genotyped 202 bats (101 females and 101 males) at the eight loci. Four additional primers (Paur03, B22, B15, G9) were tested but not used in the analysis because of sex linkage or messy PCR products (Table 1). Modified PCR conditions were applied and are given in Table 1.

Data were analysed using Genepop 3.4 (Goudet 2001: Hardy–Weinberg equilibrium, linkage disequilibrium, genetic differentiation, isolation by distance); FSTAT 2.9.3 (Raymond and Rousset 1995: gene diversity, observed heterozygosity, F statistics). Genetic isolation by distance was tested as $F_{ST}/(1 - F_{ST})$ versus log distance and using a Mantel test with 1000 permutations. SPAGeDi 1.2 software (Hardy and Vekemans 2002) was used to calculate pairwise relatedness within swarming populations, within and between-summer populations (at the individual level) and within combined sex, summer and swarming

Table 1 PCR conditions, post-PCR dilution, observed number of alleles and allele sizes for microsatellite loci used in genotyping 202 brown long-eared bats (*Plecotus auritus*) from SW Poland

Primer	Annealing temperature (°C)	DNA concen-tration (ng/ µl)	$MgCl_2$ (µl)	DMSO (50%) BSA (1.5 ng/µl)	Primer (µl)[a]	Alleles	Allele sizes observed (bp)	Post-PCR dilution	References
Paur01	60–48 (–0.5 per cycle)	1.0	2.5	0.2 µl DMSO 0.1 µl BSA	0.1	11	125-163	1:5000	Burland et al. (1998)
Paur02	60–56 (–1 per cycle)	1.0	2.0	0.1 µl DMSO	0.1	23	198–247	1:1250	Burland et al. (1998)
Paur03[b]	61	1.0	1.5	–	1.0	5	249–260 X-linked	1:5000	Burland et al. (1998)
Paur04	66–56 (–1 per cycle)	1.0	1.0	–	1.0	23	212–263	1:2500	Burland et al. (1998)
Paur05	55	1.0	1.0	0.2 µl DMSO	0.1	16	221–252	1:5000	Burland et al. (1998)
Paur06	53.4	5.0	1.0	-	1.0	30	163–228	1:1250	Burland et al. (1998)
H29	48.4	1.0	1.0	–	1.0	15	190–219	1:500	Castella and Ruedi (2000)
G30	60–48 (–1 per cycle)	1.0	1.0	–	1.0	16	129–170	1:500	Castella and Ruedi (2000)
D15	49,2	1.0	1.0	–	1.0	5	92–131	1:2500	Castella and Ruedi (2000)
B22[b]	60–48 (–1 per cycle)	1.0	1.5	–	1.0	–	Monomorphic	1:5000	Castella and Ruedi (2000)
B15[b]	60–48 (–1 per cycle)	1.0	1.5	–	1.0	–	Monomorphic	1:500	Kerth et al. (2002b)
G9[b]	51.3	1.0	1.0	–	1.0	–	Unclear	1:2500	Castella and Ruedi (2000)

[a] Stock concentration of primer 10 pmol/µl for each primer, except D15 (5 pmol/µl). [b]loci excluded from analysis, Paur03 sex-linked

populations (at the population level). At the individual level the relatedness coefficient was estimated according to Queller and Goodnight (1989): $r_{ij} = \Sigma_l \Sigma_a \Sigma_{ci} x_{lcia}(p_{jla} - p_{la})/\Sigma_l \Sigma_a \Sigma_{ci} x_{lcia} \ (p_{ila} - p_{la})$ and computed as the average relatedness coeffiecient $(r_{ij} + r_{ji})/2$, where x_{lcia} is an indicator variable ($x_{lcia} = 1$ if the allele on chromosome c at locus l for individual i is a, otherwise $x_{lcia} = 0$), p_{la} is the frequency of allele a at locus l in the reference sample, Σ_{ci} = the sum over the homologous chromosomes of individual i, and p_{ila} and p_{jla} are the frequencies of allele a at locus l in individuals i *and* j, respectively (Hardy and Vekemans 2002). At the population level the relatedness was related to F_{ST} as $relat = 2\ F_{ST}/(1 + F_{IT})$ (Raymond and Rousset 1995). For relatedness an F statistic permutation test (1000 random permutations) was performed in SPAGeDi 1.2 to compare observed values with the expected frequency distributions. In GeneClass 2.0 (Piry et al. 2004) the assignment test was performed to compute the probability that each individual from the swarming populations belongs to each reference summer colony with an assignment threshold probability of 0.01. The probability of each sampled individual from the summer populations belonging to the Skałki Stoleckie swarming population was also calculated. The assignment method was based on multilocus genotypes of representative samples from the candidate populations and of the individual to be assigned, using a Bayesian method (Rannala and Mountain 1997). A simulation (Paetkau et al. 2004) with 10,000 simulated individuals was performed.

Those individuals that were captured at both one of the swarming sites and at one of the summer colonies ($n = 25$) were included in both groups for analysis. Gene diversity, heterozygosity and F statistics were calculated for each locus and over all loci for males and females separately. Data from both sexes were combined for analysis of Hardy–Weinberg equilibrium, genetic isolation by distance and genetic differentiation.

Results

All loci used in the analysis, with the exception of D15 (5 alleles), were highly polymorphic with from 11 to 30 alleles (Table 1). Paur04 was out of Hardy–Weinberg equilibrium in one summer population, but was in equilibrium when all bats were analysed together

(Table 2), so it was included in the analysis. H29 and G30 were out of equilibrium in one population, and for all bats combined. This may be due to the presence of null alleles. All further analysis was carried out with and without these two loci, for comparison. No significant linkage disequilibrium was found in the data set.

Gene diversity and observed heterozygosity were high in all summer and swarming populations and similar in the two groups, regardless of whether six or eight loci were included in the analysis (Tables 2 and 3). F_{ST} was low in all comparisons, although significantly different from zero for summer population females, suggesting some structuring amongst summer colonies. F_{IS} at the colony level was not significant, indicating low levels of inbreeding. However, F_{IS} for swarming bats was higher than for summer colonies and significantly different from zero (Table 3). This suggests mixing between distinct groups and is discussed later. In summer colonies relatedness was greater amongst females than males and female relatedness was significantly different from zero. The relatedness amongst males from both swarming sites

Table 2 Hardy–Weinberg equilibrium *P*-value (HWE), F_{IS}, gene diversity, observed (H_O) and expected heterozygosity (H_E) for the summer (BO, BZ, HE, JE, MU, ST) and swarming populations (SS, SZ)

Population No. of individuals	BO 6	BZ 22	HE 14	JE 12	MU 22	ST 26	SS 111	SZ 14	All 227
Locus H29									
HWE	0.167	0.038^{NS}	0.848	0.715	0.241	0.147	**0.0002**	0.911	**0.004**
F_{IS}	0.286	0.160	–0.169	–0.017	0.122	0.191	0.172	–0.070	0.129
Gene diversity	0.933	0.866	0.734	0.902	0.880	0.903	0.859	0.868	0.867
Locus Paur01									
HWE	1.000	0.772	0.296	0.147	0.968	0.316	0.706	0.134	0.608
F_{IS}	–0.176	–0.102	0.146	0.154	0.019	–0.106	0.027	0.059	0.007
Gene diversity	0.850	0.784	0.585	0.689	0.788	0.800	0.797	0.835	0.766
Locus Paur05									
HWE	0.912	0.275	0.661	0.346	0.605	0.125	0.135	0.311	0.371
F_{IS}	–0.200	0.000	–0.148	0.124	–0.050	0.052	0.058	–0.009	0.021
Gene diversity	0.833	0.864	0.871	0.856	0.866	0.771	0.842	0.920	0.854
Locus Paur02									
HWE	1.000	0.377	0.311	0.609	0.957	0.998	0.355	0.176	0.814
F_{IS}	–0.091	0.085	0.020	–0.008	–0.011	–0.077	0.009	0.090	0.006
Gene diversity	0.917	0.844	0.948	0.909	0.944	0.928	0.936	0.942	0.922
Locus Paur06									
HWE	0.626	0.884	0.904	0.547	0.784	0.813	0.516	0.104	0.899
F_{IS}	0.091	0.012	0.000	–0.100	–0.013	–0.010	–0.019	0.116	–0.006
Gene diversity	0.917	0.920	0.929	0.909	0.943	0.914	0.946	0.970	0.930
Locus G30									
HWE	1.000	0.433	0.545	0.541	0.381	0.011 ^{NS}	**0.000**	0.345	**0.000**
F_{IS}	–0.176	0.047	–0.032	0.048	0.015	0.061	0.107	0.130	0.067
Gene diversity	0.850	0.763	0.761	0.788	0.785	0.819	0.717	0.739	0.778
Locus Paur04									
HWE	1.000	0.773	0.400	0.303	0.656	**0.005**	0.247	0.161	0.124
F_{IS}	–0.111	–0.070	0.027	–0.247	–0.056	0.261	0.068	0.085	0.043
Gene diversity	0.450	0.637	0.808	0.735	0.775	0.676	0.706	0.780	0.698
Locus D15									
HWE	1.000	1.000	1.000	1.000	0.668	0.039^{NS}	0.089	1.000	0.733
F_{IS}	–0.053	–0.024	–0.130	–0.146	0.099	0.352	0.097	–0.083	0.097
Gene diversity	0.317	0.089	0.253	0.364	0.504	0.415	0.302	0.198	0.306
Global-8 loci									
HWE	0.997	0.595	0.906	0.713	0.947	**0.002**	**0.000**	0.206	**0.000**
F_{IS}	–0.044	0.023	–0.031	–0.016	0.012	0.067	0.060	0.052	0.040
H_O	0.792	0.705	0.759	0.781	0.801	0.726	0.718	0.741	0.753
H_E	0.761	0.720	0.737	0.769	0.810	0.777	0.763	0.780	0.778
Global-6 loci (loci G30 and H29 excluded)									
HWE	1.000	0.923	0.841	0.578	0.992	0.017^{NS}	0.242	0.126	0.321
F_{IS}	–0.089	–0.011	–0.008	–0.027	–0.009	0.044	0.031	0.062	0.018
H_O	0.778	0.697	0.738	0.764	0.811	0.718	0.732	0.726	0.745
H_E	0.720	0.690	0.732	0.745	0.803	0.750	0.755	0.772	0.759

For a key to the site name abbreviations see Fig. 1. Populations not in HWE (after sequential Bonferroni correction) are in bold, ^{NS} = not significant

Table 3 Gene diversity (H_S), observed heterozygosity (H_O), F statistics and relatedness for females and males across colonies and swarming sites

	Females			Males			All individuals		
	Summer populations	Swarming sites	All	Summer populations	Swarming sites	All	Summer populations	Swarming sites	All
No. populations	6	2	8	6	2	8	6	2	8
No. individuals	64	54	118	38	71	109	102	125	227
H_S	0.77	0.81	0.78	0.77	0.76	0.77	0.76	0.77	0.77
							0.74	0.76	0.75
H_O	0.76	0.78	0.77	0.75	0.71	0.74	0.76	0.73	0.75
							0.75	0.73	0.75
F_{IS}	0.012	0.062**	0.036*	0.027	0.054***	0.044**	0.017	0.059***	0.040***
95% CI	–0.023–0.054	0.023–0.107	0.002–0.071	–0.029–0.089	0.003–0.117	–0.004–0.100	–0.002	0.034*	0.019
F_{IT}	0.042*	0.046	0.051***	0.038	0.059**	0.052***	0.040**	0.058***	0.053***
95% CI	0.004–0.088	–0.000–0.096	0.017–0.090	–0.018–0.098	0.005–0.119	0.004–0.108	0.025	0.033*	0.032*
F_{ST}	0.031***	–0.016	0.016***	0.011	0.006	0.008	0.024***	–0.001	0.013***
95% CI	0.018–0.047	–0.051–0.016	0.010–0.024	–0.008–0.027	–0.007–0.024	0.000–0.016	0.027***	–0.002	0.014***
Relatedness	0.059***	–0.031	0.031***	0.022	0.011	0.016	0.046***	–0.002	0.025***
95% CI	0.036–0.089	–0.098–0.030	0.019–0.045	–0.015–0.053	–0.014–0.046	0.000–0.030	0.053***	–0.003	0.027***

Note: 95% confidence intervals (CI) are given and the level of significance (testing for significant departure from zero: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p<0.001$), after 1000 permutations. In the three last columns values calculated using 6 loci (Paur01, Paur02, Paur04, Paur05, Paur06, D15, top) and 8 loci (Paur01, Paur02, Paur04, Paur05, Paur06, D15, H29, G30, bottom) are given. In all other columns results for 8 loci are given

and summer colonies was low and not significantly different from zero (Table 3). A G-test with 500 permutations for female and male gene diversity H_S, observed heterozygosity H_O, F_{IS}, F_{ST} and relatedness revealed two significant results: higher F_{ST} ($P = 0.02$) and relatedness ($P = 0.03$) in summer population females relative to swarming females.

Within-summer populations pairwise relatedness was $r = 0.024$ (8 loci) and $r = 0.029$ (6 loci) and between-summer populations $r = –0.018$ (8 loci) and $r = –0.019$ (6 loci). The observed values for within-summer populations were low but significantly different ($P < 0.001$) from simulated values (–0.020 to 0.002 with 8 loci and –0.021 to 0.005 with 6 loci analysis). The within-swarming populations relatedness was very low, $r = –0.006$ (8 and 6 loci analysis) and not different from the expected values. Computation at the population level gave similar results (Table 3), but the high average relatedness within summer populations is probably influenced by high values for females.

Genetic isolation by distance was tested for the six summer populations. The minimum distance between two populations was 5.5 km, and the maximum distance 31.5 km. There was no significant genetic isolation by distance with either six or eight loci in the analysis ($P = 0.697, 0.829$, respectively). There was also no significant increase in genetic distance of summer populations samples from the Skałki Stoleckie swarming site sample with geographical distance (regression, $P = 0.442$).

We looked for pairwise population differences between five of the summer populations and the two swarming populations. The Bobolice (BO) colony was excluded due to the small sample size. Most of the summer populations were significantly different from the Skałki Stoleckie swarming site ($n = 111$), using both six and eight loci, with two exceptions: Muszkowice, and Stolec, the two sites closest to the swarming site. Stolec was significantly different from Skałki Stoleckie using only the 8 loci analysis. Only Stolec and Bożnowice were significantly different to the second swarming site, Szklary ($n = 14$) (eight loci analysis only). The two swarming sites were not significantly genetically different. There were also significant genetic differences between almost every pair of summer populations, with either six or eight loci analysis (Table 4).

The genetic differentiation among summer and swarming populations was supported by the results of assignment tests, which estimated the probability of each summer population individual belonging to the Skałki Stoleckie swarming population. The proportion of bats from each summer population assigned (with a

Table 4 Genetic differentiation between populations of bats visiting swarming sites and bats from colonies calculated with Fisher test after applying Markov chain procedure (dememorization 10,000, batches 100, iterations per batch 5000)

Pairwise comparison between	*P*-values	
	6 loci	8 loci
Colonies		
BZ–HE	*	*
BZ–JE	*	*
BZ–ST	*	*
BZ–MU	*	*
JE–ST	*	*
JE–MU	*	NS
JE–HE	*	*
MU–ST	*	*
MU–HE	NS	NS
HE–ST	*	*
Colonies and swarming sites		
SS–BZ	*	*
SS–JE	*	*
SS–HE	*	*
SS–MU	NS	NS
SS–ST	NS	*
SZ–BZ	*	NS
SZ–JE	NS	NS
SZ–HE	NS	NS
SZ–MU	NS	NS
SZ–ST	*	*
Swarming sites		
SS–SZ	NS	NS

Note: Based on 6 and 8 loci from 5 colonies and two swarming sites (colony BO excluded, because of small sample size). For abbreviations see Fig. 1. The significance of P values is given after sequential 3 Bonferroni correction

*$P < 0.05$, NS: not significant

probability >0.01, and 10,000 simulated individuals) to the swarming site ranged from 41% to 100% and those not assigned to the swarming site from 0% to 59% (Table 5). There was a negative relationship between the proportion of bats from each summer population assigned to the Skałki Stoleckie swarming site and the distance from the swarming site, but the two were not significantly correlated (Table 5, 8 *loci analysis:* $r_s = -0.6$, $n = 6$, $P = 0.208$, *6 loci:* $r_s = -0.429$, $n = 6$, $P = 0.397$). 26.5–28.6% of the bats in the Skałki Stoleckie and Szklary swarming populations were not assigned to the sampled summer populations (Table 6). Most swarming bats were assigned to the nearest summer populations: Bobolice, Stolec and Muszkowice. There was a negative relationship between distance from the Skałki Stoleckie mine to the summer colony and assignment of swarming bats to the summer populations, but they were not significantly correlated ($r_s = -0.657$, $n = 6$, $P = 0.156$).

Discussion

This study is one of just two to date (see also Rivers et al. 2005) to investigate the small scale genetic structure of a swarming species in which field data confirm the movement of bats between the sampled summer and swarming sites. Previous studies (Kerth et al. 2003; Veith et al. 2004) used only genetic data on a larger landscape scale, sampling bats in summer and swarming populations sites not known to be related. Veith et al. (2004) did find some mtDNA haplolineages from their summer colonies at swarming sites, but an analysis of 'unrelated' summer and swarming sites may influence the conclusions drawn on population structure.

Swarming sites as hot spots for gene flow

Several indirect estimates are commonly used for measuring gene flow, including inbreeding and relatedness coefficients and population genetic structure (Lowe et al. 2004). Our calculations of all these

Table 5 Proportion of investigated individuals from summer populations assigning to Skałki Stoleckie swarming site with the probability greater than 0.01, based on 8 and 6 loci analysis, with 10,000 simulated individuals

Summer populations	Distance from swarming site (km)	Number of known swarming bats observed in each summer population	8 loci				6 loci			
			Skałki Stoleckie mine		Not Skałki Stoleckie mine		Skałki Stoleckie mine		Not Skałki Stoleckie mine	
			n	%	*n*	%	*n*	%	*n*	%
Stolec	0.5	15	21	80.8	5	19.1	20	76.9	6	23.1
Bobolice	3.0	1	6	100.0	0	0.0	5	83.3	1	16.7
Muszkowice	6.0	11	9	40.9	13	59.1	9	40.9	13	59.1
Henryków	11.5	2	11	78.6	3	21.4	7	50.0	7	50.0
Bożnowice	17.5	3	16	72.7	6	27.3	14	63.6	8	36.4
Jeszkotle	31.5	1	8	66.7	4	33.3	7	58.3	5	41.7
Together	–	33	71	69.4	31	30.4	62	60.8	40	39.2

Table 6 Proportion of swarming bats (Skałki Stoleckie and Szklary mines) genetically assigned (with probability threshold > 0.01) to known summer population of swarming bats (based on 8 and 6 loci analysis). Assigned analysis with 10,000 simulated individuals

	Swarming population	Stolec		Bobolice		Muszkowice		Henryków		Bożnowice		Jeszkotle		Neither	
		n	%	*n*	%	*n*	%	*n*	%	n	%	*n*	%	*n*	%
8 loci	Skałki Stoleckie	19	16.8	17	15.0	27	28.9	6	5.3	5	4.4	9	8.0	30	26.6
	Szklary	1	7.1	2	14.3	3	21.4	3	21.4	0	0.0	1	7.1	4	28.6
6 loci	Skałki Stoleckie	18	16.2	17	15.3	26	23.4	6	5.4	5	4.5	9	8.1	30	27.0
	Szklary	1	7.1	2	14.3	3	21.4	3	21.4	0	0.0	1	7.1	4	28.6

parameters suggest high gene flow between bats at both swarming sites and summer colonies. High gene flow is indicated by the low inbreeding coefficients, low fixation indices (F_{ST}) and generally low average relatedness in summer colonies. Low and similar F_{ST} values were also obtained for colonies of *P. auritus* in Scotland (Burland et al. 1999), in which mean colony relatedness was low and most of the offspring were fathered by males originating from a different colony (Burland et al. 2001). Relatedness between juveniles and adult females was also found to be low in a study of *P. auritus* summer colonies in Germany (Veith et al. 2004). In this study, F_{ST} and relatedness were higher in female summer populations than in swarming females, possibly due to the presence of significant numbers of mother-offspring, or other closely related pairs, within summer site samples. Although both F_{ST} and relatedness for summer colony females were low, they were significantly different from zero, indicating some population structure. Stolec was the most structured summer population, since H_O was larger than H_E and F_{IS} was greater than zero. This population consisted of two groups—bats inhabiting a building and bats found in tree hole. We did not observe any movements of bats between these roosts. This fact, supported by strong female philopatry suggests that these individuals may form two distinct subpopulations. The small sample size did not allow separate analysis. Furthermore, F_{IS} was significantly greater than zero at swarming sites, suggesting the mixing of partly distinct populations, a view supported by the observed genetic differentiation of summer populations. This is probably due to the lack of female-based gene exchange between colonies (Burland et al. 1999; Kerth et al. 2002a; Rivers et al. 2005). Therefore, the extra-colony mating is male-based, which is supported by a lower F_{ST} value for males. Even with complete female natal philopatry, many first-order male relatives will occupy different summer colonies and the probability that males from the same colony share alleles identical in state will be lower than for females (Burland et al. 1999). The difference in F_{ST} between males and females suggests that males may be less philopatric than females, and so gene flow may not be entirely dependent on mating at swarming sites. However, we must be cautious, since the result is not statistically significant, although the female F_{ST}, in contrast to that of the males, is significantly different from zero. The significant departure from zero of the F_{IS} for swarming males suggests that the population is made up of males from genetically different colonies. This in turn suggests some degree of male philopatry and a tendency to mate with the members of the same colony, presumably in the summer roosts, as observed by Stebbings (1966, 1970). The very low number of offspring sired by males from the same colony (Burland et al. 2001), the observed mating in swarming sites (Moffat 1922; Horáček 1975) and our own data suggest that swarming is the primary mating system. Similar results were also found in *M. bechsteinii* (Kerth et al. 2003), a population of *P. auritus* in Germany (Veith et al. 2004) and *M. nattereri* (Rivers et al. 2005).

Hibernacula provide an ideal opportunity for extra-colony mating, because they attract bats from many colonies during autumn swarming (e.g. Bauerová and Zima 1988; Furmankiewicz and Górniak 2002; Kerth et al. 2003; Parsons et al. 2003; Veith et al. 2004; Rivers et al. 2005, 2006). Many insectivorous bats, including *P. auritus*, form small dispersed colonies and it would be energetically expensive for bats to travel from one colony to another searching for sexually active mates. This is especially important for males that are solitary and show natal philopatry (Furmankiewicz 2004, in prep.).

That swarming is a mating event in *P. auritus* is strongly supported by the observed copulations, the sexual status of swarming males, intensive vocalization and chasing, and the fact that bats make long journeys (up to 31 km) to spend just a few hours at a swarming site (Furmankiewicz 2002, 2004, 2005, in prep.). It has also been suggested that juveniles may learn the location of hibernacula from colony adults during swarming (Veith et al. 2004). This may indeed be a secondary function of swarming, but there is no direct evidence to support the idea. Kerth et al. (2003) observed high genetic diversity in females visiting swarming sites on the same night and no mother-daughter pairs, observations that do not support information transfer as a

primary function. Furthermore, in *P. auritus*, many adult bats also visit swarming sites in spring, when information transfer about hibernacula would be unimportant (Furmankiewicz 2004, in prep.).

Burland et al. (1999) reported significant genetic isolation by distance over distances up to 100 km, in spite of low inter-colony F_{ST} estimates, suggesting the existence of a continuously distributed population, within which genes move via a stepping-stone model (Burland et al. 1999; Entwistle et al. 1996). In our study we did not detect significant genetic isolation by distance between summer colonies, but we sampled only a few colonies in relatively close proximity to each other. However, these results are consistent with the movement of bats between colonies and swarming sites. If the main function of swarming behaviour is mating, then this movement facilitates gene flow and leads to the absence of isolation by distance. Similarly, isolation by distance was not detected between summer colonies of swarming *M. nattereri* unless distances exceeded 100 km (Rivers et al. 2005). However, on a smaller scale there was a significant negative correlation between assignment of summer colony individuals to a swarming site and the distance between summer colony and swarming site, in *M. nattereri* (Rivers et al. 2005). More of the bats we captured at swarming sites were radiotracked to nearby summer roosts (Stolec and Muszkowice) than more distant roosts (Furmankiewicz 2004, in prep.). Taking all of the evidence together, bats from a given colony are most likely to visit the nearest swarming site, but bats from one colony may visit more than one swarming site, facilitating limited gene flow between swarming sites.

Strong philopatry, limited movement of bats between colonies and microgeographic genetic isolation suggest that in many species a colony behaves as a distinct subpopulation (Humphrey and Cope 1976; Burland et al. 1998; Kerth et al. 2000; Entwistle et al. 2000). The high F_{IS} values for swarming bats relative to summer colonies and female philopatry in our study suggest that different populations are mixing at swarming sites. Humphrey and Cope (1976) suggested that *Myotis lucifugus* forms demes: spatially separated local populations with limited gene flow between them. They argued that mating during swarming increases the probability of copulations between individuals from different demes, reducing inbreeding and loss of heterozygozity. If copulation occurs at swarming sites, where members of different colonies meet, mating between females and males from the same colony becomes far less likely. If the swarming populations are mixes of the bats from partly isolated summer populations, F_{IS} at the swarming sites will be similar to F_{ST} among summer populations, as we observe. This may still be compatible with random mating at swarming sites, given philopatry. However, if male philopatry is incomplete, mating is probably not random.

How many colonies visit a swarming site? Kerth et al. (2003) analysing mtDNA assumed that swarming *M. bechsteinii* come from at least three to eight nursery colonies. Veith et al. (2004) found three haplolineages occurring in both swarming populations and summer colonies, but five haplotypes appeared only in summer colonies and seven haplotypes only at swarming sites. We sampled six summer populations whose members were known to visit the Skałki Stoleckie swarming site. However there are probably more, since 27% of the swarming bats were not assigned to the sampled summer populations. *Plecotus auritus* forms nursery colonies and autumn groups of approximately 25–50 individuals (Horaček 1975; Entwistle et al. 2000, unpublished data). Autumn swarming population size of *P. auritus* at these study sites was estimated to be 550–1150 individuals (J. Furmankiewicz in prep.). Therefore swarming bats may come from at least 10 summer colonies and perhaps very many more. Rivers et al. (2006) estimated that 4,000 *M. nattereri* visited a small cluster of swarming caves in the UK, implying that 40 or more colonies may visit the site.

Genetic differentiation of summer colonies

Differentiation amongst summer colonies suggests incomplete mixing, but as discussed, mating in summer roosts and female philopatry can generate genetic differentation. Even if all mating occurs at swarming sites, Rivers et al. (2005) have shown that a low but significant F_{ST} value among summer colonies can arise when the females are philopatric, since male-mediated gene flow will not completely remove the structure generated by female philopatry. However, other mechanisms may contribute to genetic differentiation between summer colonies.

Bats from different colonies use different swarming sites

Bats do appear to show high fidelity to swarming sites (Parsons and Jones 2003; Furmankiewicz 2004; Senior at al. 2005; Rivers et al. 2006) but some bats have been shown to visit more than one site (Davis and Hitchcock 1965; Fenton 1969; Rivers et al. 2006). These sites can be close together and may be thought of as a single swarming area (Rivers et al. 2006). Rivers et al. (2006) found significant genetic differences between swarming sites 60 km apart. In the same study, assignment of

M. nattereri from summer colonies to a particular swarming site decreased as the distance between swarming site and summer colony increased. This suggests that as distance from a particular swarming site increased, bats were more likely to use other sites, closer to their roosts.

Skewed mating success

Some males could be more successful than others, but an earlier genetic study of *P. auritus* showed little or no skew in male reproductive success (Burland et al. 2001). If this is the case, random mating at swarming sites is expected. Bats in this study visited a swarming site every few days and the brief stay (usually 2–3 h, J. Furmankiewicz, in prep.) does not allow males to monopolise females, favouring random mating. However, there could be some female choice, for example through vocal flight displays or chasing. Watt and Fenton (1995) found reproductive success to be slightly skewed in swarming *Myotis lucifugus*. Swarming *P. auritus* males roost significantly closer to swarming sites than females and in the spring (the last phase of swarming in *P. auritus*) visit underground sites more often than females (Furmankiewicz 2004, in prep.).

Conservation implication

Mating at swarming sites maintains genetic diversity and outbreeding through gene flow. Outbreeding in the European bat species *Rhinolophus ferrumequinum* increases individual survival, especially of the young (Rossiter et al. 2001). Because swarming sites may support large populations from large geographical areas (Parsons and Jones 2003; Furmankiewicz 2004; Rivers et al. 2006) they need special protection. Swarming sites are often large underground hibernacula. Therefore the use of gates to protect winter bat colonies should consider their effect on swarming behaviour (Pugh and Altringham 2005).

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