Population genetic structure and gene flow in a gleaning bat, *Plecotus auritus*

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During summer the brown long-eared bat *Plecotus auritus* (Vespertilionidae) forms stable colonies, comprised of both adult females and males and young of the year. A long-term ringing study conducted in north-east Scotland has established that little movement occurs among colonies and that both sexes are recruited into their natal colony. The aim of the present study was to investigate, using microsatellite DNA markers, if genetic structure within the population reflects the spatial structure indicated by ringing. Inter-colony F_{ST} -estimates obtained for all colony members, and for females and males separately, were low (0.019, 0.026 and 0.011, respectively), but all values differed significantly from zero. These data indicate high gene flow between colonies, although some coancestry among colony members is evident in both sexes. On combining the ringing and genetic data, it is concluded that gene flow occurs via extra-colony copulation, rather than natal dispersal, and that each colony behaves as a distinct subpopulation. Microgeographical genetic isolation by distance was demonstrated for, to our knowledge, the first time in a bat species, and found to be apparent both across the entire study area and along one river valley. The results suggest that extensive macrogeographical population genetic structure may be evident across the species' range.

Keywords: Chiroptera; microsatellite; genetic structure; gene flow; isolation by distance

1. INTRODUCTION

An understanding of the population structure of a species can frequently be used to infer both past and current behavioural processes. At the macrogeographical level, characterization of population structure allows the inference of colonization and/or dispersal events, whilst the pattern of microgeographical structure within a population can be used to infer social organization. As population structure refers to the extent of spatial variation both in the distribution and the genetic composition of individuals (Hewitt & Butlin 1997), its study should combine the use of direct (e.g. mark-recapture studies) and indirect (genetic) techniques (Avice *et al.* 1992; Slatkin 1987).

In the case of the Chiroptera (bats), which consists mainly of nocturnal species, the use of direct observation to assess population structure can be problematic. In particular, temperate zone species, which form conspicuous aggregations during summer (Bradbury 1977), are frequently difficult to observe during autumn and winter, the principal mating seasons. Assessment of gene flow and the identification of distinct populations or social groups may not therefore be possible using direct observation alone.

The advent of molecular genetic techniques has provided an opportunity for questions of population structure to be more fully addressed in bats, but to date these have only been applied to a few species. Studies at the macrogeographical level have shown that migratory bat species typically show low levels of genetic differ-

entiation, even among geographically distant populations, suggesting high gene flow across these species' ranges (McCracken *et al.* 1994; Wilkinson & Fleming 1996; Sinclair *et al.* 1996; Webb & Tidemann 1996). In contrast, the non-migratory ghost bat (*Macroderma gigas*) displays extreme genetic structuring among populations over distances from 50 to 3500 km (Worthington Wilmer 1996; Worthington Wilmer *et al.* 1994). At the subpopulation level, genetic studies have largely been restricted to tropical bat species (reviewed in McCracken 1987). From such studies it is apparent that whilst individual colonies within a population may show some genetic heterogeneity due to coancestry, little genetic subdivision is apparent, possibly due to low reproductive skew or high levels of dispersal (McCracken 1987).

The brown long-eared bat *Plecotus auritus* (family: Vespertilionidae) is a palaearctic species, widespread across central and northern Europe (Stebbing 1988; Swift 1991). The species is characterized by its long ears and wing morphology (low aspect ratio and high wing loading), which together facilitate a gleaning feeding strategy, but which make fast and long-distance flight uneconomical (Norberg 1976; Entwistle *et al.* 1996). Its life cycle is typical of a temperate zone vespertilionid bat (Racey 1982), where mating occurs in autumn and winter and spermatozoa are stored in the uterus until fertilization in spring. During summer females form maternity colonies, located in tree holes, bat boxes and the attics of buildings (Swift 1991; Entwistle *et al.* 1997), where they give birth to a single young in July. *P. auritus* is rare amongst temperate zone species in that adult males are

regularly found with the females in summer colonies. The abundance of individuals detected in summer is not reflected at other times of the year as *P. auritus* roosts singly in transient roosts or hibernacula, located in buildings, tree holes, caves and mines (Strelkov 1969; Horáček 1975).

Colony composition and spatial distribution of *P. auritus* during summer in a population within north-east Scotland, as identified by direct techniques (ringing), has been described by Entwistle *et al.* (1999). Individuals of both sexes show long-term association with their summer colony. Movement among sites, both within and between years, is rarely detected, and at least some recruitment into the natal colony occurs in both sexes. Entwistle *et al.* (1999) have inferred extensive population structure for this species; however, assessment of the level of gene flow among colonies, and hence genetic subdivision within the population, is not easy to assess using ringing, for two reasons. First, not all colony members are caught on each visit (Entwistle *et al.* 1999), and therefore it is not possible to ascertain whether unringed adults are immigrants into a colony or individuals that escaped ringing as juveniles. Second, the mating period in this species extends throughout autumn and winter, the period when individuals are not evident within their summer roost. Thus, it is impossible to observe all mating behaviour that may occur, and the extent of copulation among individuals from different colonies cannot be ascertained.

In this study, microsatellite DNA markers were employed to investigate the extent to which genetic structure within the population reflects the marked spatial distribution of *P. auritus* in summer apparent from ringing. In addition, to test the prediction that *P. auritus* is non-migratory, remaining close to its summer roost site year-round (Strelkov 1969; Entwistle *et al.* 1999), the presence of a negative correlation between levels of gene flow and geographical distance (isolation by distance) was tested. By combining the ringing (direct) and genetic (indirect) data, a fuller evaluation of population structure, dispersal events and gene flow in this species was achieved than would have been possible using either technique alone.

2. MATERIALS AND METHODS

(a) Study area

The study area was situated in the Grampian and Highland regions of north-east Scotland (figure 1), at ca. 57°N. This latitude approaches the northern limit of *P. auritus* distribution, and colonies have been found almost exclusively in the attics of buildings (Entwistle *et al.* 1997). Most colonies included in the study were located within or close to the Deeside Valley (figure 1), with seven colonies situated 0.1–4.0 km apart around the town of Aboyne, ca. 35 km west of Aberdeen. Other colonies were located south of Glenshee, close to the village of Kirkmichael, and within Speyside, west of the Cairngorm region (figure 1).

(b) Sampling procedure

Bats were sampled from a total of 20 *P. auritus* colonies across the area, of which 12 were included in the ringing study (Entwistle *et al.* 1999). Roost visits were conducted during two

consecutive years (1994 and 1995). When bats were present, they were caught under licence by hand or static net and kept in cloth bird bags whilst awaiting inspection. Two 3 mm diameter wing-membrane biopsy punches were taken under licence, one from each plagiopatagium, following Worthington Wilmer & Barratt (1996). Biopsies were placed into 25% DMSO/5 M NaCl solution (Amos & Hoelzel 1991), and stored at –20 °C on return to the laboratory. The number of bats sampled from each colony and the colony locations are given in table 1. In total, 364 bats were sampled.

(c) DNA typing and genotype analysis

For each individual, DNA was extracted from a single wing biopsy using salt extraction and typed at five autosomal *P. auritus* microsatellite loci (*Paur01*, *02*, *04*, *05* and *06*) as previously described in Burland *et al.* (1998). *F*-statistics were calculated using FSTAT (v. 1.2, Goudet 1995), treating each colony as a distinct subpopulation. F_{ST} estimates were obtained for all individuals within colonies, and also separately for males (excluding colony LF, where no males were caught, table 1) and females (excluding colony LW, where only one female was sampled, table 1). To test if each *F*-statistic differed significantly from zero, 500 permutations were performed: for F_{IS} , alleles were permuted within colonies; for F_{IT} , alleles were permuted among colonies; for F_{ST} , multi-locus genotypes were permuted among colonies. Significance levels for individual locus values were adjusted to correct for multiple tests in accordance with Bonferroni procedures. Ninety-five per cent confidence intervals were calculated by bootstrapping over loci.

F_{ST} values among all pairwise colony combinations were used to test for the presence of genetic isolation by distance within the study area. A significant correlation between $\log_{10}$ geographical distance and F_{ST} was tested by a Mantel test, using the matrix comparison facility in NTSYS (v. 1.80, Rohlf 1994). Isolation by distance was considered to be present if, after 1000 permutations, the probability of obtaining the observed correlation by chance was below 5%.

3. RESULTS

F_{IS} , F_{IT} and F_{ST} values were calculated for each locus and over all loci, treating each summer colony as a separate population (table 2). No significant deviation from Hardy–Weinberg expectations was detected for any locus, with the exception of *Paur04*. Since it has previously been shown, using the method described in Beaumont & Nichols (1996), that none of the loci used in this study behave as if under selection (Burland 1998), the significant F_{IS} and F_{IT} values obtained for *Paur04* indicate that one or more null alleles are present at *Paur04*. Exclusion of *Paur04* from the analysis resulted in a mean F_{IS} which did not differ significantly from zero, whereas the overall F_{IT} remained significant (table 2).

All F_{ST} values were low, with less than 3% differentiation identified among colonies (table 2). However, values for most individual loci and the mean F_{ST} all differed significantly from zero (table 2), suggesting significant genetic subdivision within the population. Removal of locus *Paur04* from calculation of the mean overall F_{ST} had little effect, and, as expected for an F_{IS} of zero (Weir 1996), the adjusted mean F_{ST} and F_{IT} values were very similar (table 2). When the two sexes were considered

Figure 1. Diagrammatic representation of the study site showing the location of the major towns and rivers, and the 20 long-eared bat colonies included in the study. The shaded area indicates high altitude.

Table 1. Number of bats analysed (males, females and total) from each colony included in the genetic study

colony	location	females	males	total
AN	K	9	13	22
AL	K	10	6	16
BH	K	6	7	13
EL	S	11	9	20
LW ^a	S	1	9	10
TB	S	11	9	20
CR	D	6	14	20
DL	D	12	5	17
DC	D	9	9	18
DH	D	10	12	22
DV	D	7	12	19
HC	D	8	6	14
KH	D	12	5	17
LF ^b	D	18	0	18
PM	D	16	15	31
RC	D	11	5	16
SK	D	12	8	20
SH	D	7	5	12
TS	D	9	10	19
WI	D	10	10	20
total		195	169	364

^a Colony not included in females-only analysis.

^b Colony not included in males-only analysis.

D, Deeside; S, Speyside; K, Kirkmichael.

separately, the mean F_{ST} value obtained for females was twice that for males (table 2). The absence of overlap in the 95% confidence intervals for two values (table 2) suggests that these values differ significantly.

Genetic isolation by distance was tested among all colonies ($n=20$) and among those colonies located in Deeside ($n=14$). Isolation by distance was strongly supported ($p=0.001$) across the entire study area (figure 2a), where distances between colonies vary from 0.1 km to 100 km. Isolation by distance was also supported within Deeside ($p=0.045$), where distances vary between 0.1 and 40 km (figure 2b).

4. DISCUSSION

(a) Microgeographical genetic structure

The extent to which genetic structure is apparent within a population usually reflects the level of coancestry within each social group in relation to the total population. High levels of coancestry, and hence microgeographical genetic structure, are generally expected to result from high variance in reproductive success in at least one sex and low levels of gene flow between social groups (McCracken & Bradbury 1977; Chesser 1991a,b). The low F_{ST} values obtained in this study therefore suggest low reproductive skew and high gene flow among *P. auritus* colonies.

A low skew in male reproductive success may be predicted in this species from its mating behaviour. From observations based on autumn behaviour, Park *et al.* (1998) suggested a mating system based on female defense. However, mating in this species continues periodically throughout hibernation (Strelkov 1962). Thus, individual males are unlikely to prevent multiple mating by females (Entwistle 1994). Low skew is further supported by the F_{IS} and F_{IT} values. Chesser (1991a,b) has demonstrated that these values can be used as indicators

Table 2. F_{IS} , F_{IT} and F_{ST} values calculated, regarding each colony as a separate population, for each locus and mean across all loci

(Values of F_{ST} are given for all colony members and for males and females separately. The 95% confidence intervals, obtained by bootstrapping over loci, are also given. The level of significance of each value is indicated if it differs significantly from zero.)

locus	F_{IS}	F_{IT}	F_{ST}		
			all	males	females
<i>Paur01</i>	0.00	0.016	0.016**	−0.006	0.028**
<i>Paur02</i>	−0.006	0.016	0.022**	0.025**	0.021**
<i>Paur04</i>	0.213**	0.230**	0.022**	0.013	0.032*
<i>Paur05</i>	0.015	0.024	0.009	0.002	0.019*
<i>Paur06</i>	−0.012	0.012	0.024**	0.018*	0.032*
mean	0.03**	0.048**	0.019***	0.011**	0.026***
(95% C.I.)	(−0.008–0.108)	(0.014–0.125)	(0.013–0.023)	(0.021–0.031)	(0.001–0.020)
mean (excl. <i>Paur04</i>)	−0.001	0.017*	0.018***	—	—

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.002$.

Figure 2. Plot of log distance versus F_{ST} for (a) all colony pairwise comparisons and (b) those colonies located within Deeside only.

of breeding strategies, with significantly negative F_{IS} values indicative of highly polygynous mating systems (e.g. Pope 1992). For *P. auritus*, when the effects of locus *Paur04* are removed, the mean F_{IS} value estimated for summer colonies does not significantly differ from zero and F_{IT} equates to the level of genetic structure among colonies (table 2), implying little reproductive skew (Chesser 1991a).

The inference of high gene flow among colonies is in contrast to the population subdivision in summer observed from the ringing data (Entwistle *et al.* 1999). This lack of agreement may be explained in two ways. First, dispersal among colonies at a level sufficient to reduce genetic structuring, but not detectable by ringing, may occur. The lower F_{ST} values obtained for males than for females may suggest that if dispersal occurs, it is predominantly male-biased.

Alternatively, extra-colony copulation may result in considerable amounts of gene flow among colonies without permanent dispersal from the natal summer colony. Extra-group copulation has been detected in other animal species where little or no natal dispersal occurs in either sex (Amos *et al.* 1993), and may result from inbreeding avoidance. With little evidence to indicate that individuals of *P. auritus* enter other summer roost sites, even temporarily (Entwistle *et al.* 1999), contact between individuals from different colonies probably takes place during autumn and winter at transient roosts or hibernacula once the maternity colonies have dispersed. Extra-colony copulation can also explain the difference in the F_{ST} values between the sexes. Even with complete natal philopatry, many first-order male relatives (specifically, father–son dyads) will occupy different summer colonies. Thus, the probability that males from different colonies share alleles identical in state will be higher than for females (where all mother–daughter dyads will be in the same colony); F_{ST} values should reflect this accordingly.

While these two explanations are not mutually exclusive, we suggest that, when both the ringing and genetic data are considered together, natal philopatry coupled with extra-colony copulation best explains the results for two reasons. First, recruitment into the natal colony was detected by ringing in both sexes (Entwistle *et al.* 1999) and can also be inferred from the significant mean F_{ST} values obtained for females, males and all colony members (table 2). Second, assuming an island model, the mean number of migrants among colonies per generation (Nm) expected from an F_{ST} of 0.019 is 13 ($Nm = 0.25(1 - F_{ST})^{-1}$, Slatkin & Barton 1989). Whilst generation time in this species is approximately two years, only five individual inter-colony movements were recorded throughout the entire ringing study from 1978 to 1993 (Entwistle *et al.* 1999). Gene flow via extra-colony copulation, rather than dispersal, therefore appears more probable. Such data also further strengthen the suggestion that each summer colony should be considered a distinct subpopulation (Entwistle *et al.* 1999). However, the results do not discount the presence of ecologically undetectable levels of dispersal among colonies.

While the pattern of genetic subdivision identified in this population is comparable to those previously identified in other bat species (McCracken 1987), *P. auritus* is unique among those studied as gene flow in other species occurs via natal dispersal of one or both sexes. However, gene flow in *P. auritus* may still be regarded as male-mediated as males father young in different colonies, whilst females only give birth in their own colony. As such, a higher level of genetic subdivision may be expected if the female sex-linked marker, mitochondrial DNA, is investigated.

Ringed studies in *P. auritus* at different localities have revealed similar patterns as identified in north-east Scotland (Heise & Schmidt 1988; Boyd & Stebbings 1989; Park *et al.* 1998). From this, it may be predicted that similar genetic structure may also be detected. However, the behaviour of this species may vary geographically (Entwistle *et al.* 1999). In Germany, all juvenile males dispersed from the natal colony, whilst adult males were only transient colony members (Heise & Schmidt 1988). Thus, less genetic structuring in males between colonies may be predicted in this population, and gene flow may occur via male natal dispersal. Similarities in genetic structure and gene flow should therefore not be presumed for all populations of *P. auritus*.

(b) Isolation by distance

The occurrence of genetic isolation by distance within the study area, even within Deeside where no geographical barriers to gene flow appear to be present, strongly suggests that direct exchange of genes is restricted only to colonies located close by. Such localized gene flow resembles that identified in terrestrial rodents (Dallas *et al.* 1995), and may be explained, at least in part, by the wing morphology of *P. auritus*, which facilitates highly manoeuvrable, but slow, flight (Norberg 1976; Entwistle *et al.* 1996). Summer foraging distances in *P. auritus* are typically restricted to within 0.5 km of the roost for females, although males may venture further (up to 2.8 km, Entwistle *et al.* 1996). Furthermore, all inter-colony movements recorded in the ringing study occurred over a distance of less than 0.3 km (Entwistle *et al.* 1999). Whilst flight distances in some bat species may vary seasonally, the results of this study confirm the predictions of Strelkov (1969) and Entwistle *et al.* (1999) that this species is non-migratory and is unlikely to travel much further at other times of the year. Mating and winter hibernation sites are therefore probably located close to the summer roost site.

The detection of genetic isolation by distance within a single population of *P. auritus*, and the inference that this species is non-migratory, suggests that considerable macrogeographical genetic population structure occurs. Because limited gene flow is thought to stem from morphological constraints, it is likely that this would be apparent across the species' range. Such a pattern of genetic population structure has far-reaching conservation implications for this species. In particular, geographically isolated populations are likely to be genetically distinct and may require individual protection.

Genetic isolation by distance at a microgeographical scale has yet to be described in other bat species, although comparable patterns may be expected in species with

similar wing morphology and flight capabilities. However, among palaeartic bats, the two *Plecotus* species (*P. auritus* and its sister species *P. austriacus*) are distinctive in their wing morphology. As a result, it is unlikely that other palaeartic genera, which travel further from their summer roosts during autumn and winter (Strelkov 1969), will display such a marked pattern as has been described for *P. auritus*.

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