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16. GENETIC VARIATION AND POPULATION SIZE OF THE ENDANGERED FISHING BAT, *MYOTIS VIVESI*, IN ISLA PARTIDA

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Abstract

Myotis vivesi is a Mexican endemic fishing bat listed as endangered but with no recent field information about the status of its populations. We present data on the genetic diversity and population size of the largest known colony of the fishing bat on Isla Partida. The colony showed a substantial amount of genetic diversity at six microsatellite loci, with observed heterozygosity and number of alleles per locus averaging 76% and 9.5, respectively. Our estimates of effective population sizes on Isla Partida were very large, ranging from 14 170 to 68 570 individuals. Absolute population size could not be determined for the entire island because sites were not sampled simultaneously but the highest number of bats recorded at a single period was close to 8000. The colony showed substantial changes in composition. From November to March there were almost equal number of males and females. In March, however, roosts with individuals of both sexes had one male and several females. In May, males disappeared and the colony was composed almost exclusively of females with their young. Our data indicates that fishing bats at Partida have levels of genetic diversity that are similar to the values found in other widespread *Myotis* species. Further research on the genetic diversity and population size of other colonies along

Resumen

Myotis vivesi es una especie endémica de murciélago pescador enlistada como amenazada, aunque no hay información reciente sobre su status poblacional. Aquí presentamos datos sobre la diversidad genética y el tamaño poblacional de la colonia más grande conocida de esta especie en Isla Partida. La colonia mostró una significativa diversidad genética en seis loci de microsatélites con heterocigocidad y un número de alelos por locus promediando 76% y 9.5, respectivamente. El estimado del tamaño de población efectivo en Isla Partida fue muy alto, con un margen de 14,170 a 68,570 individuos. El tamaño absoluto de población no fue determinado para la isla debido a que las localidades no fueron muestreadas simultáneamente, pero el número de individuos registrados en un solo periodo fue de 8,000. La colonia mostró cambios sustanciales en su estructura y composición. De noviembre a marzo, se observó un número similar de hembras y machos. En marzo, sin embrago, los grupos mostraban un macho y varias hembras. En mayo, los machos desaparecieron y la colonia estuvo formada casi exclusivamente de hembras con crías. Los datos de esta especie en Isla Partida muestran que los niveles de diversidad genética son similares a los valores observados en otras especies de *Myotis* de amplia distribución. Estudios pos-

its distribution range are needed to evaluate the conservation status of the fishing bat.

Keywords: Conservation, effective population size, Gulf of California, *Myotis vivesi*, population genetics.

teriores sobre la diversidad genética y tamaño poblacional son necesarios para evaluar el status de conservación de esta especie.

Palabras clave: conservación, tamaño poblacional efectivo, Golfo de california, *Myotis vivesi*, genética de poblaciones.

The geographic range of the fishing bat, *Myotis vivesi* (Vespertilionidae), is restricted to the islands of the Gulf of California with a few colonies on the Sonoran and Baja California coasts (Maya 1968; Villa 1979; Herrera and Flores-Martínez 2001). *M. vivesi* is unique among members of its family in its feeding ecology and roosting habits. It eats fish, crustaceans and insects, and roosts on interstices in rockslides, crevices and caves (Maya 1968; Altenbach 1989). Fishing bats were recently listed as endangered and their few known colonies and populations appear to be decreasing (Ceballos and Navarro 1991; NOM-ECOL-059-2002). For example, *M. vivesi* has disappeared from islands where exotic rats have been introduced (Flores-Martínez, personal observations).

Successful management of rare species requires knowledge about the magnitude and distribution of genetic variation in their populations (Eizirik *et al.* 2001). Small and/or fragmented populations are prone to lose genetic variation much more rapidly than large populations (Vrijenhoek 1996). This loss of genetic variation may thwart successful adaptation to changing environments (McCue *et al.* 1996). Of more immediate concern, however, are the potential effects of inflated homozygosity in small populations. There is good evidence that high levels of homozygosity may substantially reduce individual fitness in mammals, as reflected by reduced birth weight and neonatal survival (Coltman *et al.* 1998; Coulson *et al.* 1998). Dispersal between populations, however, acts to counter the loss of genetic diversity (Vrijenhoek 1996). Because these mammals fly, fishing bat colonies may not be completely isolated and likely maintain some level of genetic exchange between islands. Thus, the vagility of this species may potentially offset (to some degree) the loss of genetic diversity due to small population size.

In this study, we present data on the genetic diversity and population size in fishing bats on Isla Partida, the largest known colony of this species. We measured genetic diversity at six microsatellite DNA loci. For the population size analysis, we did not measure absolute population size, but instead used microsatellite data to estimate effective population size. We did determine population structure and size at different sites but these sites were not sampled simultaneously during the year which precludes estimating absolute size of the population at a single time. Absolute population size is the total number of individuals in the actual population, while effective population size is defined as the number of individuals in a theoretically ideal population having the same magnitude of random genetic drift as the actual population (Hartl and Clark 1997). Effective population size is more important than absolute size in determining the rate at which genetic variation is lost (Hartl and Clark 1997). The difference between the absolute and effective population size is influenced, among other natural history traits, by unequal sex ratios and breeding structure (Frankham 1995; Sugg *et al.* 1996).

Materials and Methods

Study area. The study was conducted on Isla Partida (28°52´ N and 113°02´ W), a 1.2 km² island in the Gulf of California, Mexico. Fishing bats roost in rockslides and crevices and are the only mammal species present on the island (Maya 1968; Fig. 1).

Population size. Estimates of population size of the colony were conducted at 6 different sites on the island between November 2000 and May 2001 (Fig. 1). Most sites were not sampled during the same period; consequently, estimates of population size per period are not representative of the whole island. Using 1m² quadrants, we sampled approximately 1.6 % of rockslides used as day-roosts by the bats (Maya 1968). Quadrants were randomly selected and all rocks inside them were quickly removed and the number of bats was counted. Each quadrant was refilled to its original conditions. The total number of bats at each site was then extrapolated from the

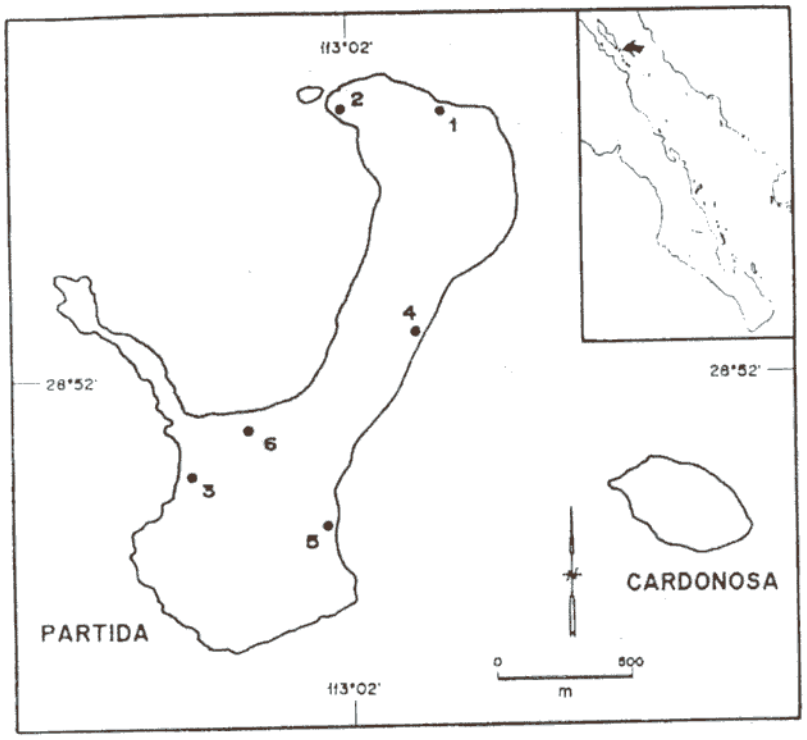

Fig. 1. Study area and sampling sites. Numbers correspond to the sites listed on Table 1. Nets were placed on site 5.

average number of bats per m² and the total roost surface. Sampling did not include roosts in crevices or under large rocks because bats in these sites cannot be trapped with the method we used. Maya (1968) had the same problem when he sampled this colony.

Genetic determinations. Approximately 10 mm² of patagial membrane were collected from 36 individuals of *M. vivesi* captured with a 12 × 2 m mist net placed on the SE of the island (site 5 in Fig 1). The samples were placed in cryogenic vials with ethanol and stored at 4 °C. Genomic DNA was extracted using Qiagen® DNeasy™ tissue kits. We followed the standard Qiagen tissue kit procedure except that we used only 1 mm² of tissue per individual and digested the tissue at 56 °C for 24 hours.

In our initial screen of potential microsatellite primers, we tested sixteen individuals from Isla Partida at 24 microsatellite loci using primers developed for *M. myotis* (Petri *et al.* 1997, Castella and Ruedi 2000) and *Plecotus auritus* (Burland *et al.* 1998). Thirteen of these loci were monomorphic and five others showed variation with poor resolution. The remaining six loci (D15, MM5, G30, E24, Paur-03, and Paur-06) showed clearly resolved variation and were, thus, chosen as the microsatellite markers for this study.

Polymerase Chain Reaction (PCR) amplifications were performed in 10mL reactions containing 1-3 ng DNA, 0.5 µM each primer, 2-3 µM MgCl, 175 µM dNTPs, and 0.5 unit of GIBCO *Taq*. Amplification conditions were 94 °C for 2 min; 30 cycles of 94 °C for 40 s, 50-55 °C for 40 s, and 72 °C for 1 min; then a final extension of 5 min at 72 °C. PCR products were separated on 5% denaturing polyacrylamide gels, stained with fluorescent dye, and visualized with a Molecular Dynamics fluorimager (Rodzen *et al.* 1998, Belfiore *et al.* 2000).

Statistical analysis. Microsatellite variability was quantified as observed and expected heterozygosity (H_O and H_E, Nei 1978) using the program TFPGA version 1.3 (Miller 1997). To confirm that each locus was an independent sample of each individual's genome, genotypic linkage disequilibrium was tested using GENEPOP version 3.1 (Raymond and Rousset 1995). Genotypic proportions were tested for agreement with Hardy-Weinberg expectations using the exact tests (Guo and Thompson 1992) and score

tests (Rousset and Raymond 1995) in GENEPOP. Effective population size N_e was calculated in two ways. One, as $N_e = \{1/[1-H_E]^2 - 1\}/8m$, where m is the average mutation rate per locus, assuming a strict stepwise mutation model (Ohta and Kimura 1973); and two, as $N_e = H_E/4m(1-H_E)$, which follows the assumption of the infinite alleles model (IAM, Kimura and Crow 1963). We assumed a value of 10^{-4} for m, since the estimated mutation rates at microsatellite loci vary between 10^{-3} and 10^{-5} (Weber and Wong 1993).

Results

Population size. Estimates of population size ranged from 500 to almost 5000 adults per site, and from 500 to almost 8000 adults per sampling period (Table 1). Because most sites were not sampled simultaneously, we could not pool them to obtain a single estimate of total population size on the island at a given time. However, they offer interesting information about the colony composition. From November to March we found both sexes at roost sites with a predominance of females. In quadrants with individuals of both sexes, there was always one male with more than one female, with a trend towards a lower male to female ratio in late March during mating (Table 1). In the two sites sampled in May, we estimated 5116 young and 7846 adults, of which almost all adults were pregnant and lactating females (Table 1). This is the highest estimate of population size that we obtained for a section of the island.

Genetic variation. Fishing bats showed substantial genetic variability at the six microsatellite loci, with the total number of alleles ranging from 3 to 15 and observed heterozygosities ranging from 0.39 to 0.94 (Table 2). None of the tests of linkage disequilibrium were significant and no departures from Hardy-Weinberg expectations were detected. Effective population size estimates varied from 14,170 to 68,570 individuals depending on the model (Table 2).

Discussion

Population size and structure. We did notice changes in the composition of the colony, although we could not estimate an absolute population size for Partida Island that included all sites sampled. In the sites sampled from November to March we found individuals of both sexes present with a slight female-biased sex ratio. This trend was much stronger in late March when we found an average of 2.80-4.40 females per male in roosts with individuals of both sexes present.

The highest population size at Partida (7846 bats) was registered in May, when we found pregnant and lactating females with their young almost exclusively in two large roosting areas. This figure is similar to the 6844 adult bats (mostly females with young) previously estimated in June and July of 1963 (Maya 1968). The real population size in this period was probably higher because sampling did not include roosts in crevices or under large rocks nor sites where we previously found bats. Adult males were not found in the sites sampled in May, which indicates that either they move to nearby islands or roost in other areas on Partida. Maya (1968) reports insemination in fishing bats to occur from late July to September, with fertilization delayed to February and March, and births from May to June.

Genetic variation. The bats showed a large amount of genetic variability in the microsatellite loci sampled. Values of observed heterozygosity (H_O) in *M. vivesi* are similar when compared with

Table 1. Population size estimates of *Myotis vivesi* in roosts on rock slides on Isla Partida. Shared roosts represent quadrants were individuals of both sexes were found. Numbers in parenthesis represent shared roosts.

Site	Date	No. of quadrants	No. of adult bats (:)	Average : in shared roost	Estimated Adult Population Size	No. of young (:)	Estimated Young Population Size
1	11/2000	9	31 (1:2.5)	1:2.2 (5)	2790	0	0
2	01/2001	10	25 (1:5)	1:1.6 (3)	500	0	0
3	03/2001	10	40 (1:5)	1:4.4 (5)	800	0	0
4	03/2001	9	31 (1:4.2)	1:2.8 (5)	2170	0	0
5	05/2001	16	42 (1:38)	1:2 (1)	2937	33 (1:1.5)	2062
6	05/2001	11	55 (0:55)	-	4909	28(1:1)	3054

2. Summary statistics of the six microsatellite loci *vivesi* on Isla Partida. H_O and H_E are observed expected heterozygosity (Nei 1978). N_E is the esti- effective population size assuming the stepwise del (Ohta and Kimura 1973)* and the infinite alleles del (Kimura and Crow 1963)**.

Locus	No. of alleles	H_O	H_E	*N_E / 1000	**N_E / 1000
D15	6	0.61	0.68	10.41	5.14
[illegible]5	8	0.79	0.86	52.57	13.90
G30	15	0.91	0.92	145.46	24.59
E24	15	0.94	0.92	135.81	23.68
Paur-03	10	0.91	0.88	64.20	15.59
Paur-06	3	0.39	0.46	2.99	2.10
Average	9.5	0.76	0.79	68.57	14.17

other microsatellite studies of related species. For example, average H_O in *M. myotis*, a common and widespread bat in the Mediterranean, is 0.67–0.78 whereas in *M. vivesi* it was 0.76 (Castella and Ruedi 2000; Castella *et al.* 2000). Similar H_O average values are also found when the same microsatellite loci are included in the comparison: average H_O of the loci D15, E24 and G30 is 0.86 in *M. myotis* and 0.82 in *M. vivesi* (Castella and Ruedi 2000). Our data suggest that the effective size of the Partida population is very large, ranging between 14,170 and 68,570 individuals.

Conservation implications. High genetic diversity and large effective population sizes suggest that the largest know colony of fishing bats is not under an immediate risk of extinction. Similarly, our highest estimate of population site is similar to the numbers described by Maya (1968) which indicates that the colony has not been reduced significantly as it has happened in other islands. Its unique roosting habits, however, makes this species very vulnerable to anthropogenic disturbances and justifies the protection of this colony in particular by the large number of females that give birth in the island. High levels of genetic diversity in Partida do not necessarily mean that the species´ risk of extinction is not significant in other parts of its distribution. For example, the genetic diversity of the greater horseshoe bat *Rhinolophus ferrumequinum* is not uniformly distributed but presents areas where heterozygosity is very low (Rossiter *et al.* 2000). Future research on the geographic distribution of the genetic diversity of the fishing bat will allow us to determine the status of the species and help design biologically sounded strategies for its conservation.

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