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ORIGINAL PAPER

Genetic relationships between roost-mates in a fission–fusion society of tree-roosting big brown bats (*Eptesicus fuscus*)

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Abstract Kin-based patterns of associations are often observed in group living mammals. Colonies of forest-living big brown bats (*Eptesicus fuscus*) exhibit fission–fusion roosting behavior and female philopatry. Within a roosting area of forest, adult females are distributed into several subgroups roosting in different trees during the day. At night, adult females leave the roost subgroups to forage and, upon return to the roosting area at dawn, both the individual composition and location of subgroups often change. Individuals exhibit nonrandom roosting associations, and we hypothesized that genetic relationships would influence roosting associations. We determined (1) whether the strength of roosting associations between pairs of bats (based on radiotelemetry) was correlated with relatedness, (2) whether individuals that roosted together in roost subgroups were more related than by chance, and (3) from roost subgroups, the pairs of bats that roosted nonrandomly and whether the proportion of related pairs was higher than expected at random. Relatedness measures were based on microsatellite genotyping and mitochondrial DNA sequences. We found from all analyses that roosting associations were not influenced by relatedness or matrilineal relationships. These results provide clear evidence that, contrary to other mammals, kinship does not mediate roosting associations within forest living big brown bats that exhibit fission–fusion roosting behavior.

Keywords Relatedness · Roosting associations · Fission–fusion · *Eptesicus*

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Introduction

One consequence and potential benefit of the formation of social groups is the opportunity for interactions between specific group members that may enhance individual fitness. Group members may interact with other individuals in their group at random or interactions may fit a nonrandom pattern if individuals tend to associate with some group members more than others. Nonrandom associations might increase the likelihood of reciprocal exchanges between specific individuals (e.g., food sharing in the vampire bat [*Desmodus rotundus*], Wilkinson 1985a). The rank and/or relatedness of group members can influence the interactions within groups of some social species (e.g., *Crocuta crocuta*, Engh et al. 2005; long-tailed macaques [*Macaca fascicularis*], de Ruiter and Geffen

1998; baboons [*Papio cynocephalus*], Silk et al. 2006a, b) while factors such as gender, age, and reproductive condition might also contribute to nonrandom associations among group members (e.g., associations based on similarity in age and sex for cowbirds [*Molothrus ater*], Smith et al. 2002). There is opportunity for kin selection to exert strong influence in groups of some social species where kinship predicts patterns of association (e.g., spotted hyaenas [*C. crocuta*], Holekamp et al. 1997; primates, reviewed by Silk 2002). Investigating the causes of nonrandom associations among group members is critical for understanding the function of group living and the evolution of social behavior.

Bats are excellent models for studying the evolution of social behavior. Bats are long-lived and exhibit a range of social structures, from solitary to highly gregarious. Moreover, flight affords individuals distributed over large geographic areas the ability to interact. In temperate-zones, stable and cohesive social groups, known as maternity colonies, form during the summer months. Maternity colonies are composed of philopatric females who return to the same roost area each spring to give birth and raise their young (Wilkinson 1992b; Burland et al. 2001; Kerth et al. 2002b; Rossiter et al. 2002). Interactions among members of a maternity colony include transferring information about roost sites and/or foraging locations (*Myotis bechsteinii*, Kerth and Reckardt 2003; *Nycticeius humeralis*, Wilkinson 1992a), allogrooming (*M. bechsteinii*, Kerth et al. 2003), allonursing (*N. humeralis*, Wilkinson 1992b), warming the roost microenvironment to increase juvenile growth and reduce energy expenditure (*Antrozous pallidus*, Trune and Slobodchikoff 1978), and babysitting (*M. thysanodes*, O'Farrell and Studier 1973). Colonies of some bat species have low average relatedness (e.g., Burland et al. 2001; Kerth et al. 2002b; Rossiter et al. 2002). Within colonies exhibiting low average relatedness, closely related females do live together in several species (*Plecotus auritus*, Burland et al. 2001; *M. bechsteinii*, Kerth et al. 2002b; *Rhinolophus ferrumequinum*, Rossiter et al. 2002). Maternity colonies consist of both unrelated and closely related members and provide an excellent opportunity to test whether patterns of association within colonies are based on kinship.

In fission–fusion societies of forest-living bats, during the day, the entire maternity colony may often be divided among several different roosting sites (often tree cavities) producing distinct spatially segregated subgroups (e.g., Russo et al. 2005; Willis and Brigham 2004; Kerth and König 1999; O'Donnell 2000). Subgroups disband at night when bats leave to forage but reform at dawn when they return to roost trees. Daily fission of subgroups at dusk and then fusion at dawn creates the potential for extensive mixing of subgroup composition. Mixing of subgroups provides each bat with the opportunity to interact with all members of the larger maternity colony, even though all members are rarely found roosting together at the same time. In this respect, fission–fusion bat social systems differ from both group-based and individual-based primate fission–fusion social systems (as described by van Schaik 1999). For bats, the entire colony may never or rarely be found together as in group-based primate fission–fusion, and similarly, bats are rarely, if ever, found roosting alone as in individual-based primate fission–fusion. Fission–fusion roosting behavior has been described in a number of bat species including *M. bechsteinii* (Kerth and König 1999), *Eptesicus fuscus* (Willis and Brigham 2004), *Chalinolobus tuberculatus* (O'Donnell 2000), *Tadarida australis* (Rhodes et al. 2006), and *D. rotundus* (Wilkinson 1985a). Bat species with fission–fusion social systems often exhibit nonrandom roosting associations among group members in which some individuals tend to associate more often than expected if all bats selected roost-mates at random (*M. bechsteinii*, Kerth and König 1999; *D. rotundus*, Wilkinson 1985a; *E. fuscus*, Willis and Brigham 2004).

Maternity colonies of tree-roosting big brown bats (*E. fuscus*) in the Cypress Hills of Saskatchewan, Canada conform to a fission–fusion social structure (e.g., Kalcounis and Brigham 1998; Willis et al. 2003, 2006; Willis and Brigham 2004). Females return to the Cypress Hills from unknown hibernacula in the spring and roost in tree cavities during summer. Adult males are never found roosting in these maternity colonies. Females exhibit strong philopatry to particular roosting areas in the forest. Within a roosting area, females roost in many of the same tree cavities within and between years and also roost in novel tree cavities (Kalcounis and Brigham 1998; Willis et al. 2003; Willis et al. 2006). Although females remain loyal to the same roosting area, frequent roost switching within this area leads to extensive mixing of individuals among subgroups. Patterns of pairwise association between individuals are not random, and the advantage of nonrandom roosting associations is not understood (Willis and Brigham 2004).

In this study, we build on the documented fission–fusion system of *E. fuscus*. Our first objective was to determine whether the strength of roosting associations between pairs of bats (based on radiotelemetry) was correlated with relatedness. Our second objective was to determine whether individuals that roosted together in roost subgroups were more related than by chance. Our third objective was to determine, from roost subgroups, pairs of bats that roosted nonrandomly and whether the proportion of related pairs was higher than expected by chance. Relatedness measures were based on microsatellite genotyping and mitochondrial DNA sequences. We predicted a positive relationship between the strength of roosting associations and relatedness, higher relatedness within roost subgroups than expected by chance, and a higher proportion of related pairs among nonrandomly roosting pairs.

Materials and methods

Study area

All field work was conducted in Cypress Hills Interprovincial Park, Saskatchewan, Canada (49°34′N, 109°53′W; Cypress Hills). For the details of the area, see Willis and Brigham (2004). The focus of this study is the most intensively studied maternity colony in the Cypress Hills resident to an area known as roosting area 1 (RA1; see Willis and Brigham 2004). RA1 consists of approximately 30–45 adult females (reproductive and nonreproductive) and young of the year. Due to the high level of philopatry, many individuals studied and marked with forearm bands by Willis and Brigham (2004) from 2000 to 2002 were the same individuals we trapped in 2003–2005. All methods were approved by the University of Regina President's Committee on Animal Care in accordance with the Guidelines of the Canadian Council on Animal Care.

Objective 1: are roosting associations of pairs of radiotracked bats correlated with measures of relatedness?

The strength of roosting associations between pairs of radiotracked bats were quantified by Willis and Brigham (2004, 2005) using a pairwise sharing index (PSI). The PSI compares the observed frequency of roosting association for two individuals radiotracked simultaneously to an expected frequency of association (see Willis and Brigham 2004, 2005 for details). Values of PSI range from −1 to +1 with positive values indicating that the pair spent more time roosting together than predicted if they selected roost-mates and roost sites at random and negative values indicating that the pair spent less time together than predicted at random. We were able to collect genetic data from 17 of the individuals for which behavioral data were presented by Willis and Brigham (2004). Different combinations of these 17 bats resulted in a total of 36 different pairs with PSI scores.

Measures of relatedness were determined from microsatellite loci and from a sequence of mitochondrial DNA. We collected and stored (in 80–95% ethanol or as in Vonhof et al. 2006) two wing punches from each bat (3 mm diameter). Samples were collected from 48 adult females, which represent the majority of adult females present in RA1 and from 41 juveniles. Additional adults (n=23) from nearby roosting areas (<4 km) were sampled for inclusion in background allele frequencies for the relatedness estimator. Total genomic DNA was extracted using a DNeasy® Tissue Extraction Kit (QIAGEN).

Nine microsatellite loci were amplified in 25 μl polymerase chain reactions (PCRs) with optimization modifications from published protocols (Table 1). PCR product was loaded into a MegaBACE® 500 sequencer, and allele size was determined in Fragment Profiler®. At least two identical runs were conducted for each individual at each locus with independent PCR amplifications. Genetic diversity indices were calculated using Cervus 2.0 (Marshall et al. 1998) based on the genotypes of adult females in RA1 (n=48) during 2002–2005. We tested whether loci conformed to the Hardy–Weinberg equilibrium expectations using GENEPOP (Raymond and Rousset 1995). Pairwise relatedness estimates were calculated in Relatedness 5.0.8 (Queller and Goodnight 1989). Calculated relatedness values range from −1 to +1 with negative values indicating pairs sharing fewer alleles than expected at random, which correspond most closely with a biological relatedness of zero. We used adults (n=68, excluding 3 juveniles that returned as adults) to determine background allele frequency. We used Cervus 2.0 (Marshall et al. 1998) to assign juveniles (n=41) to putative mothers present during the year each juvenile was born (n=26–32 candidate mothers per year) with 95% confidence and no mismatches at any

Table 1 PCR conditions for microsatellite loci amplifications and genetic diversity measures (allele size range in bp, number of alleles per locus [A], expected heterozygosity [H_e], observed heterozygosity [H_o], and null allele frequency) for each locus were calculated from adult females (n=48) in RA1

Locus	$MgCl_2$ (mM)	T_a (°C)	Allele size range (bp)	A	H_o	H_e	Null allele frequency	Source species	Source of the primers
EF1	1.5	48.4	157–217	19	0.917	0.904	−0.014	*Eptesicus fuscus*	Vonhof et al. 2002
EF6	3.0	46.1	165–195	16	0.813	0.875	+0.031	*Eptesicus fuscus*	Vonhof et al. 2002
EF14	3.0	46.1	96–138	15	0.938	0.870	−0.047	*Eptesicus fuscus*	Vonhof et al. 2002
EF15	2.0	43.0	103–146	18	0.872	0.881	+0.003	*Eptesicus fuscus*	Vonhof et al. 2002
EF20	3.0	46.1	86–115	14	0.938	0.875	−0.041	*Eptesicus fuscus*	Vonhof et al. 2002
G9	3.0	51.0	115–173	20	0.913	0.918	−0.004	*Myotis myotis*	Castella and Ruedi 2000
G25	2.0	51.0	112–140	8	0.660	0.706	+0.044	*Myotis myotis*	Castella and Ruedi 2000
BE22	3.0	53.8	131–135	4	0.479	0.531	+0.071	*Myotis bechsteinii*	Kerth et al. 2002a
TT20	2.0	46.1	180–190	7	0.688	0.639	−0.044	*Thyroptera tricolor*	Vonhof et al. 2001

locus. Our simulation included 50,000 cycles, 30 candidate mothers, 0.95 of candidate parents sampled, 0.990 of loci typed, and 0.010 loci mistyped. Putative mother–juvenile pairs (n=18) had an average pairwise relatedness of 0.49 (SD±0.09, range 0.35–0.65) which indicates relatedness was estimated accurately. The number of close relatives ($r \geq$ 0.25) for each adult within RA1 was determined for each year and then averaged over all years.

We used sequences from a portion (HVII) of mitochondrial DNA control region to determine matrilineal relationships. Mitochondrial DNA is maternally inherited (assuming no paternal leakage or mutations); therefore, individuals were considered to be from the same matriline if they shared the same sequence (as in Faulkes et al. 2003). We used primers L16517 (Fumagalli et al. 1996) and sH651 (Castella et al. 2001) to amplify the sequence for RA1 adult females (n=48). PCR amplifications were in a total volume of 25 μl and contained 12.5 ng DNA, 1.0 μM each primer, 1.5 mM $MgCl_2$, 0.2 mM dNTPS, and 1 U of *Taq* (Promega). PCR cycling conditions were 94°C for 3 min and then 30 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1.5 min. PCR product was cut from an agarose gel and purified with IsoPure™ Gel Extraction Prep Kit (Denville Scientific, Metuchen, NJ, USA). Sequencing was done using a MegaBACE® 500 sequencer and an ET Dye Terminator Cycle Sequencing Kit (GE Healthcare). We sequenced 300 bp of the segment in both orientations using forward primer L16517 and reverse primer (5′-ATGCGTATGTCCTGA GACCA-3′) that we designed. Sequences were aligned in BioEdit (Hall 1999).

We assessed whether the relatedness of pairs of bats was correlated with PSI. To determine whether the relatedness, based on microsatellite loci, between pairs of bats was correlated with PSI, we used a one-tailed Mantel test with 10,000 permutations of condensed matrices consisting of PSI values and pairwise relatedness of pairs with PSI values. To determine whether matrilineal relationships were correlated with PSI, we used a two-group randomization test (Manly 1991) to evaluate the statistical significance of difference in mean PSI between pairs within the same (n= 10 pairs) and different (n=26 pairs) matrilines. Results for all tests were considered significant at the 5% level.

Objective 2: are individuals that roosted together in roost subgroups more related than would be expected by chance?

Many roost trees had only a single exit, allowing us to use a modified harp trap or canopy mist nets (as in Kalcounis and Brigham 1998; Willis et al. 2003; Willis and Brigham 2004) to capture most, if not all, bats (approximately 95%) as they emerge from a roost tree at dusk. We refer to these bats as a roost subgroup. Every 2–3 weeks from late May to August, we attempted to capture roost subgroups. We captured 20 roost subgroups (n=3 in 2002; n=6 in 2003; n=7 in 2004; n=4 in 2005) ranging in size from 4 to 21 adults (mean= 10.75). We did not trap often to avoid excessive disturbance to the bats.

Relatedness measures from microsatellite loci and mitochondrial DNA sequences were determined as in Objective 1. We determined whether individuals in a roost subgroup were more related than expected at random by comparing the observed average relatedness of each roost subgroup to a distribution of hypothetical average relatedness for 999 randomly selected roost subgroups of equal size (similar to one group randomization with hypothetical group for comparison, Manly 1991). We determined whether individuals in a roost subgroup belonged to the same matriline more often than expected by chance using a chi-squared test. Expected values were calculated by multiplying the frequency of each matriline within RA1 by the size of the roost subgroup. We were also interested in whether females from RA1 in the same matriline had a higher average pairwise relatedness than expected by chance. To test this, we generated a hypothetical distribution of average relatedness for 999 randomly selected groups of equal size to the observed matrilineal group and compared our observed value to the hypothetical distribution.

Objective 3: within roost subgroups, of the pairs of bats that roosted nonrandomly, is the proportion of related pairs higher than expected by chance?

Relatedness measures from microsatellite loci and mitochondrial DNA sequences were determined as in Objective 1. To determine which pairs of bats associated nonrandomly, we calculated an association index (AI) between every possible pair of females in the roost subgroups from Objective 2 using the following index: $AI = (N_{AB}/(N_A + N_B + N_{AB})$ (Ginsberg and Young 1992; Archie et al. 2006). In this equation, N_{AB} is equal to the total number of times individual A and B were in a roost subgroup together, N_A is the number of times A was found in a roost subgroup without B, and N_B is the number of times B was found in a roost subgroup without A. We assessed whether a pair of bats roosted nonrandomly by comparing their observed AI to a distribution of 999 randomly generated AIs. To generate random AIs, we resampled bats into roost subgroups of equal size and number to our observed roost subgroups from the bats that were present during each year and calculated AIs based on the randomly generated subgroups. We considered pairs to be roosting nonrandomly if their observed AI was in the top 5% of the randomly generated AI distribution. By chance, we expected that 50% of pairs with nonrandom roosting associations would have a positive relatedness value and 50% would share the same matriline. We used a sign test to determine if more than 50% of pairs with nonrandom

roosting associations were positively related and to determine if more than 50% of pairs roosting nonrandomly shared the same matriline.

Results

Microsatellite loci were polymorphic with 4 to 20 alleles per locus with high expected and observed heterozygosity based on calculations from 48 adult females in RA1 during 2002–2005 (Table 1). Loci did not deviate from the Hardy–Weinberg equilibrium after a Bonferroni correction for multiple comparisons (Rice 1989), and all loci except G25 did not deviate before the correction. Average pairwise relatedness of adults within RA1 from 2002 to 2005 was −0.01. During each year, each adult (n=32 in 2002, n=26 in 2003, n=30 in 2004, n=29 in 2005) in RA1 had on average 1.84 (n=3,324 pairwise comparisons for 2002–2005) closely related ($r \geq 0.25$) adults within the roosting area. The maximum number of closely related adults for any individual was nine while the minimum was zero. Mitochondrial DNA haplotypes were determined for all RA1 adult females (n=48). Sequences were 273 or 274 bp in length with 20 variable sites that resulted from 19 transitions and 1 insertion/deletion event, which produced 6 unique haplotypes/matrilines (Table 2; see GenBank accession numbers in Table 3).

All six matrilines were present in RA1 each year, but the number of individuals within each matriline differed among years (Table 3). Some matrilines within RA1 had higher average pairwise relatedness than expected at random while others did not (Table 4). Relatedness of bats with the same matriline in RA1 ranged from 0.08 to 0.49 with an average of 0.14, which is higher than background relatedness (Table 4). Four females belonging to M16 had a much higher average relatedness than the other matrilines due to two females within this matriline possessing identical haplotypes (presumed identical twins), their mother, and a close relative of uncertain relationship to the other females.

Objective 1: are roosting associations of pairs of radiotracked bats correlated with measures of relatedness?

We found that the PSI between pairs of radiotracked bats was not correlated with relatedness (Mantel test: r=−0.069, 1,000 randomizations, P=0.574; Fig. 1). Similarly, PSI was not correlated with matriline. There was no difference (randomization test: 210 trials greater than the observed difference in the mean PSI, P=0.210) between the mean PSI for pairs of bats with the same $(\bar{x} \pm \mathrm{SD} = 0.428 \pm 0.208, n = 10)$ and different $(\bar{x} \pm \mathrm{SD} = 0.360 \pm 0.191, n = 26)$ matrilines.

Objective 2: are individuals that roosted together in roost subgroups more related than would be expected by chance?

We found that individuals that roosted together in 90% of the roost subgroups did not have higher average pairwise relatedness than expected at random (Table 5). Roost subgroups always contained females from at least two different matrilines, and females from a single matriline on average comprised 16.7% (±15.6%) of a roost subgroup. Matrilines were distributed randomly within each roost subgroup (χ^2 test: average χ^2=0.98, df=5, average P=0.98).

Objective 3: within roost subgroups of the pairs of bats that roosted nonrandomly, is the proportion of related pairs higher than expected by chance?

We found that 32 pairs of bats roosted nonrandomly within roost subgroups. These pairs included 22 out of 48 individuals that were involved in 1–6 nonrandomly associating pairs. Of the pairs of bats that roosted nonrandomly (n=32), we found that the proportion of positively related pairs (n=14) was not higher than expected by chance (sign test: Z=0.530, P=0.596), and we found that the proportion of pairs that shared the same matriline (n=9) was lower than expected by chance (sign test: Z=2.29, P=0.022).

Table 2 HVII mitochondrial DNA matrilines (haplotypes) consisted of 20 variable sites within a 273–274 bp region that was sequenced for every adult female (n= 48) in RA1

	Nucleotide position (bp)																			
	52	183	191	192	193	206	214	216	217	225	228	230	236	238	240	245	257	265	266	274
M09	G	G	C	G	G	G	C	G	T	T	–	T	T	A	G	A	A	T	A	A
M10			T					A			–						G			
M12			T								–			G						
M15			T		A	A					–									
M16			T	A							–									
M17	A	A	T	A		A	T	A	C	C	A	C	C		A	G		C	G	G

Table 3 The distribution of adult females among the six matrilines from RA1 during 2002–2005

	Matrilines with accession numbers						
	M09 EF164912	M10 EF164913	M12 EF164916	M15 EF164919	M16 EF164920	M17 EF164921	Total
RA1[a]	7	8	2	14	4	13	48
2002 RA1	4	4	2	11	2	9	32
2003 RA1	6	3	1	6	2	8	26
2004 RA1	6	5	1	6	2	10	30
2005 RA1	4	6	1	6	2	10	29

[a] Includes adult females present from 2002 to 2005.

Discussion

Our study provides evidence that patterns of association between roost-mates within fission–fusion bat colonies are not based on preferences for relatives. The strength of roosting associations between pairs of radiotracked bats (PSI) was not correlated with genetic relatedness or matrilineal relationships. The average pairwise relatedness of 18 out of 20 roost subgroups did not exceed values predicted with random roost-mate selection, and individuals within roost subgroups did not roost with bats from the same matriline more often than predicted by chance. Of the pairs of nonrandomly associating bats in roost subgroups, there was not a higher proportion of related pairs than expected by chance, and fewer pairs shared the same matriline than expected by chance. Our results provide strong support that, in contrast to the fission–fusion social systems of many mammals (e.g., African elephants [*Loxodonta africana*] Archie et al. 2006; African lions [*Panthera leo*] Packer et al. 2001; spotted hyenas [*C. crocuta*] Van Horn et al. 2004; Wahaj et al. 2004), individual *E. fuscus* do not preferentially associate with kin.

Our results contribute to a growing body of evidence suggesting that the absence of a relatedness effect on within-colony associations is a general feature of bat colonies (but see mother–offspring preferences in Brigham and Brigham 1989; Rossiter et al. 2002; Rossiter et al. 2005; Kerth et al. 2003). One potential benefit of forming roosting associations within bat colonies may be the sharing of information. *Myotis bechsteinii* transfers information about new roost sites to roost-mates and *Nycticeius humeralis* transfers information about foraging and roost locations to roost-mates independent of relatedness (Kerth and Reckardt 2003; Wilkinson 1992a). At our study site, *E. fuscus* switches roost sites about every 2 days leading us to over 35 different roost trees within RA1 (Willis and Brigham 2004). Consequently, as for *M. bechsteinii* and *N. humeralis*, knowledge about potential roost sites is likely an important resource, although we have no direct data on information transfer about roost sites in *E. fuscus*.

Social thermoregulation is likely an important benefit of group living for *E. fuscus*, especially for adult reproductive females and for young juveniles not yet capable of thermoregulation (e.g., Racey and Swift 1981; Wilde et al. 1999; Lausen and Barclay 2003; Thomas et al. 1990; Hollis 2004, Willis and Brigham 2007). Subgroup size affects local heating of the roost cavity for *E. fuscus* and, as a result, roosting in a larger subgroup would result in a

Table 4 Average pairwise relatedness of adult females within each matriline in RA1 during 2002–2005

Matriline	Average pairwise relatedness	*n*	*P*
M09	0.08	7	0.043
M10	0.17	8	<0.001
M12	0.09	2	0.216
M15	0.02	14	0.115
M16	0.49	4	<0.001
M17	0.01	13	0.156
Average	0.14	8	0.002

P was determined from 1,000 randomizations.
n: number of adult females in RA1 with the designated matriline

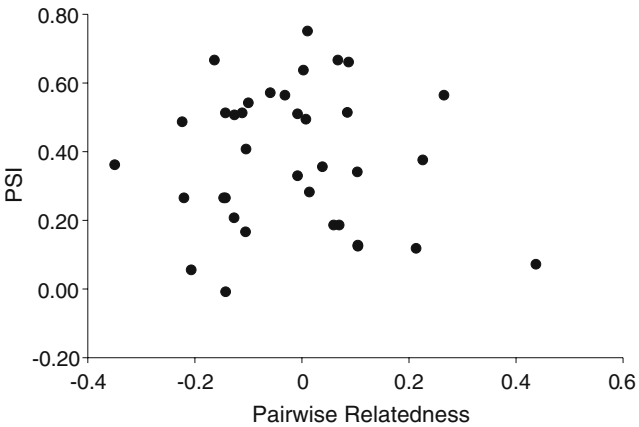

Fig. 1 A scatter plot demonstrating the relationship between PSI value and pairwise relatedness. PSI values are taken from Willis and Brigham (2004) based on data from 36 pairwise combinations of 17 different adult females from RA1 during 2000–2002

Table 5 Average pairwise relatedness of adult females within each roost subgroup

Roost subgroup	Average pairwise relatedness	*n*	*P*
TG1	0.00	19	0.216
TG2	−0.04	4	0.590
TG3	−0.03	4	0.588
TG4	0.01	8	0.213
TG5	−0.03	17	0.832
TG6	−0.02	10	0.549
TG7	−0.11	9	1.000
TG8	−0.01	7	0.401
TG9	0.00	9	0.343
TG10	−0.04	5	0.604
TG11	−0.02	21	0.606
TG12	0.02	14	0.136
TG13	−0.01	14	0.469
TG14	−0.03	19	0.819
TG15	0.04	9	0.095
TG16	−0.02	6	0.570
TG17	−0.01	16	0.366
TG18	0.07	9	0.029
TG19	0.07	10	0.010
TG20	0.06	5	0.151
Average	−0.01		

P was based on 1,000 randomizations.
n: sample size

thermoregulatory energy savings (Willis and Brigham 2007); although, as suggested by Kalcounis and Brigham (1998), *E. fuscus* roosting within tree cavities have the potential to raise the temperature above the thermoneutral zone, which might limit the optimal size of a subgroup. In addition to energetic savings, lactating females that stagger their return from foraging to the roost site at night could keep juveniles warm throughout the night and/or guard juveniles (similar to *A. pallidus*, Trune and Slobodchikoff 1978; *M. thysanodes*, O'Farrell and Studier 1973). If the number of adult females within a roost site is too low, then juveniles might be left unattended during the night and suffer decrease in fitness due to the decrease in body temperature and/or increased predation.

If subgroup size is important (e.g., to reduce energetic costs for social thermoregulation or facilitate information transfer), then subgroup size should remain relatively stable. Fission–fusion sociality might provide a mechanism to stabilize the subgroup size even if the number of individuals within the colony fluctuates. Bats in a large colony, which are divided into smaller subgroups on a daily basis, are able to buffer changes in total colony size and maintain a relatively constant subgroup size. As total colony size decreased in *M. bechsteinii*, the number of subgroups formed per day also decreased while subgroup size remained relatively stable, which suggests that the size of subgroups is important and not a by-product of total colony size (Kerth and König 1999). In general, as the size of a group increases, average relatedness decreases, and individuals face a trade-off between the size of a subgroup vs the kin composition of a subgroup (Avilés et al. 2004; Lukas et al. 2005). In our study area, if females only roosted with matrilineal females, then subgroup size would range between 1 and 11 individuals (Table 3); this is less than the average subgroup roosting size of 18.1 bats observed during 2000–2002 by Willis and Brigham (2004). This suggests that for *E. fuscus* females, roosting in a larger subgroup likely provides more benefits than roosting with close relatives.

Variation in mating systems and life history traits help to explain the low overall relatedness of bat colonies. Mating behavior of many species may prevent high levels of relatedness from accumulating within colonies even in species exhibiting high female philopatry because many males from outside the group sire offspring within the group and females share reproduction within the group (Burland and Worthington Wilmer 2001). Bats have an unusual life history among small mammals with low reproductive rates and long lifespan (Jones and MacLarnon 2001; Barclay and Harder 2003; Maurer et al. 2004). *Eptesicus fuscus* may live 19 years in the wild and reproduces at a rate of only one or two offspring per year with only 10–30% of volant, immature females returning to the natal roost the following spring (Kurta and Baker 1990). Low fecundity, small litter size, long lifespan, low reproductive skew, and high juvenile return coupled with low adult mortality likely reduce group relatedness in other bat species (e.g., Wilkinson 1985b). These factors might explain why females from some matrilines do not have a higher average relatedness than expected at random (Table 4). Regardless, our relatedness estimates would be improved by long-term monitoring of this colony to construct a detailed pedigree that could (1) be used to verify that mitochondrial DNA haplotypes correspond with matrilines and (2) confirm that pairwise relatedness estimates fall within the expected ranges based on known familial relationships.

Although the ultimate benefits of nonrandom associations within fission–fusion bat societies remain unclear, our data provide strong evidence that these associations are not influenced by relatedness or matrilineal relationships. Inclusive fitness benefits gained by preferentially interacting with kin at roost sites do not appear to explain roosting associations between individuals. However, we were not able to observe interactions between individuals inside roost cavities, and it is possible that behavioral interactions inside the roost are concentrated between closely related individuals or females of the same matriline. An important priority for future research should be the direct observation

of individuals inside roost structures using imaging technologies to clarify whether associations among individuals within the roost are influenced by relatedness or other factors such as dominance hierarchies, reciprocity, and/or reproductive condition.

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