

When genes move farther than offspring: gene flow by male gamete dispersal in the highly philopatric bat species *Thyroptera tricolor*

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Abstract

For species characterized by philopatry of both sexes, mate selection represents an important behaviour for inbreeding avoidance, yet the implications for gene flow are rarely quantified. Here, we present evidence of male gamete-mediated gene flow resulting from extra-group mating in Spix's disc-winged bat, *Thyroptera tricolor*, a species which demonstrates all-offspring philopatry. We used microsatellite and capture–recapture data to characterize social group structure and the distribution of mated pairs at two sites in southwestern Costa Rica over four breeding seasons. Relatedness and genetic spatial autocorrelation analyses indicated strong kinship within groups and over short distances (<50 m), resulting from matrilineal group structure and small roosting home ranges (~0.2 ha). Despite high relatedness among-group members, observed inbreeding coefficients were low ($F_{IS} = 0.010$ and 0.037). Parentage analysis indicated mothers and offspring belonged to the same social group, while fathers belonged to different groups, separated by large distances (~500 m) when compared to roosting home ranges. Simulated random mating indicated mate choice was not based on intermediate levels of relatedness, and mated pairs were less related than adults within social groups on average. Isolation-by-distance (IBD) models of genetic neighbourhood area based on father–offspring distances provided direct estimates of mean gamete dispersal distances (\hat{r}) > 10 roosting home range equivalents. Indirect estimates based on genetic distance provided even larger estimates of \hat{r} , indicating direct estimates were biased low. These results suggest extra-group mating reduces the incidence of inbreeding in *T. tricolor*, and male gamete dispersal facilitates gene flow in lieu of natal dispersal of young.

Keywords: disc-winged bat, extra-group mating, gamete dispersal, gene flow, neighbourhood area, philopatry

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Introduction

Behavioural patterns of natal dispersal, social group formation based on kinship and mate choice have been shown to directly shape population genetic structure in many mammalian species (Chepko-Sade & Halpin 1987; Storz 1999; Holekamp *et al.* 2012). These factors interact, as dispersal is often associated with kin competition

and inbreeding avoidance (Moore & Ali 1984; Pusey 1987; Costello *et al.* 2008), and mate choice has been shown to be influenced by levels of social association and genetic relatedness (Pusey & Wolf 1996; Cohas *et al.* 2008). Study of the population genetic structure of social mammals can provide a more complete understanding of these three related processes and their interactions.

Natal dispersal has long been proposed as an adaptation for close inbreeding avoidance (Greenwood 1980; Johnson & Gaines 1990) and the primary mechanism of

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gene flow between populations of organisms (Wright 1931), yet some species are characterized by extreme philopatry. In these situations, offspring of both sexes continue to reside on or near the natal territory beyond the age of reproductive maturity (e.g. Waser & Jones 1983; Amos *et al.* 1993; Stockley *et al.* 1993; Sillero-Zubiri *et al.* 1996; Peacock 1997; Blumstein & Armitage 1999; Clutton-Brock *et al.* 2001). For species that maintain solitary territories, this results in high relatedness among nearest-neighbours (Peacock & Smith 1997; Winters & Waser 2003). In social (i.e. gregarious) species, the result is group formation based on kinship (Greenwood 1980), often typified by cooperative breeding where offspring assist their parents in raising young (Emlen 1995). Yet, extreme philopatry can occur in noncooperatively breeding mammals when fitness benefits are accrued by associating with close kin (Amos *et al.* 1993), or when ecological or morphological constraints limit dispersal (Burland *et al.* 1999; Miller-Butterworth *et al.* 2003).

Highly philopatric species typically display low rates of inbreeding due to various mating behaviours, including mate choice based on relatedness (Hoogland 1982; Dobson *et al.* 1997; Peacock & Smith 1997), extra-pair copulations (Goossens *et al.* 1998; Fietz *et al.* 2000; Roemer *et al.* 2001) and mating outside the typical home range or social group (Amos *et al.* 1993; Winters & Waser 2003). Within the context of gene flow, mate selection based on group membership or geographical location distributes male gametes among social groups, or over greater distances than young disperse. This phenomenon has been referred to as 'gamete dispersal' (Waser & Elliott 1991) creating patterns of genetic spatial structure analogous to plants in which pollen dispersal occurs over large distances yet seed dispersal is limited (Crawford 1984).

Spix's disc-winged bat (*Thyroptera tricolor*) is a gregarious species that displays all-offspring philopatry, forming social groups consisting of one or more reproductive females and offspring of both sexes from multiple years (Chaverri & Kunz 2011a). This bat is morphologically specialized to roost within the furled, developing leaves of *Heliconia* and *Calathea* plants (Wilson & Findley 1977). Groups exhibit fidelity to small roosting home ranges encompassing one or more patches of plants that continually produce new leaves (Vanhof *et al.* 2004; Chaverri & Kunz 2011b). Group cohesion is maintained through highly specific vocalizations between members during the location of roosts (Chaverri *et al.* 2010, 2013). We sought to investigate the effect of natal philopatry among both sexes and highly limited spatial movement on the population genetic structure of *T. tricolor*, and determine whether behaviours associated with mate choice reduce the potential for close inbreeding and facilitate gene flow.

For gregarious, highly philopatric species, the implications of mate choice should be assessed within the context of both the social organization and spatial genetic structure of the breeding population. Parentage analysis and relatedness estimates, performed in conjunction with capture–recapture surveys, can describe the spatial distribution, genetic relationships and social interactions between mated pairs and their offspring (Winters & Waser 2003; Vignieri 2007). Genetic spatial autocorrelation analysis (Epperson & Li 1996) is highly effective for detecting detailed patterns of spatial genetic structure in animals (Peakall *et al.* 2003). A useful model for estimating the effect of dispersal (gamete or natal) on gene flow is that of isolation by distance (IBD) (Wright 1943). Populations are described as collections of panmictic groups (i.e. genetic neighbourhoods), and the scale of gene flow, or neighbourhood area, can be estimated directly from observed dispersal distances. An IBD modelling approach also allows for indirect, genetic estimates of gene flow (Rousset 1997), as geographically closer individuals are anticipated to be more similar genetically. Comparisons of direct and indirect estimators have been successfully applied to studies of movement and gene flow in other mammalian species (Rousset 2000; Broquet *et al.* 2006; Selonen *et al.* 2010), with the benefit of addressing potential biases associated with direct estimates of dispersal from field data.

In this study, we investigate patterns of mate selection and resultant gene flow in a highly philopatric, gregarious bat. We chose to evaluate mate choice both in a social and spatial (i.e. population-level genetic structure) context on account of this species' strong social group cohesion and philopatry to small roosting home ranges. We follow a behavioural-to-genetic level approach by using a combination of capture–recapture and microsatellite data to identify mated pairs and their genetic, social and spatial relationships. We combine home range data with individual-, group-, and population-level genetic analyses to: (i) estimate relatedness within social groups, (ii) determine whether positive spatial genetic structure occurs at the individual or group level, (iii) evaluate patterns of mate choice in relation to geographical distance and levels of relatedness, (iv) evaluate the collective evidence for mate choice behaviour to reduce the incidence of close inbreeding and facilitate gene flow, and (v) infer the spatial scale of gene flow, by comparing direct (demographic) and indirect (genetic) estimates of gamete dispersal distances within an isolation-by-distance model context. Our results suggest that extra-group mating represents a behavioural adaptation for close inbreeding avoidance in *T. tricolor* and that gamete dispersal facilitates gene flow in this species, in lieu of natal dispersal of young.

Materials and methods

Study species

Spix's disc-winged bat is a small (3–4 g) insectivorous species found in Neotropical forests from central Mexico to southern Brazil (Wilson & Findley 1977). Social groups are comprised of individuals that regularly share the same roost. Groups are typically of mixed sex, consisting of 2–12 individuals (Findley & Wilson 1974). Morphological data suggest that ovulation occurs during July–August in southwestern Costa Rica (G. Chaverri and T. J. Orr, unpublished data), and long-term studies show that females give birth to one pup per year during a single, synchronized breeding event (i.e. seasonal monoestry; Chaverri & Vonhof 2011). The patterns of male reproductive success are currently unknown. Males reach sexual maturity within their first year, whereas females are characterized by a second developmental (i.e. subadult) year. Young-of-the-year of both sexes and subadult females can be readily distinguished from adults, as pelage colour changes from dark grey to light brown within the first year and sexual characteristics become conspicuous (e.g. descended testicles, keratinized nipples, lactation, pregnancy, etc).

Field methods and study populations

This study was conducted at two sites within southwestern Costa Rica (Fig. 1). The Km23 site (8°38'N, 83°05'W; 93.6 ha) is located within a matrix of primary and secondary wet tropical forest and agricultural lands, with abundant *Heliconia imbricata* and *Calathea lutea* present in the understory. The Sirena site (8°28'N, 83°35'W; 104.0 ha) is located within Corcovado National Park and is composed of continuous stands of primary and late secondary forests, with *Heliconia* spp. patches scattered in the understory.

Field surveys were conducted in 2008, 2009 and 2011 within preidentified patches of habitat, every 2–4 weeks between the months of May to September. No surveys were conducted in 2010. Groups were captured at the roost, and bats were fitted with individually numbered metal wing bands (Porzana Ltd.), sexed, aged and reproductive condition assessed. Age was classified as juvenile (young-of-the-year), subadult (females only) and adult. A 3-mm biopsy punch of skin tissue was taken from each wing and stored in 5 M NaCl with 20% dimethyl sulfoxide solution for use in genetic analyses. All protocols for capturing and handling bats, and for collecting and exporting DNA, were approved by the Costa Rican government (Permit # R-008-2009-OT-CONAGEBIO).

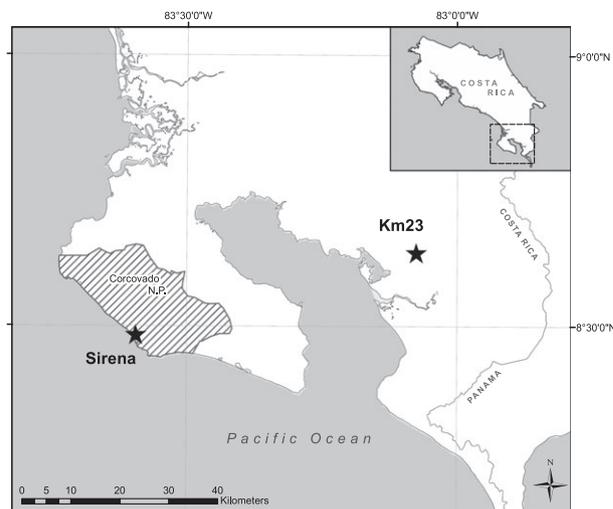


Fig. 1 Map of the two study sites, Km23 and Sirena, located in southwestern Costa Rica.

We captured 768 individuals within the two sites, including 379 bats from Km23 and 389 bats from Sirena. Sex ratios [M/(M + F)] were not significantly different from parity [Km23 = 0.54 ($X^2 = 2.22$, $P = 0.136$), Sirena = 0.48 ($X^2 = 0.51$, $P = 0.477$)]. Of our total captures, 279 bats (139 from Km23 and 140 from Sirena) were first sampled as juveniles or female subadults and were thus of known age. Social units (i.e. groups, as defined by roost sharing) were identified by applying Newman's (2004) modularity modified for weighted networks to all individuals captured at a site across all years. Modularity has an expected value of 0.0 for randomly assigned groups and equals 1.0, if there is no association among members of different groups. Analyses were performed within the program SOCPROG (Whitehead 2009), resulting in 48 groups from Km23 (modularity = 0.95) and 67 groups from Sirena (modularity = 0.94). This approach allowed us to characterize social interaction among-group members across years. We further subdivided social group structure by year, identifying those members present within each group during each breeding season. This allowed for comparisons within and between groups, while accounting for annual changes in composition (e.g. reproduction, mortality, etc).

All capture locations were recorded on a hand-held GPS unit. Leaves in usable condition, as well as those that had recently been used (i.e. guano still present), were also recorded to facilitate habitat mapping at each site. Group roosting home ranges were constructed using the combined capture locations for all individual group members. For groups with three or more capture locations, the 100% minimum convex polygon (MCP) was constructed in ArcGIS 10.0 (ESRI Redlands, CA). The centroid for each home range was used in all pair-

wise comparisons of geographical distance. Mean roosting home range size for both sites (~0.20 ha) indicated high fidelity to small areas, similar to previously documented estimates for this species (0.19 ha in Vonhof *et al.* 2004; 0.14 ha in Chaverri & Kunz 2011b). There was no difference in the size of male and female individual home ranges (*t*-test: $t_{189} = 1.97$, $P = 0.767$). For groups with fewer than three captures, either single capture locations or the centre point between two capture locations was used for distance comparisons. We estimated study area size for each site using the Minimum Bounding Geometry tool in ArcGIS to construct the convex hull around all recorded habitat locations and calculated the area of that polygon.

Molecular analysis

DNA was extracted from wing tissue using Qiagen® DNeasy Blood & Tissue Kits. All samples were genotyped at nine polymorphic microsatellite loci (Table 1) developed by Vonhof *et al.* (2001). Polymerase chain reaction amplifications were performed on a PTC-200 Peltier Thermal Cycler (MJ Research), and microsatellite fragment length was resolved on an ABI 3730 capillary electrophoresis system with an internal size standard and scored using GeneMarker 1.9 (Softgenetics®).

Allele frequencies, and observed and expected heterozygosities, were calculated for each locus. We tested deviations from Hardy–Weinberg equilibrium in GENEPOP 4.2 (Raymond & Rousset 1995) using the exact probability test, with Markov chain parameters set to 100

batches with 1000 iterations per batch. Nei's estimator of F_{IS} (Nei 1987) was calculated across all loci for each population, and within each social group, using FSTAT 2.9.3 (Goudet 2001). The number of alleles per locus observed and expected heterozygosities, the probability of excluding an incorrect parent when no parents are known (Jamieson & Taylor 1997), and F_{IS} values are shown in Table 1. We confirmed the presence of a null allele at locus Tt13 using the program ML-RELATE (Kalinowski *et al.* 2006), estimated its frequency at 0.05 and adjusted our calculations of parentage and relatedness accordingly (see below).

Population structure

We performed analysis of spatial autocorrelation among individual breeding-age adults using the genetic distance-based, multiallele, multilocus approach developed by Smouse & Peakall (1999). Geographical and genetic pairwise distance matrices were calculated in GenAlEx 6.5 (Peakall & Smouse 2012). The 95% confidence interval around the estimate of the genetic autocorrelation coefficient, r_c , for each distance class was constructed via bootstrapping as described in Peakall *et al.* (2003). Significance tests by random permutation of the data provided 95% confidence intervals about the null hypothesis of no spatial genetic structure ($r_c = 0$). To examine genetic similarity between social groups within the context of potential mate choice, we used GenAlEx to perform spatial autocorrelation analysis at the group level. For a given pair of social groups, the individual-by-individual genotypic distances were calculated

Table 1 Genetic diversity and exclusionary power of nine microsatellite loci for Spix's disc-winged bat (*Thyroptera tricolor*) within two populations expressed as (N) the number of alleles (H_O) observed and (H_E) expected heterozygosities per locus, (F_{IS}) the inbreeding coefficient and the probability of excluding an incorrect parent when no parents are known (P_{E-1st}). Mean within social group F_{IS} estimates are shown below population totals

Locus	Km23					Sirena				
	N	H_O	H_E	F_{IS}	P_{E-1st}	N	H_O	H_E	F_{IS}	P_{E-1st}
Tt2	16	0.863	0.856	-0.008	0.88	17	0.785***	0.805	0.025	0.82
Tt5B	8	0.765	0.777	0.016	0.79	11	0.714	0.742	0.039	0.75
Tt8	19	0.902	0.910	0.009	0.95	23	0.875	0.879	0.004	0.92
Tt10	15	0.900***	0.876	-0.027	0.92	16	0.880	0.890	0.010	0.92
Tt13	15†	0.789**	0.885	0.108	0.92	19†	0.677***	0.916	0.260	0.95
Tt30	18	0.942	0.905	-0.040	0.94	21	0.912	0.892	-0.023	0.93
Tt33	17	0.858	0.881	0.027	0.92	17	0.865	0.842	-0.027	0.88
Tt34	5	0.517	0.491	-0.054	0.38	7	0.523	0.517	-0.011	0.36
Tt37B	21	0.821**	0.840	0.023	0.87	18	0.833	0.840	0.009	0.88
		Overall		0.010	1.0				0.037	1.0
		Within-group		-0.117					-0.084	

Deviation from Hardy–Weinberg equilibrium in a single locus indicated by ** $P \leq 0.01$, *** $P \leq 0.001$.

†Number of alleles includes a null allele identified during analysis.

across all pairs of breeding-age adults, representing the specific among-group contrasts, and the average was calculated for this set. Matrices of mean genetic distances and geographical distances among-group home range centroids (described above) were used as inputs for the analysis. Selection of distance classes for all autocorrelation analyses was based on the multimodal distribution of pairwise group distances within each site (Fig. S1, Supporting information), similar to Beck *et al.* (2008). Both individual- and group-level autocorrelation analyses were performed for each year of the study and included only breeding-age individuals captured within a site during that year. This resulted in 12 analyses overall. Sample sizes for each analysis are provided in the Supporting information (Tables S1 and S2). Patterns of both the individual- and group-level spatial autocorrelation analyses were similar across years, allowing us to average the output for each type of analysis from each site and simplifying the presentation of results.

Parentage analysis

Parents were assigned to individuals of known age (i.e. juveniles and subadult females) using PARENTE (Cercueil *et al.* 2002). For our candidate parent data sets, we included all individuals captured within a site over the entire study, and we limited our assignments to individuals of known age to guarantee the directionality of the parent/offspring relationship. The proportion of each population sampled was parameterized based on capture–recapture estimates of population size (see below), and typing error rate was parameterized to 0.01 to account for potential scoring errors. Triad assignments made with 90% confidence and a maximum of one mismatch per parent were retained for further analyses of mated pairs. When available, field observations of preweaning mother–offspring relationships (i.e. pup still attached to mother’s nipple) were used to verify maternal assignment.

Simulation studies have demonstrated that null alleles with a frequency of 0.2 or less introduce inconsequential bias during molecular parentage analysis by underestimating the exclusion probability (Dakin & Avise 2004). The estimated frequency of the null allele at locus Tt13 was 0.05. The exclusion probability for this locus was 0.935, while the overall exclusion probability of all markers combined was 1.0 (Table 1). We therefore felt we were not assigning parents with a false level of confidence. To avoid falsely excluding parents due to the null allele, we required that exclusions be based on mismatches at more than one locus. Running the analysis twice, adjusting the number of allowable mismatching loci from one to two, resulted in identical assignments across the two analyses.

Within-group relatedness and natal philopatry

We used both genetic and capture–recapture methods to ascertain the level of natal philopatry and relatedness within social groups. This was carried out to assess the suitability of group members as potential mates and to confirm previous observations of all-offspring natal philopatry in *T. tricolor* (Chaverri & Kunz 2011a). Maximum-likelihood estimates of pairwise relatedness, *R*, among all individuals within each population were calculated using ML-RELATE, while accounting for the presence of a null allele at locus Tt13. We then wrote a script in R 2.15 (R Development Core Team 2012) to calculate the mean pairwise relatedness of each breeding-age adult to all other adults (of both sexes) present within the same social group for each breeding season (or year). For statistical purposes, analyses were limited to groups containing three or more adults. Comparisons were made for 233 individuals belonging to 36 social groups from Km23 and 223 individuals belonging to 47 social groups from Sirena. The number of bats of each sex used for the calculations, as well as the number of groups represented, is summarized in Table 2. Bootstrap resampling of the relatedness of each individual to its group mates was conducted 10 000 times with replacement to generate 95% confidence intervals around the mean. To generate a distribution for the null hypothesis of no relatedness between an individual and its group mates, we randomly sampled a number of nongroup members (without replacement) equal to the size of the social group in question. Mean pairwise relatedness of the focal individual to the nongroup members was calculated, and this was repeated 10 000 times. The 0.025 and 0.975 quantiles of this distribution were used to provide a test of significance. Results were subsequently averaged within each sex for a particular breeding season for comparison.

We specifically assessed the level of natal philopatry within groups using two methods. First, we identified juveniles born in 2008 and 2009 for which maternity

Table 2 Yearly summaries of the number of adult bats (by sex) and groups used to calculate the mean pairwise relatedness of each individual to all other members of a social group at two study sites in southwestern Costa Rica. Results of analyses are shown in Fig. 2

Site	Year	Females	Males	Total	No. of groups
Km23	2008	45	54	99	19
	2009	41	68	109	21
	2011	29	41	70	17
Sirena	2008	34	40	74	18
	2009	50	72	122	31
	2011	27	46	73	20

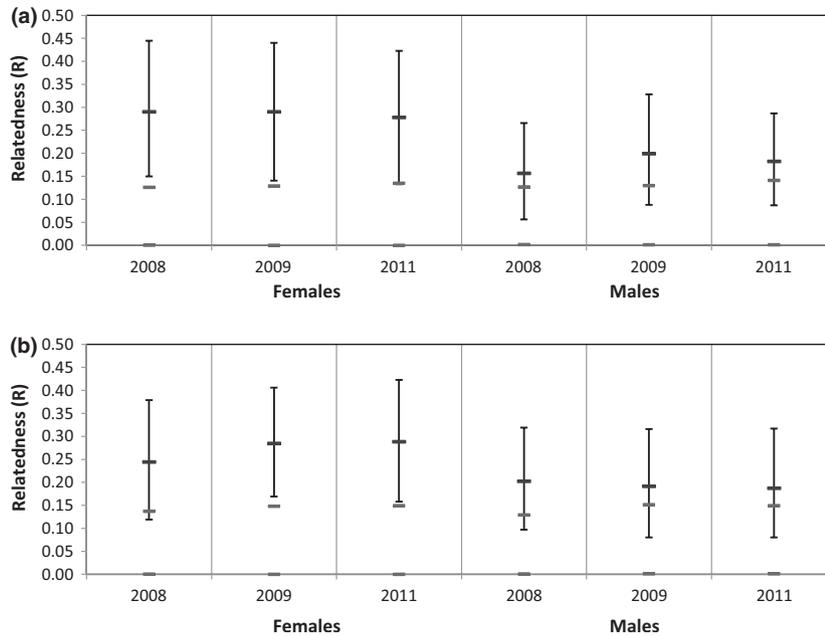


Fig. 2 Mean pairwise relatedness estimates of adult bats to all other adult members of the same social group for both (a) Km23 and (b) Sirena organized by sex and breeding season. Grey bars represent 95% confidence intervals around the null hypothesis of zero relatedness. Error bars represent bootstrapped confidence intervals around the mean.

could be assigned via parentage analysis. Of those, we identified individuals with adequate survival and recapture histories to determine their specific group mates before and after reaching breeding age. We determined the proportion of individuals that continued to roost with their mother and/or natal group mates (i.e. members of the social group present the year that bat was born) hence exhibiting natal philopatry, vs. those that were roosting with new bats and possibly dispersed. We interpreted association with natal group mates but not the mother to indicate mortality of the mother and still considered this natal philopatry. This allowed for quantification of natal philopatry among breeding-age adults of both sexes. Second, because males are typically the dispersing sex within mammals (Greenwood 1980; Dobson 1982), we compared the pairwise relatedness of fathers identified via parentage analysis to their group mates, both before successfully siring a pup and after the reproductive event. Such an approach provides genetic confirmation of whether reproductive males are truly philopatric and continued to roost with close kin after mating, or whether delayed dispersal occurs in association with reproductive maturity.

Simulated random mating

To determine whether nonrandom mating based on relatedness preferences was occurring, random mating was simulated in R 2.15 using a resampling scheme with 10 000 iterations. In each iteration, pairwise relatedness values were selected randomly from a matrix

comparing all adult males with all adult females. The number of simulated ‘matings’ drawn in each iteration was equal to the number of mated pairs identified at each site during the study, and mean relatedness was calculated for each iteration to estimate the 95% confidence limits. The distribution of pairwise relatedness values resulting from simulated random mating was then compared with the observed distribution for mated pairs identified through parentage analysis. Observed relatedness values between mated pairs were also compared with the mean overall pairwise relatedness estimate from each population, as well as the mean values from within social groups (see above) using a Mann–Whitney *U*-test.

Direct estimate of gamete dispersal distances

Under all-offspring philopatry, offspring will belong to the same social group as their mother, with gene flow limited to dispersal of male gametes during mating. Patterns of mate choice therefore have important implications for population genetic structure. We estimated gamete dispersal distance, both directly (demographic) and indirectly (genetic), to quantify the effect of mate choice on the spatial extent of gene flow at each site. In the absence of natal dispersal, Wright’s equation for neighbourhood area is expressed as $4\pi\sigma^2/2$, where σ^2 represents the mean square axial parent–offspring dispersal distance and the $\frac{1}{2}$ accounts for the fact that gene flow only occurs through the movement of male gametes (Crawford 1984). Therefore, σ^2 is equivalent to the squared axial distances between fathers and

offspring. Assuming symmetrical dispersal in two dimensions, $\sigma^2 = \frac{1}{2} \times r^2$ where r is the Euclidean distance between fathers and offspring (See Appendix 1 in Sumner *et al.* 2001 for detailed explanation). We substituted the home range distances between fathers and offspring (see above) for r , squared and summed these distances for all n pairs, and calculated $\sigma^2 = [1/(2n)] \sum r^2$ (Waser & Elliott 1991). The resultant model estimate of mean gamete dispersal distance within a population is equivalent to the radius of the calculated neighbourhood area or \hat{r} .

Indirect estimates of gamete dispersal distance

Direct methods of estimating neighbourhood area draw upon observed father–offspring distances and are subject to potential sampling biases, primarily resulting from under-representation of long-distance gamete dispersal events. This makes indirect genetic-based estimates of dispersal distance particularly useful for comparison. Rousset (1997) demonstrated that genetic neighbourhood area can be estimated from the slope, b , of the regression of pairwise genetic distances $F_{ST}/(1-F_{ST})$ on the logarithm of geographical distances, d , between all individuals or groups within a population. The inverse of the slope of the regression line is an estimate of the product $4D\pi\sigma^2$, where D is the density of breeding adults in the population. Thus, with a density estimate, one can calculate genetic neighbourhood area indirectly.

We used GENEPOP 4.2 to estimate b and compute the 95% confidence interval based on an advanced Bayesian computation bootstrapping procedure. Population size was then estimated via capture–recapture data analysis in MARK (White & Burnham 1999). The Cormack–Jolly–Seber model based on live recaptures in an open population (Lebreton *et al.* 1992) was used to estimate recapture rate, p . We then followed the methodology of Hoyle *et al.* (2001) and estimated the population size N_i for each capture occasion i , as n_i/p_i , where n_i is the number of bats captured on occasion i and p_i is the estimated recapture rate for that occasion. Approximately 95% confidence intervals were calculated as $\hat{N}_i \pm 2se(N_i)$, where $se(N_i)$ is the standard error of the estimate given by the equation $se(\hat{N}_i) = n(se[p])/p^2$ (Lettink & Armstrong 2003). We then calculated a single population size for each site by weighting each estimate by the standard error and calculating the weighted mean. We estimated adult population size by multiplying the above estimate by the proportion of bats captured each year that were reproductive adults. Adult population size was then divided by site area (see above) to estimate the density of breeding adults (adult/m²).

Results

Genetic variation, group relatedness and population structure

Alleles per locus ranged from 5 to 23 resulting in 100% power of parentage exclusion (Table 1). Population F_{IS} values were low (overall values: 0.010 for Km23; 0.037 for Sirena), and negative within-group F_{IS} values indicated social groups were outbred (−0.117 for Km23 and −0.084 for Sirena). Mean pairwise relatedness of female adults to their group mates during the 3 years of sampling was between the secondary (half-siblings) and primary (e.g. parent–offspring, full-sibling) level, ranging from 0.278 to 0.290 for Km23, and 0.244 to 0.288 for Sirena (Fig. 2). Mean pairwise relatedness of males to their group mates was between the tertiary (e.g. first cousins, avuncular, etc.) and secondary level, ranging from 0.156 to 0.199 for Km23 and 0.187 to 0.202 for Sirena (Fig. 2). This places the average level of relatedness among adults (both sexes) and their group mates at approximately the secondary level (0.233) for both sites. Mean pairwise relatedness estimates between individuals and their group mates exceeded the null distribution and were significantly greater than zero for both sexes in all years (Fig. 2).

Patterns of spatial autocorrelation averaged over the three breeding seasons confirmed the presence of significant positive spatial structure among individuals over short distances (group mates), but not among groups (Fig. 3) for both sites. Significant positive structure is present among individuals at distances of <50 m (Fig. 3a, b). Group-level spatial analysis revealed no significant local positive autocorrelation at any distance (Fig. 3c, d). Site-specific results for each annual analysis are provided in the Supporting information (Tables S1 and S2). The combined relatedness and genetic spatial autocorrelation analyses suggest that (i) bats within social groups are frequently related and (ii) are generally unrelated to bats in neighbouring groups. This suggests that mating options within social groups are largely limited to close kin, whereas extra-group mating, even with nearest-neighbours, poses no risk of inbreeding.

Parentage analysis and mated pair identification

We were able to assign at least one parent to 203 offspring (108 for Km23 and 95 for Sirena), and both parents to 34 offspring (22 for Km23 and 12 for Sirena), identifying as many mated pairs. Relatedness estimates between parent–offspring dyads conformed to the expectation of 50% of alleles identical by descent (mean $R = 0.52$) and

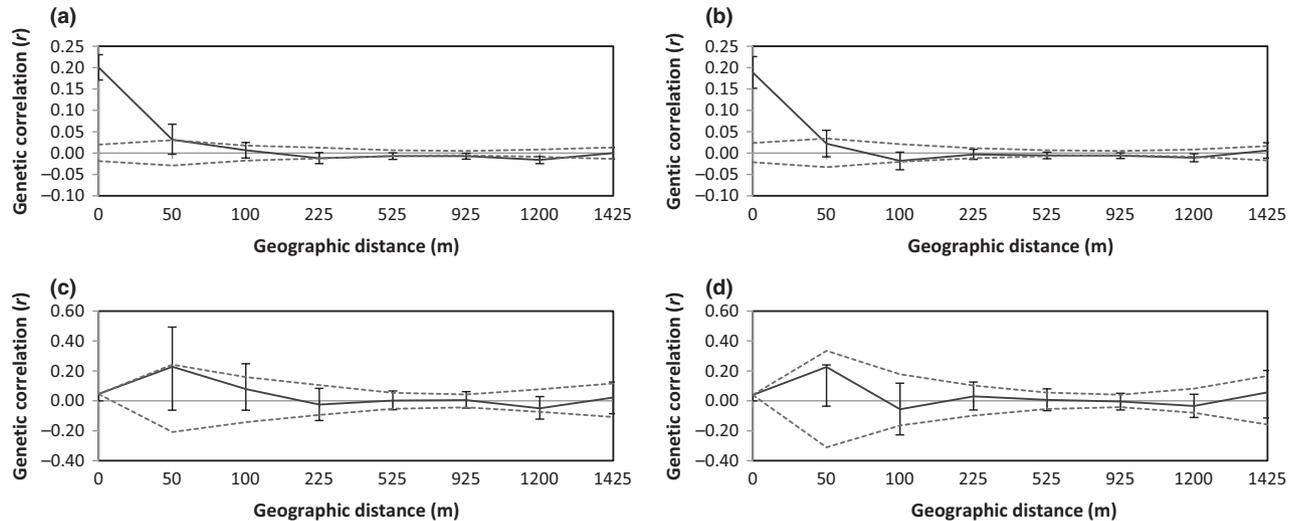


Fig. 3 Correlogram plots of the spatial autocorrelation coefficient r_c as a function of geographical distance for adult individuals at Km23 (a) and Sirena (b), as well as adults by group at Km23 (c) and Sirena (d). Results within sites represent averages of three survey years. Upper and lower bounds for the 95% confidence interval about the null hypothesis of no spatial structure ($r_c = 0$) and the upper and lower 95% error bars about r_c as determined by bootstrap resampling are shown.

genetically assigned mothers matched field-assigned mothers in all cases. All identified mother–offspring dyads belonged to the same social group. In 13 instances, fathers were identified but mothers were not, due to unsuccessful capture or low assignment probabilities among genetically compatible mother–offspring dyads. In these cases, we used the offspring’s home range as a surrogate for the unidentified mother and compared fathers and their offspring instead. A total of 47 comparisons were made (31 for Km23 and 16 for Sirena) for the purpose of determining group membership and geographical distance between mated pairs.

Mated pair group membership and spatial distribution

No mated pair belonged to the same social group, but in three cases, where the mother could not be identified, offspring and fathers were members of the same group. This indicates that mating among group members does potentially occur, but we have no estimate of relatedness between mated individuals in these instances. Mated pairs rarely (3 of 47) had roosting home ranges that overlapped, with the majority of pairs separated by large distances when compared to average home range size. Mated individuals at Km23 were separated by 43–1419 m with a mean of 516 (median = 480; Fig. 4a). Distances between mates at Sirena were similar, ranging between 1 and 1057 m with a mean of 499 (median = 457; Fig. 4b). Mean distance between nearest-neighbouring social groups was 49 m (SE = 4.9) at Km23 and 47 m (SE = 4.2) for Sirena (Fig. 4). These results indicate that reproductive individuals selected

mates farther away than nearest-neighbours the majority of the time (Kolmogorov–Smirnov test, $P < 0.001$ for both sites).

Mated pair relatedness

Mean relatedness between mated pairs was 0.037 (bootstrap 95% CI 0.018–0.058) for Km23 and 0.073 (0.026–0.130) for Sirena (Fig. 5). Mean relatedness between mates did not fall outside the simulated distribution of possible random matings [0.052, (0.021–0.092) for Km23 and 0.052, (0.012–0.107) for Sirena] nor the 95% confidence distribution of the population means [0.052, (0.048–0.057) for Km23 and 0.052, (0.047–0.056) for Sirena], indicating that mates are no more or less related than random. Mean relatedness between mated pairs was also significantly lower than the average relatedness of adults to the other members of their social group [0.233, (0.111–0.366); Mann–Whitney U -test, $P < 0.01$ for each site; Fig. 5].

Confirmation of all-offspring philopatry

Our capture–recapture data allowed us to follow group-mate associations for 37 bats ($n = 21$ for Km23 and $n = 16$ for Sirena) from birth to breeding age (Table 3). Of the 21 bats from Km23, 100% of males ($n = 12$ of 12) continued to roost with their mother and/or other members of the natal group, while 89% of the females ($n = 8$ of 9) did the same. Thus, 95% of bats of both sexes still associated with members of their natal group after reaching breeding age, and only one female was

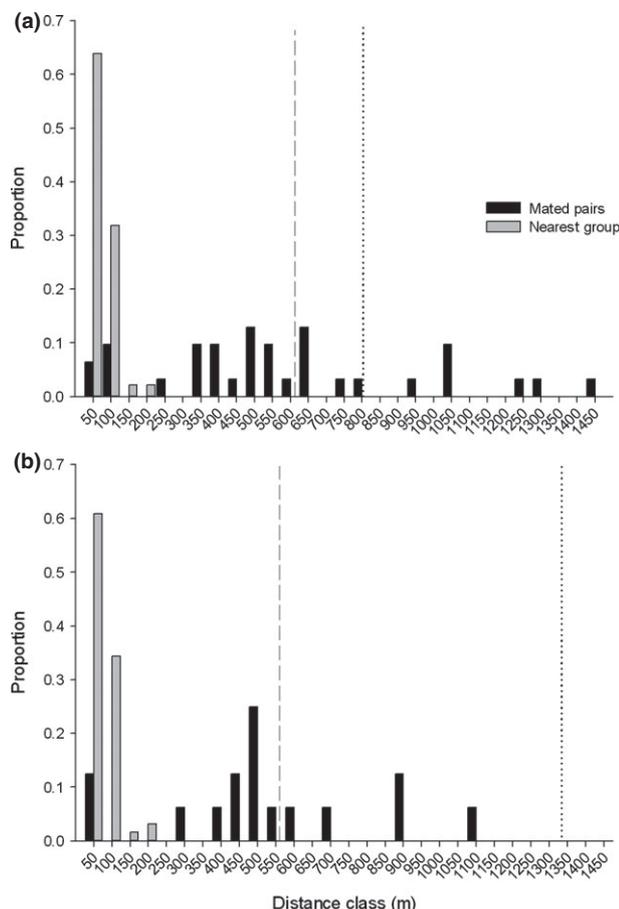


Fig. 4 Frequency distributions as proportions for the pairwise geographical distance between nearest-neighbouring groups (grey) and mated pairs (black) at (a) Km23 and (b) Sirena. The distributions are significantly different for both sites based on Kolmogorov–Smirnov tests ($P < 0.001$ for each). Dashed lines represent direct estimates of mean gamete dispersal distance (\hat{r}), while dotted lines represent indirect estimates.

observed roosting with new, non-natal group bats. At Sirena, 57% of males ($n = 4$ of 7) were still roosting with their mother or natal group members, as were 89% of females ($n = 8$ of 9), resulting in 75% of bats of both sexes still associating with members of their natal group. It should be noted that for those bats observed in new groups, natal group members were no longer captured within the study sites. This pattern suggests that these missing group mates may have permanently emigrated outside the study site boundaries, were not successfully captured again but were present, or had died, and does not necessarily suggest group switching associated with natal dispersal. These results confirm that individuals of both sexes maintain social bonds with members of their natal group beyond the age of reproductive maturity.

In addition, we found no significant difference in pairwise relatedness of the 47 identified fathers to their

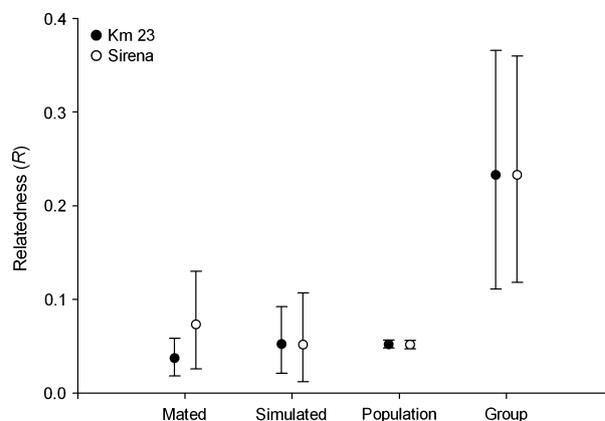


Fig. 5 Mean pairwise relatedness, R , between mated pairs (mated), simulated random mating based on resampling of reproductive adults (simulated), all adults sampled at each site (population), and within groups (group) for Km23 (●) and Sirena (○). Bars are 95% confidence intervals based on bootstrap resampling (10 000 iterations) of the data.

group mates pre- and postreproduction (Mann–Whitney U -test, $P = 0.455$). Relatedness estimates of breeding males to the bats they were observed roosting with both pre- (mean = 0.233) and postreproduction (0.193) were comparable to the mean relatedness of males to group mates ($R = 0.156$ – 0.202) for both sites (see Fig. 2). These results suggest that reproductive male *T. tricolor* continue to roost with close kin after first breeding.

Gamete dispersal distance and genetic neighbourhood area

Direct estimates of mean axial square dispersal distance σ^2 based on pairwise father–offspring distances from Km23 resulted in a genetic neighbourhood area of 1.19×10^6 m² (119 ha) and mean gamete dispersal distance \hat{r} of 615.1 m (Table 4). Direct estimates for Sirena were slightly less, with a neighbourhood area of 1.02×10^6 m² (102 ha) and mean gamete dispersal distance of 569.3 m. These values likely represent underestimates resulting from finite study area sizes and the potential inability to detect dispersal events at the upper end of the distribution of distances.

ArcGIS calculations of site area resulted in 9.36×10^5 m² (93.6 ha) for Km23 and 1.04×10^6 m² (104 ha) for Sirena (Table 5). Recapture rate estimates, p , from the Cormack–Jolly–Seber model ranged from 0.10 to 0.54 at Km23 and 0.48 to 0.80 at Sirena. These recapture rates resulted in adult population size estimates of 144.2 ± 23.3 for Km23 and 151.2 ± 14.2 for Sirena, and adult density estimates of 1.54×10^{-4} and 1.45×10^{-4} adult/m², respectively (Table 5). A small but positive slope, b , of the regression of $F_{ST}/(1-F_{ST})$ vs. $\ln(\text{distance})$ was observed at both sites (Fig. 6). The

Table 3 Proportion of breeding-age adults observed roosting either with or without their assigned mother and/or natal group members. Results are for bats born in 2008 and 2009 for whom maternity could be assigned and group associations determined after reaching breeding age. Numbers in parentheses represent sample sizes

Association with natal group	Km23			Sirena		
	Males (12)	Females (9)	Total (21)	Males (7)	Females (9)	Total (16)
Roosting with mother or natal group members	1.0 (12)	0.89 (8)	0.95 (20)	0.57 (4)	0.89 (8)	0.75 (12)
Not roosting with mother or natal group members	0.0 (0)	0.12 (1)	0.05 (1)	0.43 (3)	0.11 (1)	0.25 (4)

Table 4 Summary for direct demographic and indirect genetic methods of estimating neighbourhood area, mean square axial parent-offspring dispersal distance σ^2 , mean gamete dispersal distance \hat{r} and the ratio of indirect/direct estimates of gamete dispersal distance

Site	Km23		Sirena	
	Direct	Indirect	Direct	Indirect
σ^2 (m ²)	1.89×10^5	—	1.62×10^5	—
Neighbourhood area (m ²)	1.19×10^6	2.04×10^6	1.02×10^6	5.63×10^6
\hat{r} (m)	615.1	806.4	569.3	1.34×10^3
Ratio	1.31		2.35	

Table 5 Isolation-by-distance (IBD) and density estimates for the two study populations. IBD slope is the slope of the IBD regression with bootstrap confidence intervals, Mantel test *P*-value (10 000 permutations) and *D* adult density estimate

Site	Km23	Sirena
IBD		
IBD slope (CI)	3.18×10^{-3} (-2.29×10^{-3} , 5.99×10^{-3})	1.22×10^{-3} (-3.81×10^{-3} , 8.07×10^{-3})
<i>P</i> -value	0.065	0.104
Density		
Study site area (m ²)	9.36×10^5	1.04×10^6
<i>N</i> (adults)	144.2 ± 23.3	151.2 ± 14.2
<i>D</i> (adult/m ²)	1.54×10^{-4} (1.29×10^{-4} , 1.79×10^{-4})	1.45×10^{-4} (1.32×10^{-4} , 1.59×10^{-4})

slope estimate for Km23 was 3.18×10^{-3} , 95% confidence interval (-2.29×10^{-3} to 5.99×10^{-3}). For Sirena, *b* was estimated at 1.22×10^{-3} (-3.81×10^{-3} to 8.07×10^{-3}). Mantel test results based on 10 000 permutations indicated that the slopes were marginally significant (*P*-values of 0.065 and 0.104 for Km23 and Sirena, respectively) and lower confidence intervals were negative in both cases, which translates into an infinite dispersal estimate. Such results are not unexpected in populations with localized dispersal, as the expected patterns of isolation by distance are often weak resulting in low power for the Mantel test to reject the null hypothesis (Rousset 2008). Several studies utilizing the same methodology have found similar patterns of IBD (i.e. small slope, large scatter) and have demonstrated that estimates of *b* provide biologically meaningful inference (Sumner *et al.* 2001; Broquet *et al.* 2006; Puebla *et al.* 2012). The indirect estimate of

neighbourhood area for Km23 was 2.04×10^6 m² (204 ha), resulting in a mean gamete dispersal distance of 806.4 m. The indirect estimate of neighbourhood area for Sirena was 5.63×10^6 m² (563 ha), resulting in a \hat{r} value of 1340 m. Indirect methods resulted in larger estimates of neighbourhood area and mean gamete dispersal distance for both sites (Table 4; Fig. 4), with the indirect estimates of \hat{r} being 1.31 times the direct estimate at Km23 and 2.35 times the direct estimate at Sirena.

Discussion

Within-group and population genetic structure

Estimates of mean pairwise relatedness between breeding-age adults and their group mates revealed that the majority of social groups sampled were comprised of

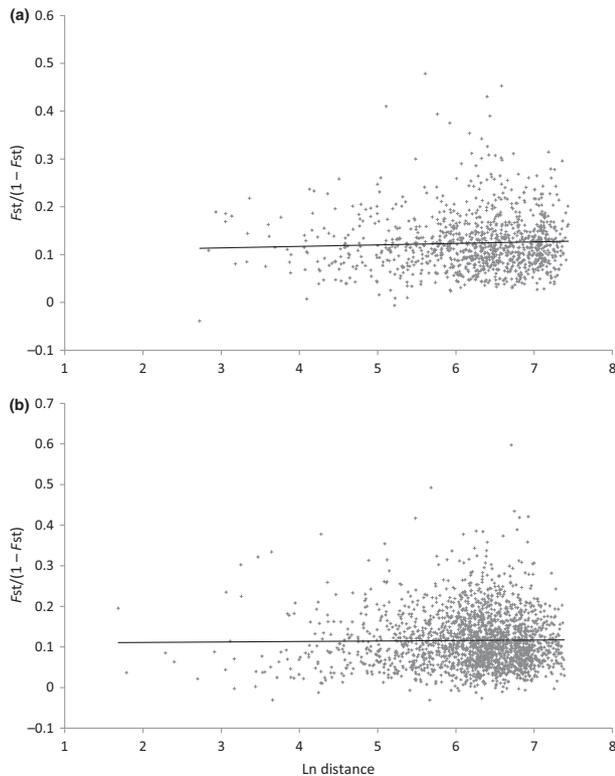


Fig. 6 Patterns of isolation by distance (IBD) at (a) Km23 and (b) Sirena. Regression analyses of genetic distance $F_{ST}/(1-F_{ST})$ on the logarithm of geographical distance, d , are characterized by small slopes and a large scatter as expected, owing to the random nature of mutation and drift. For Km23, $F_{ST}/(1-F_{ST}) = 0.104 + 0.00318 \ln(d)$. For Sirena, $F_{ST}/(1-F_{ST}) = 0.109 + 0.00122 \ln(d)$.

close kin. Females showed higher within-group relatedness than males. Although such results are often attributed to sex-biased dispersal, we attribute this finding to the matrilineal group structure within *T. tricolor*, which should result in higher female–female relatedness. If offspring of both sexes remain within the social group of the mother, while fathers reside in different groups, over time females will end up roosting with both their mother ($R = 0.5$), their offspring (potentially from multiple years, $R = 0.5$) and any half-siblings ($R = 0.25$) or tertiary relatives (first cousins, aunts, uncles, etc., $R = 0.125$). Males on the other hand will be roosting with their mother, but their offspring will belong to other social groups. Males will have a relatedness value of 0.5 with their mother, while the remaining group members will be related to that male at a level of 0.25 (if half-siblings) or less. Thus, female group members on average will share a higher proportion of alleles which are identical by descent than males.

The individual-level spatial autocorrelation analysis exhibited strong positive genetic correlation within the scale of a typical group home range. This is not

surprising given that spatial autocorrelation estimates of r_c and relatedness estimates are strongly correlated (Banks *et al.* 2005). Both study populations showed non-significant genetic correlations among groups over all distance classes, suggesting that matings between nearest-neighbours should pose no risk of close inbreeding. Spatial autocorrelation analyses and the high relatedness estimates between adults and their group mates, particularly for reproductive males both pre- and post-reproductive event, all suggest highly limited natal dispersal. Genetic analyses were corroborated by capture–recapture observations of breeding-age adults of both sexes roosting with either their mother or members of their natal social group. These findings further support previous observations of all-offspring philopatry in *T. tricolor*. The pattern of population genetic structure we detected appears to reflect a combination of high levels of philopatry, formation of kin-based social groups representing distinct lineages unrelated to neighbouring groups and group fidelity to small roosting home ranges.

Patterns of mate choice

This study provides evidence that extra-group paternity in *T. tricolor* is common. Successful mating occurred almost exclusively between individuals residing in different home ranges and mating between group members could only be inferred from three instances where father and offspring belonged to the same group, but mothers were not identified. High mean heterozygosities and low to negative F_{IS} values suggest that successful mating between close kin is rare. Furthermore, mated pairs rarely (6%) had roosting home ranges that overlapped, and the majority of females successfully mated with non-neighbouring males separated by relatively large distances (Fig. 4). Simulated random mating suggests that mate selection was not based on preferences for intermediate levels of relatedness (i.e. optimal inbreeding) as has been observed in some small mammals (Hoogland 1982; Peacock & Smith 1997), and pairwise relatedness between mated pairs was significantly lower than mean relatedness levels between group members. Therefore, the mating system of *T. tricolor* is characterized by reproduction between unrelated individuals from different social groups separated by several roosting home ranges.

Although we have identified mated pairs, and the spatial distribution of their roosting home ranges, we have no direct evidence regarding the mechanism by which mates encounter one another. It is possible that one or both sexes could expand their roosting home ranges temporarily in association with the breeding season. However, this would have to occur during short

periods (<14 days), as a long-term study which sampled *T. tricolor* groups twice a month demonstrated that individuals reside within a consistent roosting home range year round (Chaverri & Kunz 2011a). Distances between mated pairs were significantly greater than necessary to avoid close inbreeding (i.e. the distance between unrelated nearest-neighbours of the opposite sex), suggesting mate choice is not simply based on genetic similarity. In addition, the spatial scale over which *T. tricolor* mated pairs are distributed, the observation that mating appears random with regard to distance (i.e. flat distribution in Fig. 4), and the low success rate for sampling fathers may suggest that mate selection could be occurring independent of individual roosting home range distributions. Mate encounters resulting from temporary *en masse* aggregations associated with common resources or display sites could result in the distribution of mated pairs we observe. Lekking or lek-like mating behaviour with temporary male display sites has been documented in a number of bat species (Wickler & Seibt 1976; Bradbury 1977; McWilliam 1988; Berry & Brown 1995; Murray & Fleming 2008), with displays consisting of various forms of calling behaviour combined with visual and/or olfactory displays. Vocalizations are important for maintaining group cohesion in *T. tricolor* (Chaverri *et al.* 2010), yet it is unknown if vocalizations serve a function in mating. Alternatively, extra-group mating could simply occur in association with nightly activities, such as foraging or night roosting. There is currently no information regarding these behaviours for *T. tricolor*. Knowledge of foraging home range size, whether group and nongroup members forage together, and the distribution and use of night roosts could aid in predicting the frequency and spatial scale over which suitable mates regularly encounter one another. It is important to recognize that regardless of the mechanism by which mate choice occurs the effects on population genetic structure are ultimately realized at the scale of the roosting home range. Dispersal of male gametes well beyond the spatial extent of permanent individual movement maintains gene flow and shapes population genetic structure in *T. tricolor*.

Comparison to mating systems in other species

The social structure of *T. tricolor* appears to be unique among bats in that offspring of both sexes are highly philopatric, social groups are of mixed sex, and these groups show strong fidelity to small roosting home ranges year round (Vonhof *et al.* 2004; Chaverri & Kunz 2011a). Further, our data demonstrate that males are rarely found in the same social group as their offspring, and despite high levels of philopatry, male gametes are dispersed across wide areas. This suggests one or both

sexes are temporarily leaving their roosting home range and encountering mates that normally reside some distance away.

Among year-round resident tropical bats, the majority of species that have been studied have been characterized as having polygynous mating systems where males defend territories or groups of females (McCracken & Wilkinson 2000). Similar to *T. tricolor*, several species of bats form year-round groups composed of both males and females, but in these cases, males mate with female group mates and one sex typically disperses (e.g. Wilkinson 1985; Nagy *et al.* 2007, 2013). One problem with the characterization of these mating systems is that little is known about the frequency of mating that may take place away from roost sites, and short-term movements for the purposes of mating cannot be ruled out in most cases. There is considerable variation in the stability of observed polygynous mating groups in bats, and in some cases, females in socially polygynous species may visit multiple males in a given breeding season, leading to more fluid social structure and promiscuous genetic mating systems (e.g. Campbell 2008; Chaverri *et al.* 2008; Garg *et al.* 2012). In short, associations between males and females in socially polygynous systems are tied to mating, and long-term associations between sexes similar to those observed for *T. tricolor* do not occur (see McCracken & Wilkinson 2000).

Females of a number of temperate bat species exhibit strong philopatry to breeding habitats that they occupy during the reproductive period in summer (often single roosts or patches of habitat; e.g. Kerth *et al.* 2000; Rossiter *et al.* 2000; Kerth *et al.* 2002; Kerth & Petit 2005), with one species exhibiting philopatry by both sexes (*Plecotus auritus*; Entwistle *et al.* 2000; Burland *et al.* 2001). However, male (and sometime female) dispersal is common in temperate bats (McCracken & Wilkinson 2000; Vonhof *et al.* 2008), and mating often takes place away from maternity colonies during seasonal movements between summer and overwintering habitats. The result is that high levels of gene flow may occur between individuals originating from different summer populations at temporary mating aggregations in swarming sites or mating territories (Zahn & Dippel 1997; Kerth *et al.* 2003; Veith *et al.* 2004). Unlike *T. tricolor*, individuals of temperate bat species do not maintain cohesive social groups year round, and mating outside of summer maternity colonies typically results in low levels of genetic differentiation across large spatial scales (Burland *et al.* 1999; Petit & Mayer 2000; Rossiter *et al.* 2000; Castella *et al.* 2001; Bryja *et al.* 2009; Turmelle *et al.* 2011).

There are few analogues to the social and mating system we have observed in *T. tricolor*. The African banana leaf-roosting bat *Neoromicia nanus* uses similar leaf roosts to *T. tricolor*, but has a dissimilar social structure with

groups consisting of multiple females with young, while males roost solitarily and establish small home ranges (Happold & Happold 1996). Mating in *N. nanus* takes place in a prolonged mating season during which males establish themselves in leaf roosts and are visited briefly by 1–5 different females at any given time (Happold & Happold 1996). Similar seasonal patterns of individual males roosting alone with labile groups of females, or consistent transient associations between males and females, have not been observed for *T. tricolor* at any time of year (Vonhof & Fenton 2004; Chaverri & Kunz 2011a). We argue that extra-group matings are therefore likely to take place during nightly foraging bouts, followed by a return to the normal roosting home range during the daytime. Whether these movements outside of the home range consist of individual males establishing temporary territories, grouped displays such as leks, or brief interactions between individual males and females during foraging is an open question.

Natal philopatry of both sexes has been observed in a limited number of mammalian species, along with various mating behaviours which minimize inbreeding. Cooperative breeding and suppression of subordinate reproduction have been shown to reduce inbreeding in meerkat (*Suricata suricata*) colonies consisting of close kin (Clutton-Brock *et al.* 2001). Other species appear capable of distinguishing suitable mates from close kin, either based on social familiarity as in the banner-tailed kangaroo rat (*Dipodomys spectabilis*; Waser *et al.* 2012) or from evidence of nonrandom mating based on relatedness such as in pikas (*Ochotona princeps*; Peacock & Smith 1997). Similar to *T. tricolor*, extra-group copulations have been observed in other mammals, including Ethiopian wolves (*Canis simensis*; Sillero-Zubiri *et al.* 1996) and common shrew (*Sorex araneus*; Stockley *et al.* 1993). However, these two species fail to share either the same level of social group cohesion or site fidelity as *T. tricolor*. Social structure and mate choice patterns in *T. tricolor* most closely resemble those of pilot whales (*Globicephala melas*; Amos *et al.* 1993). Both sexes of *G. melas* are philopatric, pods represent matrilineal, and breeding is thought to occur during short periods when males leave their natal pod in search of mates. The similarities in kin-group formation and mate selection in such divergent lineages raises interesting questions regarding potential evolutionary convergence on certain reproductive and dispersal strategies in response to similar ecological constraints, despite drastic differences in life history.

Gamete dispersal distances and genetic neighbourhood size

Comparisons of direct and indirect estimates of genetic neighbourhood area demonstrate the spatial scale over

which male gamete dispersal is facilitating gene flow in *T. tricolor*. The result is a pattern of spatial genetic structure in *T. tricolor* which is quite unique among mammals and more closely resembles plants with limited seed dispersal, where gene flow is primarily mediated through dispersal of pollen (Crawford 1984). Despite microsatellite loci providing reasonable power to detect parent–offspring relationships, and capture–recapture analyses indicating high percentages of each population recaptured annually (53.4–95.8%), our limited success with paternal assignment (~12%) suggests that many fathers resided outside the study areas. We therefore conclude that our direct estimates are biased low and underestimate mean gamete dispersal distance, \hat{r} . This expectation is supported by larger indirect (i.e. genetic) estimates for both sites (Fig. 4). Direct and indirect estimates for Km23 showed reasonable agreement, with indirect estimates of \hat{r} being 1.31 times direct estimates (Table 4). If we treat the direct and indirect estimates as upper and lower bounds on the extent of gene flow, assuming an average home range area of 0.2 ha (see Materials and methods) \hat{r} estimates (615.1–806.4 m) suggest mated individuals will be separated by distances equivalent to 13–18 roosting home ranges on average. Similarly, genetic neighbourhood area estimates (1.19×10^6 – 2.04×10^6 m²) suggest that the mating system of *T. tricolor* acts to disperse male gametes across an area representing 595–1020 home range equivalents.

Estimates for Sirena showed less agreement than for Km23, with indirect estimates of \hat{r} 2.35 times direct estimates. Direct estimates of neighbourhood area and \hat{r} were comparable to Km23, likely resulting from our attempt to standardize study area size. However, indirect estimates were much larger for Sirena, resulting in a genetic neighbourhood area (5.63×10^6 m²) approximately five times the size of the study area. This would suggest direct estimates are strongly biased low and would account for the lower success in paternity assignment for Sirena, as the majority of fathers would be expected to reside outside the study area. Such a large estimate of neighbourhood area suggests the effect of mate choice on gene flow may be even more pronounced. The resulting estimate of \hat{r} (1340 m) indicates mated individuals are separated by 30 roosting home range equivalents on average. Similarly, the genetic neighbourhood area estimate suggests dispersal of male gametes across an area equalling 2815 home range equivalents. This estimate is more than twice the neighbourhood size predicted for Km23 and could have implications regarding habitat constraints on gene flow. The Sirena site is located within Corcovado National Park and is characterized by continuous stands of forest with abundant roosting habitat (i.e. *Heliconia* plants) in

the understory. The Km23 site is an intact stand of forest surrounded by a matrix of agricultural land with limited connectivity to adjacent undisturbed areas. Habitat distribution, and resulting group roosting home range distributions, might influence the spatial scale over which breeding adults encounter one another, ultimately determining the extent of gene flow. A better understanding of the relationship between habitat suitability, social group distribution, foraging behaviour and mate choice could prove critical for the future conservation of *T. tricolor*, and other highly philopatric species.

Conclusion

Few studies have examined the implications of extreme philopatry for mate choice and gene flow in noncooperatively breeding mammals (Amos *et al.* 1993; Burland *et al.* 2001; Winters & Waser 2003; Waser *et al.* 2012). Here, we present a compelling example of how mate choice based on social group membership and geographical proximity can reduce close inbreeding and facilitate gene flow. Spix's disc-winged bat displays a level of natal philopatry and site fidelity (i.e. small roosting home ranges) unique among even highly philopatric mammalian species. This study demonstrates that *T. tricolor* avoids close inbreeding by mating with nongroup members separated by relatively large distances. This pattern of mate choice results in the dispersal of male gametes, moving genes well beyond group roosting home ranges, and maintaining gene flow in the absence of natal dispersal. All-offspring philopatry, small home range size and male gamete dispersal create a pattern of spatial genetic structure in *T. tricolor* that more closely resembles plants with limited seed dispersal, where gene flow is primarily mediated through dispersal of pollen. Extra-group mating also has significant implications for the social structure of this species by removing some of the underlying causes for natal dispersal (i.e. risk of inbreeding and competition for mates). As a result, both *T. tricolor* males and females appear able to benefit from long-term associations with close kin.

The results of this study indicate that male gamete dispersal is an important form of gene flow for this, and potentially other highly philopatric, gregarious species. Broadening our understanding of mating behaviours that can reduce close inbreeding will improve our understanding of the variety of adaptive strategies naturally occurring across species that are philopatric, naturally fragmented or dispersal-limited. Future work should examine the relationship between mate choice and habitat distribution to predict the

potential genetic consequences of habitat loss for this species.

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Data accessibility

Capture location, microsatellite genotype and demographic data are deposited at Dryad: doi:10.5061/dryad.46h6j. R Scripts (version 2.15) used in analyses have been placed in the archive as well.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Combined frequency distributions of (a) nearest-neighbour distances among groups and (b) pairwise distances among groups for both sites.

Table S1 Yearly outcomes and the overall mean of genetic spatial autocorrelation of individual adult *T. tricolor* at two sites in southwestern Costa Rica.

Table S2 Yearly outcomes and the overall mean of genetic spatial autocorrelation of social groups of adult *T. tricolor* at two sites in southwestern Costa Rica.