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RESEARCH ARTICLE

Conserving the endangered Mexican fishing bat (*Myotis vivesi*): genetic variation indicates extensive gene flow among islands in the Gulf of California

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Abstract The endangered Mexican fishing bat, *Myotis vivesi*, appears to have suffered widespread extinction and population decline on islands throughout the Gulf of California, largely due to predation by introduced cats and rats. To restore populations of fishing bats and other native species, conservation efforts have focused on eradicating introduced vertebrates from several Gulf islands. These efforts assume that individuals from existing populations will recolonize islands and that continued dispersal will help sustain vulnerable populations thereafter. However, the extent of inter-island dispersal in fishing bats is unknown. In this study we analyzed patterns of genetic variation to gauge the extent of gene flow and, thus, potential dispersal among islands. DNA was sampled from 257 fishing bats on 11 Gulf islands (separated by ca. 6–685 km of open water), and individuals were genotyped at six microsatellite loci and haplotyped at a 282 bp fragment of the mtDNA control region. With microsatellites, we found weak population genetic structure and a pattern of isolation by distance, while with mtDNA we found strong structure but no isolation by distance. Our results indicate that island subpopulations separated by large expanses of open water are nonetheless capable of maintaining high genetic diversity and high rates of gene flow. Unfortunately, little is known about the spatial patterns of dispersal or mating system of fishing bats, and these behavioral factors, in particular female philopatry, might reduce the probability of the species recolonizing Gulf islands.

Keywords Dispersal · Gene flow · Gulf of California · Vespertilionidae

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Introduction

Most of the historically recorded extinctions have occurred on islands (Ricketts et al. 2005), and many of the presently threatened species persist only in insular habitat fragments (e.g., Garner et al. 2005; Loewl et al. 2005). When insular populations go extinct, efforts often are made to restore habitat at those sites in the hope that dispersers from extant populations will recolonize empty patches (Scott et al. 2001). Continuing dispersal thereafter may be needed to sustain vulnerable populations by boosting population size (Hanski 1999) and replacing genetic diversity lost to genetic drift (Frankham et al. 2002). Thus, the effectiveness of restoration efforts depends largely on the dispersal behavior of the threatened species (Scott et al. 2001). However, accurate information on dispersal is often lacking because of the difficulty of tracking animal movements (Macdonald and Johnson 2001).

One of the many threatened island-dwelling species is the Mexican fishing bat (*Myotis vivesi*), a vespertilionid

found almost exclusively on small islands in the Gulf of California (Maya 1968; Villa 1979). Fishing bats generally roost within rock crevices in talus, making them easy prey for introduced cats (*Felis catus*) and rats (*Rattus* spp., Villa 1979; Donlan et al. 2003). These exotic vertebrates have decimated populations of many native species on Gulf islands (Mellink 2002; Tershy et al. 2002; Wood et al. 2002; Vázquez-Domínguez et al. 2004) and have contributed to the apparent extinction and decline of fishing bat populations in the Gulf (Ceballos and Navarro 1991). Recent surveys of eight Gulf islands with historical records of *M. vivesi* found no sign of the species (Flores-Martínez 2005; J. J. Flores-Martínez and L. G. Herrera, unpublished data). On at least three of these islands (Encantada, Granito, and San Pedro Mártir, Mellink 2002) cats and rats are present. These issues prompted the Mexican government and IUCN to list the fishing bat as endangered (SEMARNAT 2002) and vulnerable (IUCN 2006), respectively.

In an attempt to restore fishing bats and other native fauna, conservation groups recently began eradicating exotic vertebrates, including cats and rats, from some of the Gulf islands (Mellink 2002). The restoration programs assume that, in lieu of artificial translocation, the threatened species will recolonize restored islands by dispersal from other islands (or the mainland) and that continued dispersal will thereafter sustain vulnerable populations (Scott et al. 2001). However, the extent of dispersal by fishing bats is unknown, and documenting these movements using direct methods would be problematical due to the volant, nocturnal activity of the species. Recently, however, molecular markers such as microsatellite loci and mitochondrial DNA sequences and have become valuable tools for inferring the extent of dispersal, based on models that link genetic structure (differentiation of allele frequencies among populations) to gene flow, an outcome of dispersal (Waples and Gaggiotti 2006).

In this study, we used microsatellite and mitochondrial markers to measure genetic structure of fishing bats in the Gulf of California. Our objective was to use this information to infer the potential for reestablishment and persistence of fishing bats on restored islands. Microsatellite studies of other vespertilionids (*Plecotus auritus*, Burland et al. 1999; *M. myotis*, Castella et al. 2000, 2001; *M. bechsteinii*, Kerth et al. 2002a) in Europe and N. Africa found only weak genetic structure among mainland colonies separated by distances ranging ca. 0.1–770 km, indicating extensive gene flow in these bats. Racey et al. (2007) found that the presence of sea channels ca. 20–90 km wide (English Channel and North Sea) did not significantly affect the degree of genetic differentiation in pipistrelle bats (*Pipistrellus* spp.), which indicated extensive over-water movement. However, microsatellite analyses of two closely related *Myotis* species, *M. myotis*, located in southern Spain, and *M. punicus*, in northern Morocco, suggested that neither species had ever bred on the opposite side of the intervening Gibraltar Strait, despite its narrow width (14 km, Castella et al. 2000). We propose that the much greater distances separating islands in the Gulf of California might present a considerable impediment to fishing bat dispersal and, thus, to conservation of this species. To gauge the extent of inter-island gene flow and, thus, potential dispersal by fishing bats in the Gulf, we sampled DNA from fishing bats on Gulf islands and analyzed the distribution of genetic variation within and among islands. If inter-island dispersal by fishing bats were rare, then we would expect to find substantial genetic differentiation, reflecting a divergence of allele/haplotype frequencies among islands due to low levels of gene flow (Bohonak 1999; Hisheh et al. 2004). Because gene flow replaces alleles lost to genetic drift (Hartl 2000), we would also expect to find low genetic diversity relative to that found in non-insular populations (e.g., Telfer et al. 2003). Finally, if inter-island gene flow is restricted by distance then we should find a positive relationship between genetic distance and geographic distance, in which more closely situated populations are genetically more similar than those farther apart (Hutchison and Templeton 1999).

Materials and methods

Study locations, tissue collection, and DNA extraction

Fishing bats were sampled from 11 islands in the Gulf of California during March–July 2003 (Fig. 1). Nine of these islands had no introduced fauna or other environmental perturbations before or at the time of sampling. On Estanque cats were eradicated in 1999 (B.R. Tershy, personal communication), but they still persist on Salsipuedes (Mellink 2002). Fishing bats apparently can survive on islands with cats if alternative roosting sites on steep cliffs are available (Maya 1968) or predator densities are very low (Vázquez-Domínguez et al. 2004). Minimum distance (Euclidean) between sampled islands ranged from ca. 6 to 685 km; minimum distance between an island and the nearest of the other sampled islands ranged from ca. 6 to 154 km (Table 1). Sampled islands ranged in size from 80 to 1,300 ha.

Total number of individuals sampled was 257, consisting of 161 females (117 adults, 41 juveniles, three unknown age), 90 males (73 adults, four juveniles, 13 unknown age), and six individuals of unknown sex and age. Bats were captured using 12 m mist nets placed near bat colonies during 18:00–06:00. To obtain genetic material, a 3 mm diameter piece of wing membrane was removed with a sterile biopsy-punch and stored in a cryogenic vial with 95% ethanol (Worthington Wilmer and Barratt 1996). Genomic

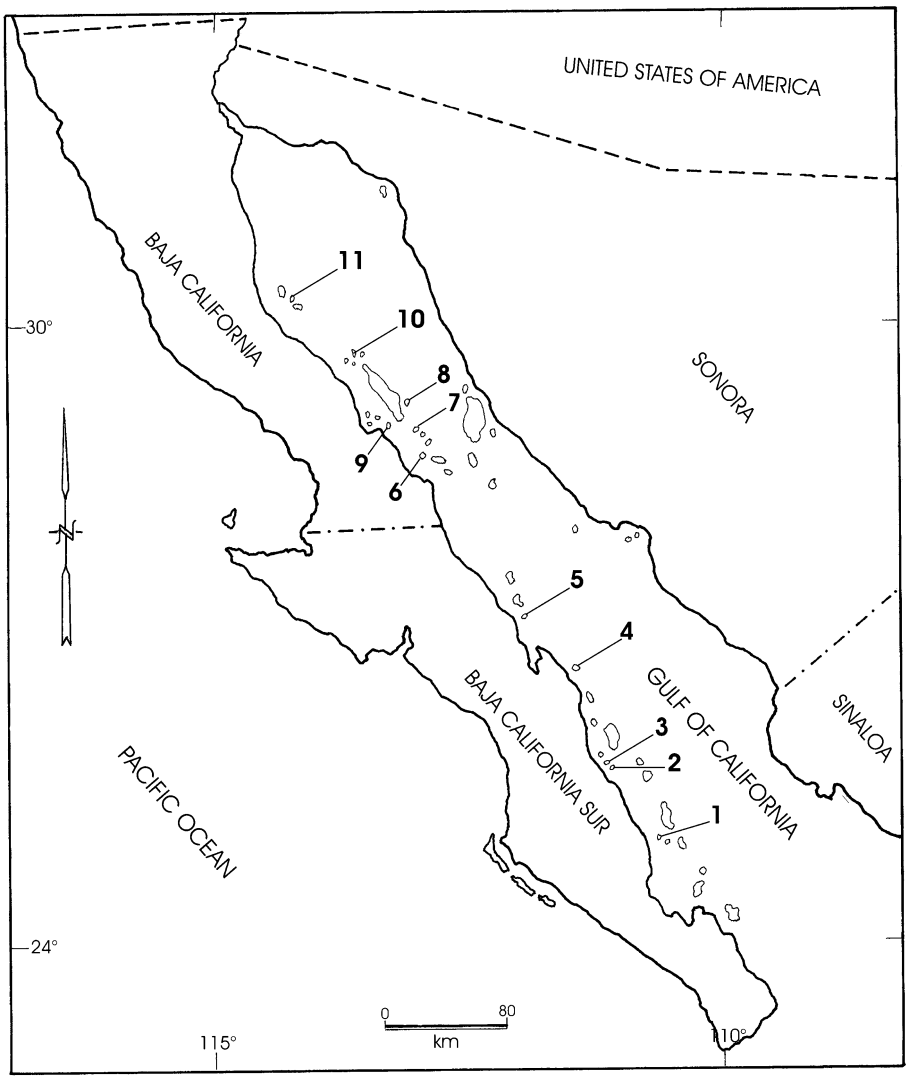

Fig. 1 Islands in the Gulf of California where genetic samples of Mexican fishing bats (*Myotis vivesi*) were collected. *1* Cayo, *2* Tijeras, *3* Colorado, *4* Santa Inés, *5* San Rafael, *6* Salsipuedes, *7* Partida Norte, *8* Estanque, *9* Piojo, *10* Muela, and *11* Encantada

DNA was extracted using GenElute (Sigma–Aldrich, St. Louis, MO, USA) tissue kits. We followed the manufacturer's extraction procedure except that we used only 1 mm^2 of tissue per individual and digested the tissue at 55°C for 24 h while turning continuously.

Laboratory analysis of genetic markers

mtDNA sequencing

We analyzed variation in a 282 bp fragment of the mtDNA control region amplified in a random subsample of 134 individuals from the eleven islands. The fragment was amplified using the primers L16517 (5′-CATCTGGTTCTT ACTTCAGG-3′; Fumagalli et al. 1996) and V23 (5′-AACT TGTTCAGCACTTTAGAT-3′, modified from H00651; Kocher et al. 1989). Polymerase chain reaction (PCR) amplifications were performed in 50 µl reactions containing 5–10 ng DNA, 0.5 µM each primer, 3.0 mM MgCl, 175 µM dNTPs, and 1 U each of FastStart *Taq* DNA polymerase and buffer (Roche, Indianapolis, IN, USA). Amplification conditions were 95°C for 4 min; 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 2 min; then a final extension of 7 min at 72°C. Double-stranded amplicons were purified using QIAquick PCR purification kits (Qiagen, Hilden, Germany) and sequenced in both directions by using BigDye chain terminators on an ABI 377 automated DNA sequencer (Applied Biosystems, Inc., Foster, CA, USA).

Microsatellite genotyping

We tested 24 microsatellite loci for polymorphism in eight fishing bats using primers developed for *M. myotis* (Petri et al. 1997; Castella and Ruedi 2000) and *P. auritus* (Burland et al. 1998). Thirteen of these loci were monomorphic and four others showed variation with poor resolution. The remaining seven loci (D9, D15, MM5, G30,

Table 1 Pairwise matrix of genetic distance (above diagonal) and geographic distance (below diagonal; km) between islands in the Gulf of California, where genetic samples were collected from Mexican fishing bats (*Myotis vivesi*) in 2003

Islands[a]	Ca	Ti	Co	SI	SR	Sa	PN	Es	Pi	Mu	En
Ca	–	0.135	0.008	0.763*	0.121	0.365	0.197	0.115	0.078	0.374*	0.250
		0.008	0.000	0.001	0.001	0.017	0.008	0.022	0.012	−0.003	0.009
		0.229	−0.057	0.187	0.052	0.341	0.374	0.586	0.276	−0.042	0.360
Ti	113.2	–	0.041	0.458*	0.000	0.066	0.000	0.000	0.000	0.084	0.006
			−0.003	0.001	0.009	0.016	0.005	0.017	−0.006	−0.003	0.010
			−0.296	0.004	0.038	0.326	−0.048	0.427	−0.513	−0.304	0.062
Co	118.8	5.6	–	0.666*	0.020	0.251	0.094	0.021	0.000	0.265	0.145
				−0.001	0.001	0.028	0.002	0.011	−0.004	−0.004	0.001
				−0.031	0.062	0.571	0.000	0.411	−0.104	−0.186	−0.086
SI	272.3	159.6	154.1	–	0.471	0.311*	0.398*	0.492*	0.571*	0.310*	0.369*
					−0.008	0.010	0.001	0.008	−0.006	−0.008	0.002
					−0.355	0.194	0.153	0.177	−0.302	−0.298	0.154
SR	300.0	187.2	181.6	28.5	–	0.134	0.017	0.000	0.000	0.152	0.054
						0.008	0.001	0.004	−0.012	−0.004	−0.002
						−0.027	0.149	−0.017	−0.553	−0.087	0.080
Sa	487.9	375.2	369.6	215.7	188.0	–	0.011	0.091	0.148	0.000	0.000
							0.010	0.011	0.005	0.014	0.017
							0.413	0.063	−0.221	0.158	0.470
PN	504.0	391.3	385.7	231.8	204.1	16.1	–	0.034	−0.289	−0.189	−0.013
								0.001	−0.006	−0.029	−0.002
								0.004	−0.006	−0.003	−0.001
Es	524.5	411.9	406.4	252.3	224.8	76.7	21.1	–	−0.482	0.095	0.256
									−0.007	0.006	0.006
									−0.006	0.008	0.010
Pi	537.7	424.6	419.0	266.0	237.8	55.0	42.2	33.6	–	−0.490	−0.356
										−0.009	−0.007
										−0.008	−0.008
Mu	597.3	484.5	478.9	325.1	297.2	109.5	93.5	73.8	63.0	–	−0.305
											−0.004
											−0.003
En	684.7	571.5	565.9	414.0	385.6	202.3	187.4	169.8	148.6	98.8	–

The three values above the diagonal are, from top to bottom, mitochondrial (282 bp fragment of mtDNA control region, 134 individuals) F_{ST}, microsatellite (six loci, 257 individuals) F_{ST}, and microsatellite D_{LR} measures (Paetkau et al. 1997). Statistical significance (available only for F_{ST} values) following adjustment for multiple comparisons ($\alpha = 0.05$; Dunn-Sidak method; Gotelli and Ellison 2004) is indicated by an asterisk (*)

[a] Islands: Cayo (Ca), Tijeras (Ti), Colorado (Co), Santa Inés (SI), San Rafael (SR), Salsipuedes (Sa), Partida Norte (PN), Estanque (Es), Piojo (Pi), Muela (Mu), Encantada (En)

E24, Paur-03, and Paur-06) produced consistently interpretable variation. However, Paur-03 was sex-linked, leaving six loci to be used as the microsatellite markers for this study. PCR amplifications were performed in 10 µl reactions containing 5–10 ng DNA, 0.5 µM each primer, 2–3 mM MgCl, 175 µM dNTPs, and 1 U each of *Taq* DNA polymerase and buffer (GIBCO BRL, Gaithersburg, MD, USA). Amplification conditions were 94°C for 2 min; 40 cycles of 94°C for 40 s, 50–57°C for 40 s, and 72°C for 1 min; then a final extension of 5 min at 72°C. PCR products were diluted in 98% formamide loading dye, separated on 5% denaturing polyacrylamide gels, stained with fluorescent dye, and visualized with a Molecular Dynamics FluorImager (Belfiore and May 2000).

Analysis of genetic variation

Mitochondrial sequences were edited by eye and aligned in CLUSTAL_X (Thompson et al. 1997). We used ARLEQUIN 3.01 (Excoffier et al. 2005) to quantify within-island haplotypic variability as gene (h) and nucleotide (π; Nei 1987) diversity. For microsatellite loci, we quantified within-island

genetic diversity by calculating observed heterozygosity (H_O) and Nei's (1987) unbiased estimate of average expected heterozygosity (H_E), using the program TFPGA (ver. 1.3, Miller 1997). Microsatellite allelic richness was estimated using FSTAT (ver. 2.9.3, updated from Goudet 1995; http://www2.unil.ch/popgen/softwares/fstat.htm). In order to detect heterozygote deficiency, as might result from the presence of non-amplifying ("null") alleles, we tested one-tailed probabilities of departure from Hardy–Weinberg (HW) equilibrium, using the Markov chain exact test in GENEPOP (web version <http://genepop.curtin.edu.au>; Raymond and Rousset 1995). To test for linkage disequilibrium we used the Markov Chain approximation in GENEPOP, which estimates the probability of genotype independence for each pair of microsatellite loci within each island. Significance level was adjusted for multiple comparisons using the Dunn-Sidak method ($\alpha = 0.05$, Gotelli and Ellison 2004).

Genetic structure and gene flow

We used multiple approaches to measure population genetic structure of fishing bats in the Gulf (for convenience we sometimes refer to the different islands as "subpopulations" within the greater Gulf population of fishing bats). First, we used ARLEQUIN 3.01 (Excoffier et al. 2005) to quantify the magnitude of genetic differentiation (F_{ST}) among subpopulations for mtDNA haplotypes and microsatellite alleles (Weir and Cockerham 1984). We tested for statistical significance of differentiation estimates by bootstrapping over loci (20,000 permutations) to generate 95% confidence intervals and jackknifing over loci to obtain standard errors. We tested for overall heterogeneity in microsatellite allele frequencies using the Markov Chain approximation in GENEPOP to estimate the one-tailed probability of allelic differentiation under the null hypothesis of no difference among subpopulations.

Second, we measured the relationship between genetic distance and geographic distance among subpopulations using GENEPOP, which regresses genetic distance measures on geographic distance (ln km), calculates Spearman rank correlation coefficients (r), and uses a Mantel (1967) permutation procedure (1,000,000 permutations) to establish 95% confidence intervals for r. We used two genetic distance measures: linearized F_{ST} values ($F_{ST}/1-F_{ST}$; Slatkin 1985) and the genotype likelihood ratio distance, D_{LR} (for microsatellites only), which measures differences between likelihood values calculated as the expected probabilities of each individual's genotype in each subpopulation (Paetkau et al. 1997). To avoid biasing the likelihood values, the test individual was excluded from its sample subpopulation when estimating allele frequencies and assigned a frequency value of 0.01 if this subtraction resulted in a probability of zero (Paetkau et al. 1997). We used GENECLASS2 (Piry et al. 2004) to calculate likelihood values for D_{LR}.

Third, as an alternative to traditional F_{ST} methods, which specify subpopulations a priori, we used the Bayesian clustering method in STRUCTURE (ver. 2, Pritchard et al. 2000; Falush et al. 2003) to measure population structure of microsatellite variation. This program estimates, without regard to sampling location, the number of genetic subpopulations (K), as deduced by posterior probabilities [LnP(D)]. STRUCTURE assigns genotypes to genetic clusters (subpopulations) such that linkage and HW disequilibrium are minimized within clusters. Using the admixture model and correlated allele frequency parameters, five replicates of each run from $K = 1$ (a single panmictic population) to $K = 11$ (each island representing a different subpopulation) were performed. Each replicate was run for 200,000 Markov chain Monte Carlo (MCMC) generations with an initial burn-in of 20,000 generations. For the K with the highest likelihood, we determined for each sampled individual its probability of membership in each genetic cluster based on its degree of ancestry (q) that could be attributed to that cluster (Pritchard et al. 2000).

Results

Genetic variation

We recovered 39 mtDNA haplotypes and deposited the sequences in GenBank under accession nos EF119455–EF119493. Haplotype diversity ranged from 0.000 to 0.945, averaging 0.543, while nucleotide diversity ranged from 0.000 to 0.017, averaging 0.009 (only one island, Cayo, lacked diversity; Table 2). Microsatellite variation averaged over subpopulations was high, with the number of alleles per locus ranging from 3 to 19, and heterozygosity (H_E) estimates ranging from 0.36 to 0.92. Only one locus, MM5, showed significant heterozygote deficiency ($P < 0.009$). Averaged over loci, allelic richness ranged from 6.8 to 8.0; mean H_E per island ranged from 0.69 to 0.79, averaging 0.76 (Table 2). Significant heterozygote deficiency was detected in only one of the 11 subpopulations (La Muela, $P < 0.005$). When analyzed within islands, significant linkage disequilibrium was found in only one of the 165 comparisons (E24/P06 on Partida Norte, $P < 0.0004$). Analyzed across islands, no significant linkage disequilibrium among loci was detected among any of the 15 possible pairwise comparisons.

Genetic structure and gene flow

We found much greater subpopulation differentiation for mtDNA haplotypes than for microsatellite loci. For

Table 2 Mitochondrial (282 bp fragment of mtDNA control region, 134 individuals) and microsatellite variation (six loci, 257 individuals) for Mexican fishing bats (*Myotis vivesi*) sampled from eleven islands in the Gulf of California

Site	*N*-mt	*H*	*h*	π	*N*-mc	*A*	H_O	H_E
Cayo	12	1	0.00	0.000	26	7.3	0.70 (0.23–0.96)	0.72 (0.22–0.92)
Tijeras	14	6	0.604	0.017	18	7.9	0.77 (0.39–0.94)	0.75 (0.32–0.94)
Colorado	11	6	0.181	0.004	22	7.4	0.71 (0.23–0.91)	0.76 (0.27–0.92)
Santa Inés	13	4	0.423	0.012	19	7.5	0.75 (0.42–0.95)	0.76 (0.39–0.91)
San Rafael	12	3	0.439	0.010	20	7.8	0.74 (0.30–0.90)	0.75 (0.26–0.92)
Salsipuedes	11	9	0.945	0.011	11	6.8	0.68 (0.27–1.00)	0.69 (0.33–0.94)
Partida Norte	13	7	0.730	0.011	59	7.7	0.74 (0.39–0.93)	0.77 (0.45–0.93)
Estanque	13	5	0.538	0.015	17	7.7	0.85 (0.59–1.00)	0.79 (0.51–0.93)
Piojo	10	3	0.378	0.003	16	8.0	0.84 (0.50–1.00)	0.78 (0.39–0.92)
Muela	12	9	0.939	0.012	20	7.7	0.69 (0.30–0.89)	0.76 (0.34–0.93)
Encantada	13	7	0.794	0.007	29	7.4	0.75 (0.34–0.97)	0.75 (0.34–0.91)
Mean	12	5	0.543	0.009	23	7.6	0.75 (0.37–0.91)	0.76 (0.36–0.92)

N is number of individuals sampled for mitochondrial (-mt) and microsatellite (-mc); *H* is the number of mtDNA haplotypes; *h* and π are gene and nucleotide diversity, respectively; *A* is allelic richness; H_O and H_E are observed and expected heterozygosity, respectively (range across six loci in parentheses)

mtDNA, F_{ST} ranged from 0.00 to 0.76 (Table 1) averaging 0.15. For microsatellites, exact tests revealed statistically significant differentiation of islands over all six loci ($X^2 = 22.6$; $df = 12$; $P < 0.04$). However, the magnitude of differentiation was extremely low, with F_{ST} values ranging from −0.029 to 0.028 (Table 1), averaging 0.002 (SE = 0.002; 95% CI: −0.001–0.007). Only 8% of the alleles (6/75 alleles at six loci) were private (i.e., unique to a particular island), two on Partida Norte and one each on Muela, Estanque, San Rafael, and Tijeras.

For mtDNA there was no relationship between genetic distance and geographic distance ($r = 0.11$; $P = 0.12$; Fig. 2). This contrasted with our result for microsatellite loci, for which we found a positive (i.e., isolation by distance) relationship using the D_{LR} ($r = 0.32$, $P = 0.03$; Fig. 2) distance measure and a marginally significant relationship using F_{ST} ($r = 0.26$; $P = 0.07$).

Bayesian analyzes using STRUCTURE found no genetic structure in our samples. The highest mean likelihood was found for $K = 1$ (Ln = −5,907.640). For $K = 9$ (i.e., the model with nine subpopulations), one of the five replicates produced a slightly higher likelihood (Ln = −5,902.100); however, the proportion of membership assigned to each of the nine subpopulations was roughly symmetric ($q = 0.111$), and the likelihood values varied considerably among the replicates (from −5,902.1 to −8,730.2; mean = −6,724.6). Conversely, for $K = 1$, the likelihood values among the five replicates did not vary substantially (from −5,904.2 to −5,909.6). Thus, according the cluster analysis, the mostly likely model for fishing bats on the Gulf Islands sampled in our study is that of a single panmictic population (Pritchard et al. 2000; Evanno et al. 2005).

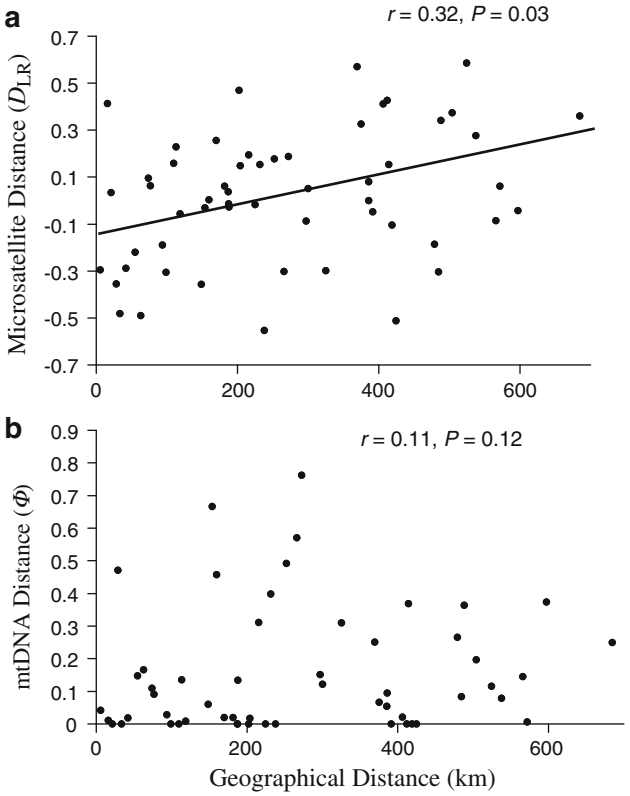

Fig. 2 Relationship between genetic distance and geographic distance (km) among 11 island populations of fishing bats (*Myotis vivesi*) sampled in the Gulf of California in 2003. *r* values are Spearman rank correlation coefficients; *P* values were determined using Mantel (1967) tests. (**a**) relationship for six nuclear microsatellite loci (257 individuals) using the D_{LR} measure (Paetkau et al. 1997). A similar relationship was found using linearized F_{ST} values ($F_{ST}/1-F_{ST}$; $r = 0.26$; $P = 0.07$). (**b**) Relationship for mtDNA haplotypes (282 bp fragment of mtDNA control region, 134 individuals) using linearized F_{ST}

Discussion

Results of our microsatellite analyzes point to extensive inter-island gene flow, and thus potential dispersal, by fishing bats in the Gulf of California. First, genetic diversity was high. Mean heterozygosity ($H_E = 0.76$) was greater than that found in populations of *M. myotis* in mainland Spain and *M. punicus* in mainland Morocco ($H_E = 0.71$ and 0.56, respectively, Castella et al. 2000), and was similar to that in colonies of other vespertilionids in northern/central Europe: *M. myotis* ($H_O = 0.78$, Castella et al. 2001), *M. bechsteinii* ($H_O = 0.83$, Kerth et al. 2003), *P. auritus* ($H_E = 0.79$, Veith et al. 2004), and *M. nattereri* ($H_E = 0.79$, Rivers et al. 2005). High levels of genetic diversity would be expected if dispersal among islands were extensive, because gene flow replaces alleles lost to genetic drift (Hartl 2000). Extensive dispersal should also prevent the buildup of private alleles (Slatkin 1985). Indeed the frequency of private alleles in our study was only 8%.

Second, we found only slight population structure at microsatellite loci. Mean overall F_{ST} was nearly zero (0.002), and even between the most distantly separated of sampled islands (Cayo and Encantada, ca. 685 km apart), F_{ST} values were negligible (0.009). None of the between-island F_{ST} estimates were higher than 0.030. Our F_{ST} estimates are lower than those found among mainland (i.e., non-insular) colonies of vespertilionids in Europe: *M. myotis* colonies separated by 130-580 km in Spain, and *M. punicus* colonies separated by 270–770 km in North Africa ($F_{ST} < 0.02$, Castella et al. 2000); *M. nattereri* colonies separated by ca. 5–150 km in England (mean $F_{ST} = 0.017$, Rivers et al. 2005). Our mean F_{ST} was also lower than that found among European colonies of *Pipistrellus* spp., which included populations separated by the English Channel and North Sea ($F_{ST} = 0.024$–0.044, Racey et al. 2007).

Third, there was positive relationship between microsatellite genetic distance and geographic distance; this isolation by distance pattern is consistent with dispersal among islands being limited by geographic distance, such that gene flow is increasingly less likely at greater and greater distances. Our results ($r = 0.26$–0.32) were similar to those found in colonies of *M. bechsteinii* ($r = 0.20$; Kerth and Petit 2005) and *M. myotis* ($r = 0.26$–0.30; Ruedi and Castella 2003), both distributed across hundreds of kilometers in Europe.

Finally, Bayesian clustering/MCMC analyses by STRUCTURE revealed a lack of genetic structure and very high inter-island gene flow estimates, respectively. According to these analyses the island subpopulations of fishing bats sampled in the Gulf of California could not be distinguished from a single panmictic population.

In contrast to our results from microsatellite analyzes, we found strong mtDNA haplotype differentiation among islands (ca. 75 times greater than with microsatellites). This combination of strong mtDNA versus weak microsatellite structure has been found in several other vespertilionids (e.g., *Nyctalus noctula*, Petit and Mayer 1999; *M. myotis*, Ruedi and Castella 2003; *M. bechsteinii*, Kerth et al. 2008) and is generally attributed to strong female philopatry and extensive, male-biased dispersal (Chen et al. 2008). In addition, we found no isolation by distance pattern with mtDNA markers. The strong mtDNA structure and lack of isolation by distance suggest that female dispersal among islands was too rare to overcome genetic drift.

Conservation implications

Our results show both positive and negative implications for efforts to conserve Mexican fishing bats. On the one hand, our results indicate that island subpopulations separated by large expanses of open water are nonetheless capable of maintaining high rates of gene flow; thus, the maintenance of genetic diversity does not appear to be strongly limited by distance from potential sources of immigrants. In this respect, the prospects seem more favorable for conserving populations of fishing bats than for less vagile island-dwelling species (e.g., some lizards on islands of New Zealand, Towns 2002). On the other hand, results from our mtDNA analyzes suggest that this high gene flow might not translate into high probabilities that restored islands will be recolonized. Strong female philopatry seems to be the rule in vespertilionid bats (e.g., Petit and Mayer 1999; Castella et al. 2001; Ruedi and Castella 2003; Chen et al. 2008); and when dispersal is rare, colonization of empty habitat might be even rarer (Kerth and Petit 2005). In addition, inter-island gene flow can occur in ways other than individuals immigrating to an island and reproducing there. Among European colonies of *M. bechsteinii*, *M. nattereri*, and *P. auritus*, for example, much of the gene flow appears to occur via mating swarms, which are sites (located 10–500 km from the maternal colonies) where the sexes meet and copulate during the late summer/fall (Kerth et al. 2003; Veith et al. 2004; Rivers et al. 2005). In maternal colonies of the endangered *M. bechsteinii*, female philopatry is almost complete (i.e., females stay in their natal colonies to rear pups), and thus new colony formation apparently is very rare (Kerth et al. 2002a). The greatest source of new genetic diversity in maternal colonies of *M. bechsteinii* apparently comes from foreign males encountered at mating swarms, leading Kerth et al. (2003) to urge strict protection of swarming sites. Unfortunately, little is known about the spatial pattern of dispersal or the mating system of fishing bats, so we do not know to what degree the high genetic connectedness found in our study represents successful mating by immigrant females.

Habitat characteristics may also play a large role in determining whether an immigrant female stays and successfully reproduces on an island. All 11 islands in our study area support substantial densities of fishing bats (e.g., >10 000 individuals on Partida Norte; Herrera and Flores-Martínez 2002; J. J. Flores-Martínez and L. G. Herrera, unpublished data) and thus presumably contain suitable habitat. However, other islands may be deficient in one or more key habitat characteristics. For example, on the island of San Jorge, where fishing bats appear to be rare, large amounts of bird guano have accumulated in the interstices of the talus, making it inaccessible to fishing bats (J. J. Flores-Martínez and L. G. Herrera, unpublished data). Thus, it is possible that frequent movement through the interstices is required to keep the talus open to bat roosting. Restored islands may also be deficient in cues (e.g., sounds of bat calls) indicating the presence of conspecifics. Recently, conservation biologists have become increasingly aware of the importance of conspecific attraction in habitat selection (Reed 1999). For example, loggerhead shrikes preferentially settle near conspecifics, thus reducing the probability that unoccupied patches of habitat will be recolonized (Etterson 2003). Higher densities of conspecifics in some species can provide protection from predators or facilitate reproduction (Stephens and Sutherland 1999). Fishing bats in the Gulf of California form large aggregations (Maya 1968; Herrera and Flores-Martínez 2002; Flores-Martínez 2005; Flores-Martínez et al. 2005); thus, low densities or the absence of conspecifics on restored islands may reduce the probability of immigrants settling there.

Conclusions

In sum, our genetic study of fishing bats on islands in the Gulf of California revealed high genetic diversity on islands and extensive inter-island gene flow. However, gaps in our knowledge of fishing bat natural history, especially dispersal and mating system, need to be filled in order to effectively restore and protect populations. Specific questions that need to be answered include: When and where does copulation occur? To what degree are males and females philopatric? What is the spatial/temporal distribution of dispersal? What habitat characteristics do dispersing individuals prefer or what cues do they use in selecting habitat? Answering these questions will be logistically difficult and expensive, possibly involving an intensive mark-recapture and radio-tracking program. However, in the meantime, density and distribution of fishing bats should be carefully monitored via systematic surveys in order to determine the trajectory of populations. Particularly on islands targeted for restoration, surveys should be conducted before and after removal of exotic predators. If fishing bats do not recolonize or if densities decrease (or fail to increase sufficiently), managers should consider habitat modification, such as making available more roosting areas or enhancing their social context. The latter approach is gaining more attention as a potential tool in bird conservation; namely, the use of playback calls to attract conspecifics to an unoccupied but suitable habitat patch (Ward and Schlossberg 2004; Hahn and Silverman 2007). If fishing bats are attracted to conspecific calls (e.g., as found with spear-nosed bats, *Phyllostomus hastatus*, Wilkinson and Boughman 1998; but see Kerth et al. 2002b), then installing bat call playback systems in colonies or potential colony sites might be used to increase densities. Innovative and experimental approaches may be necessary to ensure the long-term persistence of Mexican fishing bats in the Gulf of California.

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