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ORIGINAL ARTICLE

Relatedness, parentage, and philopatry within a Natterer's bat (*Myotis nattereri*) maternity colony

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Abstract

Given their cryptic behaviour, it is often difficult to establish kinship within microchiropteran maternity colonies. This limits understanding of group formation within this highly social group. Following a concerted effort to comprehensively sample a Natterer's bat (*Myotis nattereri*) maternity colony over two consecutive summers, we employed microsatellite DNA profiling to examine genetic relatedness among individuals. Resulting data were used to ascertain female kinship, parentage, mating strategies, and philopatry. Overall, despite evidence of female philopatry, relatedness was low both for adult females and juveniles of both sexes. The majority of individuals within the colony were found to be unrelated or distantly related. However, parentage analysis indicates the existence of a number of maternal lineages (e.g., grandmother, mother, or daughter). There was no evidence suggesting that males born within the colony are mating with females of the same colony. Thus, in this species, males appear to be the dispersive sex. In the Natterer's bat, colony formation is likely to be based on the benefits of group living, rather than kin selection.

Keywords Chiroptera · Kinship · Natal philopatry · Parentage assignment

Introduction

The formation of social groups within species is classically explained through the concept of kin selection and inclusive fitness (Hamilton 1964). Though cooperation between non-kin in social groups has been also been observed in a number of species (reviewed in Clutton-Brock 2009). The latter phenomena are supported by the hypothesis that mutualism can maintain the structure of social groups if any group based fitness loss is outweighed by the benefits of group membership e.g., co-operation, mutualism, and/or information sharing (Fletcher and Zwick 2006; Bergmüller et al. 2007; Rankin et al. 2007).

The formation of social groups is particularly evident within bat species, with all temperate species adopting similar life history patterns in response to the seasonal variations of the environment (Schober 1984). Thus, following a period of hibernation, females tend to gather into groups, commonly referred to as maternity roosts or maternity colonies (the subject of study in this paper), in which they birth, feed, and rear a single offspring. Maternity colony formation is followed by dispersal, breeding, and hibernation throughout the remainder of the year (Altringham 2001). Chiropteran social behaviour is highly variable (e.g., obligate and facultative monogamy/polygyny, harems, mating territories, aggregations, and leks), even within closely related species (Racey and Entwistle 2000). When combined with the fact that females spend a significant proportion of their lives within a group, bats make ideal study organisms for research on group behaviour (Bradbury and Vehrencamp 1977; McCracken et al. 2000). Roost and/or habitat choice for the formation of the colony is firstly dependent on time of year, with maternity and bachelor colonies usually being formed in the late spring/summer months. Within certain species large aggregations of bats exhibiting swarming behaviour are common in autumn, with hibernation taking place during the winter (Altringham 2001). Several hypotheses with associate predictions have been proposed to explain the formation of these colony groups. These range from kin selection

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benefits—wherein a higher degree of relatedness would be expected within colonies (Wilkinson 1984; Wang 2011), to cooperation and mutualism (potentially between non-kin) based on benefits such as information sharing (Kerth et al. 2000; Burland and Worthington Wilmer 2001), thermoregulation (Neuweiler 2000), and to group rearing and defence (Clutton-Brock 2009). An alternative hypothesis is that the formation of these colony groups could simply be associated with resource limitation, with limited numbers of appropriate dwellings being available (Kunz 1982; Kunz and Fenton 2006).

Individuals of species that form social groups often show fidelity to a particular location or site. This philopatric behaviour is common in mammals as a whole (Johnson and Gaines 1990) and can provide a host of benefits (reviewed in Clutton-Brock 2009) including, for instance: dwelling, habitat and foraging locations; cooperation and mutualism benefits (i.e., information transfer, offspring care and grooming); and possible kin selection benefits, which are particularly common when individuals have a temporal dispersal mechanism (Wilkinson 1984). Previous studies have shown high female natal philopatry in many bat species (Petri et al. 1997; Petit and Mayer 1999; Kerth et al. 2000, 2002; Castella et al. 2001; Boston et al. 2012). It should be noted, however, that individuals within a colony site may cycle between a number of roosting sites within a given area, in a 'roost network', with philopatry possibly being to the network or colony rather than one specific roosting spot (Smith 2000; Lundy et al. 2012).

While natal philopatry would be expected to lead to genetic substructuring at multiple levels (Di Fiore 2003; Dixon 2011), molecular based studies within several bat species suggest the opposite, with genetic structuring in summer roosts being quite low, though often significantly greater than zero (Kerth et al. 2003; Rivers et al. 2005; Furmankiewicz and Altringham 2007). The low level of genetic population structure observed in bats is believed to be associated with a high level of male-biased gene flow (Kerth et al. 2003; Rivers et al. 2005). Male-biased gene flow has been reported in the closely related noctule (*N. noctula*; Petit and Mayer 1999) and in Bechstein's bat (*Myotis bechsteinii*; Kerth and Konig 1999). This male behaviour, combined with temporary female dispersal for mating, would lead to low within-colony relatedness, as females are unlikely to breed with relatives, thus avoiding the drawbacks associated with philopatry i.e., inbreeding and loss of genetic diversity (Chesser 1991; Radespiel et al. 2009).

Natterer's bat (*Myotis nattereri*), is a typical temperate bat species, and as with some other species of the genus, exhibits swarming behaviour (Altringham 2001; Rivers et al. 2005) in which breeding is believed to occur. Stebbings et al. (1991) showed that maternity colonies within the species can consist of up to 200 females, with few or no males, while bachelor colonies are often comprised of much smaller numbers (two up to approximately 20 or 30) of male individuals only (Swift 1997). Females gather into maternity colonies from spring, with offspring being born during the summer months. High female philopatry has also been exhibited in *M. nattereri* (Parsons et al. 2003; Rivers et al. 2005, 2006).

Building on current knowledge and research on chiropteran group behaviour, this study aims to test the hypotheses that a *M. nattereri* maternity colony site consists of adult females and their offspring with few or no adult males present, with low overall relatedness but multiple maternal lineages (with female philopatry and male dispersal). With this hypothesis we would expect (1) females of *M. nattereri* show natal philopatry to their maternity site, with individuals returning to the same grouping in consecutive years (though this may be to a 'roost network' than a single site); (2) overall roost relatedness is low, though consists of multiple maternal lineages i.e., does relatedness and assignment show data consistent with grandparent–parent–offspring relatedness? (3) There is no evidence of local (born in the roost) male parentage or philopatry due to male-biased dispersal behaviour.

This hypothesis was addressed through the examination of an entire maternity roost, sampled consecutively over 2 years, and utilising the analysis of nuclear and mitochondrial DNA (mtDNA) data. Using a combination of mtDNA and microsatellite data permits a full test of the above hypotheses, capitalising on the benefits of multiple characters. The combination of mtDNA and nuclear DNA analysis is highly informative and useful in the analysis of population and social patterns, particularly when those populations are characterised by a disparity in male and female dispersal and breeding mechanisms.

Materials and methods

Sampling

As part of an ongoing project mapping the distribution of *M. nattereri* in Ireland (by the Centre for Irish Bat Research under funding from NPWS Ireland), a colony, located in Glengarriff Forest, County Cork, Ireland (51°45′29″N, 9°34′06″W) was selected for this study based upon its large number of individuals and ease of access, and the presence of 12 identical bat boxes in a small area (approximately 300 m diameter) would allow us to test the 'roost network' hypothesis. Samples were collected over a 2 year period in two separate extensive samplings as follows: in July 2009 members of the colony were captured and sampled from one of the bat boxes in the forest. The same colony was sampled again in July 2010 from another bat box located nearby (approximately 50–100 m). On both years, sampling

was carried out late in the nursing season to allow for sufficient maturation of juveniles thus minimising disturbance. All individuals present were sampled on both occasions, with this being carried out by a team of four to ensure all bats were placed back into their bat box within 30 m of removal. A total of 118 individuals were sampled, with 53 in the first year and 65 in the second year. Individuals representing two additional colony sites, which have also been comprehensively sampled in County Kilkenny ($n = 26$) and County Kerry ($n = 14$), were also included in the some of the analyses for population-based comparisons.

Wing-biopsy tissue samples were taken from all bats caught, under licence from the National Parks and Wildlife Service (Licence No. 74 C/2008) and the Northern Ireland Environment Agency (Licence No. TSA/12/08), within the Republic of Ireland and Northern Ireland. Sex, age, and reproductive status were recorded by examining individuals for characteristic identifiers, and a 3 mm biopsy of wing tissue taken and stored in 90% ethanol until extraction.

DNA extraction and molecular screening

DNA extraction was carried out using the Quiagen DNeasy Blood and Tissue Kit (Quiagen). On average, a total of 1 µg total DNA was obtained from each wing biopsy. DNA was resuspended to a final concentration of approximately 20 ng/µl. DNA quality and concentration was checked on an ethidium bromide (0.3 ng/ml) stained 0.8% $0.5 \times$ TBE agarose gel. All resulting DNA was found to be suitable for molecular analysis.

Microsatellite screening was based on a panel of 16 marker loci (Table 1). This included both microsatellites for closely related species and markers developed specifically for *M. nattereri* from an enriched microsatellite library (Scott et al. 2013). Screening was carried out using the Applied Biosystems 3730×l DNA analyser, and genotypes were scored using GeneMapper v4.1 (Applied Biosystems, TM), with the methodology outlined in Scott et al. (2013).

All individuals were also genetically screened for an approximately 366 bp section of the mitochondrial D-loop HVII using primers L16517 (Fumagalli et al. 1996), and SH651 (Castella et al. 2001), starting at the 3′ end of the central conserved block and ending before the R2 tandem repeats (Fumagalli et al. 1996). PCR amplifications were carried out in 50 µl reaction volumes under the following conditions: 3 µl of template DNA (approximately 20 ng / µl), 2.5 mM $MgCl_2$, 10 pM/µl of each primer 200 µM of each dNTP, and 1 U of *Taq* DNA polymerase (Invitrogen) with $1 \times$ of the corresponding PCR buffer. Thermal profiles started with an initial denaturation stage at 95 °C for 3 min, followed by ten touchdown 60–50 °C cycles consisting of 45 s at 94 °C, 45 s at 60 (− 1/cycle) °C, 1 min at 72 °C, followed by 30 cycles at 50 °C with the same initial. Resulting PCR products were purified and prepared for sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit, following manufacturer's protocol. Sequencing was carried out using the ABI3730XL DNA analyser (Applied Biosystems) with the raw sequence data being subsequently processed using Sequencing Analysis Software V5.4 (Applied Biosystems, TM). Processed sequencing data was checked for consistency and ambiguities using ChromasPro 1.5 (http://www.technelysium.com.au). Resulting checked sequences were then aligned using ClustalW (Thompson et al. 1997) within Bioedit (Hall 1999).

Table 1 Basic indices including number of alleles (N_a), observed (H_O) and expected (H_E) heterozygosities, average non-exclusionary probability for the first parent (NE-1P), average non-exclusionary probability for second parent (NE-2P), and HWE significance (*significant at the 0.05% level; NS—not significant; ND—non determinable)

Locus	Source	N_a	H_O	H_E	NE-1P	NE-2P	HWE
A2	Jan et al. (2012)	12	0.785	0.778	0.504	0.332	NS
EF15	Jan et al. (2012)	8	0.507	0.480	0.871	0.715	NS
G2	Jan et al. (2012)	5	0.655	0.692	0.751	0.587	NS
G31	Jan et al. (2012)	11	0.723	0.750	0.645	0.468	NS
Mnatt-1	Scott et al. (2013)	8	0.792	0.717	0.601	0.423	NS
Mnatt-2	Scott et al. (2013)	13	0.764	0.745	0.764	0.575	NS
D15	Castella and Ruedi (2000)	22	0.689	0.641	0.413	0.26	NS
D9	Jan et al. (2012)	16	0.781	0.833	0.371	0.227	NS
E24	Castella and Ruedi (2000)	8	0.856	0.842	0.733	0.554	NS
F19	Jan et al. (2012)	19	0.509	0.659	0.309	0.182	ND
G30	Castella and Ruedi (2000)	10	0.815	0.848	0.655	0.469	NS
H23	Jan et al. (2012)	5	0.759	0.699	0.85	0.692	*
Mnatt-3	Scott et al. (2013)	11	0.292	0.517	0.617	0.432	*
H19	Castella and Ruedi (2000)	4	0.670	0.751	0.938	0.838	NS
H29	Castella and Ruedi (2000)	8	0.358	0.358	0.617	0.438	NS
Global	–	10.67	0.664	0.687	0.000676	4.86E-06	NS

Data analysis

Genotypic microsatellite data was assembled into an Excel database, and formatted for several input formats using the Microsatellite toolkit add-on (Park 2001). Missing data at more than two loci was selected as a cut-off point for sample data inclusion. Analyses of locus information used all available individuals. The remaining analyses primarily used individuals from the focal site in Cork (both years).

GENEPOP 4.1 (Raymond and Rousset 1995) was employed to produce basic population genetics statistics including: allelic frequencies; mean number of alleles per locus (A); observed and unbiased expected heterozygosity (H_O and H_E respectively). SMOGD (Crawford 2010) was used to calculate per locus estimations of D_{est} (Jost 2008), with harmonic means being used for multilocus estimates. GENEPOP 4.1 was used to calculate F_{ST} values of each grouping and overall, using methods derived from Cockerham (1973) and Weir and Cockerham (1984).

The 'identity analysis', implemented in GenCap 1.3 (Wilberg and Dreher 2004) was used to identify returning individuals in the second year of sampling. This software was also used to estimate the likelihood, Sib P (ID), that two individuals within the population could share the same genotype by chance (Wilberg and Dreher 2004). The unbiased linkage disequilibrium approach of Waples (2006) implemented in LDNe V1.7 software (Waples and Do 2008) was utilised to estimate effective population size (N_e), in order to provide support to the 'roost network' hypothesis, with P crit values ranging from 0.05 to 0.01 as suggested in Waples and Do (2010). N_e estimates were based on juvenile specimens only to avoid bias resulting from analysing individuals from overlapping generations.

CERVUS 3.0 (Marshall et al. 1998) was used for juvenile parentage assignment within the Glengarriff roost over the 2 years. Log likelihood ratio probabilities of parentage for each candidate mother were calculated, taking into account genotyping errors, which have been shown to increase successful assignment (Kalinowski et al. 2007). Maternal assignment was carried out for each year, with potential mothers in the second year including adult females caught in 2011, as well as females (adult and juvenile) caught in 2010. Juveniles from 2010 were included as potential candidate mothers because of the short sexual maturation time of microchiropteran bats (Altringham 2001; Cheng and Lee 2002). No paternal assignment was carried out for the 2010 samples due to the lack of adult males. Parentage assignment in 2011 was based upon data from juvenile males from 2010. Parentage assignment confidence was estimated using log likelihood ratio probabilities of parentage for each candidate mother, taking into account genotyping errors and the presence of other potential mothers, following Kalinowski et al. (2007). To validate individual assignments results, 100,000 simulations were carried out to assess the significance of the difference between the most likely mother and the next most likely mother, with confidence intervals of 80% (relaxed) and 95% (strict) based on a default error rate of 1% per locus.

Relatedness between individuals was calculated using GenALEx 6.4 (Peakall and Smouse 2006), following Queller and Goodnight's (1989) pairwise relatedness estimator due to small sample sizes (as recommended in Wang 2002), and utilising 1000 permutations to gauge confidence intervals, and using whole sample library to calibrate. Relatedness was calculated using the 15 microsatellite loci for adults and juveniles at three levels: (1) combined years, (2), year 1 (2010), and (3) year 2 (2011).

To investigate population substructuring within the data, the non-spatial Bayesian clustering method implemented within STRUCTURE (Pritchard et al. 2000a) was used. The Glengarriff samples were analysed using a burn-in of 5×10^5 followed by 10^6 iterations over 20 replicated runs. Outputs from independent replicate runs were subsequently joined and relabelled using CLUMPP (Jakobsson and Rosenberg 2007), Using the LargeKGreedy algorithm and G statistic with 1,000,000 random input orders over ten runs. The 'ad hoc' method described in Evanno et al. (2005) was used to attempt to identify the minimum number of genetic clusters (k) explaining the genetic data. Visual examinations of resultant STRUCTURE plots were also carried out, in order to identify any subtle trends and aid in determination of k.

Mitochondrial DNA data was matched to the sites utilised in the microsatellite analysis, to allow for direct comparison between the nuclear and mitochondrial data. Two nearby sites were used for comparison (in Co. Kilkenny, and Co. Kerry—GPS available on request). Basic analyses and counts of haplotypes were calculated for each of the maternity sites involved in the study.

Results

The study consisted of 158 unique samples (i.e., excluding recaptures) across three locations (as detailed in Table 2). The two Cork locations were within 100 m of each other, with 53 in year one (30 adults, 23 juveniles—12 males, 11 females) and 65 (41 adults, 24 juveniles—11 males, 13 females) in year two. All adults present within the Glengarriff maternity colony over the 2 years were females, while only one and two adult males were observed in Kerry and Kilkenny respectively. The recapture of six females was noted through scarring on the wings and subsequent genetic comparison.

Table 2 Summary of statistics and measures of differentiation for each population, consisting of number of specimens (n), observed (H_O) and expected (H_E) heterozygosities, mean number of alleles (A), significance of HWE deviation (HWE) and the measures of differentiation D_{est} and F_{ST}

Site	n	H_E	H_O	A	HWE	D_{est}/F_{ST}
Glengarriff (Cork) 2010	53	0.68	0.61	8.79	NS	−0.005/−0.005
Glengarriff (Cork) 2011	65	0.68	0.59	8.79	NS	0.000/−0.001
Kilkenny	26	0.73	0.70	8.00	NS	0.000/−0.004
Kerry	14	0.66	0.65	5.79	NS	−0.001/−0.015
Global	158	0.69	0.62	8.12	NS	0.026/0.012

Roost structure

Microsatellite genotypic data was obtained for over 95% of the loci screened; i.e., 158 (96%) samples (including samples from the two comparison sites in Kilkenny and Kerry). All loci were found to be polymorphic with four to 22 alleles segregating per locus/sample (Table 1). Combined (across all loci), samples were found to conform to Hardy–Weinberg equilibrium (HWE). Over all samples per loci, however, (i.e., assuming a panmictic population), two of the 15 loci were shown to deviate from HWE ($P < 0.05$). Mean observed heterozygosity ranged from 0.36 to 0.84 (avg. 0.69). Overall, there were no major differences observed between observed and expected heterozygosities.

On average, 90% of all specimens were successfully sequenced for the approximately 366 bp (bp) region of the mitochondrial *HVII* segment of the D-Loop. Analysis of resulting sequencing data identified three individual mtDNA haplotypes.

The combined non-exclusionary probability of successful assignment of a juvenile parent, based on all loci, calculated by maternity simulation assignments was 0.9997 (2.76×10^{-4}) for the first parent, increasing to 0.9999 (3.21×10^{-6}) for the second parent, when the first was known. These represent the average probability of not excluding a single randomly chosen unrelated individual from parentage at one or more loci, assuming no typing errors (Marshall et al. 1998).

Mean number of alleles were in a similar range of 8–8.79, with the exception of Kerry (5.79; Table 2). No variation was noted between temporal samples (Glengarriff). Genetic diversity, as measured by heterozygosity (H_E), was found to be highly consistent across all samples. No deviations from HWE were observed within samples. In contrast, all maternity colonies were found to be fixed for a single mtDNA haplotype. For all site groupings, effective population size could not be distinguished from infinity; i.e., there was no evidence for any disequilibrium caused by genetic drift due to a finite number of parents.

Parentage

Initial identity analysis identified three individuals in the second year which displayed identical microsatellite multilocus genotypes. Parentage assignment of juveniles caught in Glengarriff in 2010 and 2011 is displayed in Table 3. For 2010, maternity assignment was carried out for 53 bats comprising 23 offspring (i.e., juveniles) and 30 putative mothers (i.e., adults). Overall maternity was assigned for nine juveniles (39%); seven of these juveniles were assigned with a high probability (95% confidence) and the remaining two with 80% confidence, matching high relatedness estimates for these individuals. No paternal assignments were made due to absence of available potential fathers in this cohort.

For 2011, two approaches were used for maternity analysis. In the first case only individual adult females observed

Table 3 Summary of results from parentage assignment analysis using samples caught in Glengarriff in 2010 and 2011

	2010		2011		
	Maternity	Paternity	Maternity	Maternity (Inc.)	Paternity (Inc.)
Offspring assigned	23	–	24	24	24
Candidate parents	30	–	41	64	12
Assignment 95%	7 (30%)	–	16 (67%)	18 (75%)	0 (0%)
Assignment 80%	9 (39%)	–	17 (71%)	21 (88%)	1 (4%)
	Sib P (ID)	6.06×10^{-06}	HW P(ID)	5.96×10^{-15}	

Number/percentage of assignments at strict (95%) and relaxed (80%) confidence (based on log-likelihood analysis) shown per year for maternal and paternal assignments. Inclusion of 2010 potential parents shown by (Inc.). Probabilities of identity Sib P(ID) and HW P(ID) are also displayed

in 2011 were considered as potential mothers of 2011 juveniles. In the second approach, all female individuals (i.e., adults and juveniles) observed in 2010 were added to the 2011 putative mothers. Thus, the potential number of mothers in 2011 ranged from 41 (excluding 2010 females) to 64 (inclusive) respectively. Maternity was assigned to 17 (71%) and 21 (88%) juveniles for each of the two approaches. The confidence of the assignment in each case varied from 16 (67%) and 18 (75%) at the 95% confidence level with the remainder at 80% confidence level.

Paternity analysis of 24 offspring in 2011 with 12 candidate males (i.e., 2010 male juveniles) yielded no assignments at the 95% level, and one assignment at 80% level.

Relatedness

The overall pairwise relatedness was consistently low (< 0.05) among all groups of Glengarriff individuals (Table 4). This suggests that members were either unrelated or distantly related. Pairwise relatedness values above 0.5 (that expected of a parent/full sibling) were rare ($n = 17$, 0.7%), though most individuals displayed pairwise relatedness values around or above 0.025; i.e., half-sibling/grandparent level (Fig. 1).

Overall, relatedness estimates for adults were higher (− 0.003 95% CI [− 0.01, 0.003]) than juveniles (− 0.020 95% CI [− 0.03 to − 0.011]), though still at levels indicating low relatedness. Although confidence intervals just overlap, relatedness appeared to show a decrease in adults in the second year, with mean R values of 0.016 and − 0.007 for 2010 and 2011 respectively.
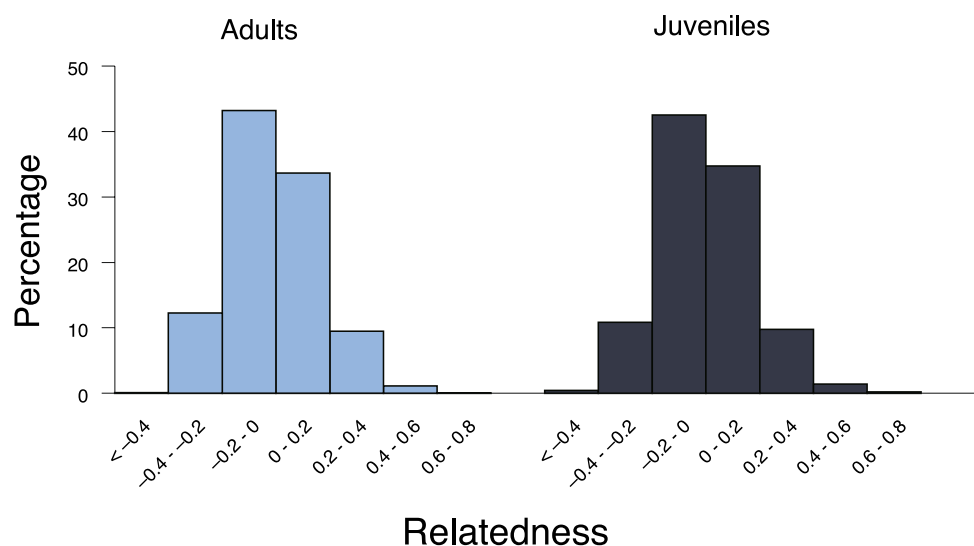
Structuring

Each of the three nearby sites involved in this study were characterised by complete make-up of a single, unique (i.e., distinct), mtDNA haplotype at each of the three geographical locations, with no within-region variation. This includes all 117 samples in Cork (Glengarriff) taken over the 2 years. Cluster analysis in TESS yielded no clear evidence of any genetic substructuring within the Glengarriff site, which is unsurprising considering the low level of differentiation, though higher levels of k yielded higher DIC (deviance information criterion) values ($k = 8+$), which could be indicative of the presence of many separate (maternal) lineages within the roost.

Table 4 Overall relatedness at multiple levels including all adults and all juveniles per year, and over both years, using Queller and Goodnight's (1989) estimator

	Adults	Juveniles	Adults 2010	Adults 2011	Juveniles 2010	Juveniles 2011
Mean R	− 0.003	− 0.020	0.016	− 0.007	− 0.019	− 0.008
Uv	0.003	− 0.011	0.031	0.005	0.004	0.010
Lv	− 0.010	− 0.030	0.002	− 0.017	− 0.039	− 0.031

UV and Lv indicate the upper and lower 95% confidence intervals calculated by bootstrap resampling

Fig. 1 Relative frequency (percentage) of pairwise relatedness values between all adults, and all juveniles. Relatedness values based on the Queller and Goodnight (1989) estimator

Discussion

Roost structure

The absence of males is consistent with other studies of maternity colony makeup, including *M. nattereri* (Park et al. 1998) and other species e.g., Leisler's bat (*Nyctalus leisleri*; Boston et al. 2015). The focal study site in Glengarriff consisted of 53 and 65 individuals in 2010 and 2011 respectively. These numbers are high for a roost box, with Park et al. (1998) finding a maximum group size of 37 for *M. nattereri* in a similar study. Despite this, the number of individuals in colonies are likely to be much larger than those present within any particular roosting site. A number of studies have suggested that individuals within a colony make use of a 'network' of roosts. Lundy et al. (2012), using radio tracking of *M. nattereri*, has shown that one particular individual relocated to a nearby (1.2 km) roost during the course of the study. This behaviour has also been reported in other bat species including Serotine bats (*Eptesicus serotinus*; Catto et al. 1996), *M. bechsteinii* (Kerth and König 1999), and the whiskered bat (*M. mystacinus*; Buckley et al. 2013). Movement between different roosts may be more pronounced post-nursing, as maternity roost sites are likely a compromise between favourable maternity colony habitat, and favourable foraging habitat (Lundy et al. 2012). Though linkage disequilibrium based estimates to calculate population size failed to produce sub-infinity estimates (likely due to a maternity site not representing a distinct Mendelian population), simple estimates based on the assignment percentage and number of females present in the roost produce an estimate of 61 adults for 2010 and 100 adults in 2011. The ease of access to multiple appropriate candidate dwellings (bat boxes) may also have an impact on the formation of these wider networks.

Although the data is insufficient to provide a high degree of support, the movement of the colony among nearby boxes supports previous evidence that individuals in a single *M. nattereri* natal population may utilise a network of nearby roosts, with the return of a small number of individuals and lack of differentiation confirming that it was members of the same grouping recaptured in the second year. This low recapture rate may suggest the grouping is much larger than those bats present in the roost box, and provides further evidence in favour of the 'roost network' or potentially a fission–fusion hypothesis (though further study will be required to support this).

Parentage

Maternal assignment within years was quite low, with only 30% of juvenile parents assigned in the first year at 95% confidence. One explanation for this could be the time of sampling, with catches being carried out late in the nursing season (to minimise colony disturbance) when numbers tend to decline as dispersal to autumn breeding or swarming locations occurs (Dietz and Pir 2009). The unassigned juveniles present in the roost are likely weaned, with their maternal parent possibly having dispersed to breeding grounds (Neuweiler 2000). Parents could also be in a nearby roost within a 'roost network', particularly when offspring become less dependent, with other roosts possibly providing better access to optimum foraging grounds than those chosen to favour rearing of offspring (Lundy et al. 2012). As total assignment using all possible female parents in the second year led to 75% assignment, both these scenarios are strongly supported (either separately or a combination of both). Interestingly, one of the 2010 captures assigned as a parent to a 2011 juvenile was recorded as a juvenile when caught initially, confirming that *M. nattereri* can reach sexual maturity within their first year. This is similar to the findings of Boston et al. (2015) with the Leisler's bat (*Nyctalus leisleri*) and Cheng and Lee (2002) with the leaf nosed bat (*Hipposideros terasensis*) though the latter found this only in male individuals.

No males were assigned as parents at the 95% assignment level, though one was assigned at the 80%. Close examination of the microsatellite multi-locus genotypes of the two individuals indicate that they possessed very common genotypes. Thus, it is likely that these observed matches do not reflect true parentage, but a chance event/close relation (Marshall et al. 1998). Therefore, it is unlikely that any males born in the roost in 2010 fathered offspring in 2011 with any of the females present therein. Also, if males were breeding with females present in the roost, a high overall relatedness would be expected; this provides good support for our prediction of male dispersal prediction. Male dispersal is common in mammals (Greenwood 1980), and these results are in concordance with earlier findings within this species (Kerth et al. 2003; Rivers et al. 2005; Furmankiewicz and Altringham 2007) and other bats; e.g., the noctule (*N. noctula*; Petit and Mayer 1999) and *M. bechsteinii* (Kerth and König 1999).

Philopatry and relatedness

High levels of geographical structure are evident from the mtDNA data (i.e., presence of a single unique haplotype in each colony). This supports female philopatry, one of our initial hypotheses, and corroborates what has been found in this (Park et al. 1998; Rivers et al. 2006) and many other

bat species (Petri et al. 1997; Petit and Mayer 1999; Kerth et al. 2000, 2002; Castella et al. 2001; Boston et al. 2012). Philopatric behaviour is common in mammals (Johnson and Gaines 1990) and is likely utilised by *M. nattereri* for benefits including: knowledge of known suitable roosts, habitat, and food availability (Greenwood 1980). Although recapture rates were low, identity analysis also supports female philopatry, as do the females caught in year 1 assigned to juveniles in year 2. The low number of individuals resampled does not necessarily indicate a low level of philopatry. Rather, it is believed to be a consequence of there being a roost network, with overall philopatry being to the network or local area rather than a specific roosting spot.

Overall levels of relatedness in maternity sites were consistently low. This is similar to findings in other bat species; e.g., the brown long-eared bat (*Plecotus auritus*; Burland and Worthington Wilmer 2001) and *N. leisleri* (Boston et al. 2012), though high relatedness within colonies has been displayed in *M. bechsteinii* (Kerth et al. 2000). The underlying reason for group formation, therefore, is believed to be due to the benefits derived through mutualism or reciprocity, such as thermoregulation, information transfer and group rearing (as reviewed in Clutton-Brock 2009). This is consistent with findings of Wilkinson (1984, 1988), wherein familiarity, rather than relatedness to an individual, is the primary promoter of mutualism and reciprocity. Lower relatedness within juveniles suggests that males born in this roost or nearby do not father offspring with females in the same colony or area, and supports the lack of significant male assignment in supporting male biased dispersal. The low differentiation, therefore, must be caused by high male geneflow, with the significant difference from zero due to the fact that high gene flow cannot completely counteract high female philopatry (Kerth et al. 2002; Rivers et al. 2005).

Though overall relatedness is low, the fact that pairwise relatedness estimators showed that most individuals had several individuals at grandparent or half-sibling level of relatedness shows that the roost contains maternal lineages and multiple generations and/or maternal-siblings. This is supported by the large number of clusters being suggested based on Bayesian assignment and supports our initial hypotheses of both female philopatry and within-roost maternal lineages.

Conclusions

The evidence indicates that maternity colonies do not represent a true Mendelian population, but a grouping of mostly unrelated, female individuals and their offspring. Colonies are believed to be made up of a number of different roosts, between which individuals or groups of individuals may cycle. Therefore, group stability is thought to be due to the mutual and reciprocal benefits of group living, and the additional benefits of philopatry. Male dispersal is likely a necessary compromise, allowing for all the benefits of philopatry, yet escaping the problems associated with it, such as inbreeding, drift, and the consequent loss of diversity (and therefore population/species health and adaptability).

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