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Effective population size dynamics of *Myotis vivesi* during the Pleistocene and Holocene climatic changes

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Myotis vivesi (Fish-eating Myotis) is an endemic species of the Gulf of California, Mexico. In this study, a 282 bp fragment of the mtDNA control region and six microsatellites loci were used to reconstruct its demographic history using summary and coalescent based statistics. Our results suggest that *M. vivesi* experienced a demographic population expansion between 230,000 to 50,000 years ago. After this expansion, *M. vivesi* experienced a slight reduction in the effective population size between 30,000 to 5,000 years ago and a spatial expansion in the last 5,000 years. Population changes observed in *M. vivesi* could be related to climatic changes that occurred in the Gulf of California in the Pleistocene and Holocene periods.

Key words: *Myotis vivesi*, Gulf of California, mismatch distribution, BSP, msvar

INTRODUCTION

The effect of Pleistocene glaciations on the genetic diversity of several taxa has been widely documented (Hewitt, 2000). Studies that have evaluated the possible effect of Holocene climatic changes on diversity and effective population size (*Ne*) have increased in recent years (Storz and Beaumont, 2002; Lucchini *et al*., 2004; Heller *et al*., 2008; Okello *et al.*, 2008). The estimation of *Ne* is crucial information in the establishment of conservation programs, thus, it is important to assess *Ne* through time to track if its current value is a product of recent or historical events (Johnson *et al*., 2009). One approach to assess if a population has experienced a bottleneck is to evaluate the levels of genetic diversity in contemporary and historical samples (Wandeler *et al.*, 2007). However, this strategy is not always possible. An alternative is to use the patterns of genetic variation in contemporary populations to infer the extent and timing of the historical demographic changes (Pearse and Crandall, 2004). Traditionally, the methods available to infer bottlenecks or population expansion were based on summary statistics of genetic diversity (Cornuet and Luikart, 1996; Fu, 1997; Garza and Williamson, 2001; Ramos-Onsins and Rozas, 2002). Recently, a group of methods based on the coalescent theory has made it possible to time major demographic changes, such as declines or expansions, and to estimate the magnitude and severity of such events (Storz and Beaumont, 2002; Drummond *et al.*, 2005).

In spite of its importance as one of the most productive basins of the world, the Gulf of California has received little attention to evaluate the possible effects of Pleistocene–Holocene climatic changes on the genetic diversity of taxa (Pfeiler *et al*., 2005; Hurtado *et al*., 2007; Pfeiler *et al*., 2008). In this paper, we analyzed the data previously published by Floyd *et al.* (2010) to evaluate the effect of Pleistocene-Holocene climatic changes on the demographic history of *Myotis vivesi* (Vespertillionidae)

in the Gulf of California using both summary statistics and coalescent based approaches.

Material and Methods

A 282 bp fragment of the second hypervariable domain (HVII) of the mtDNA control region (134 individuals) and six nuclear microsatellites markers (257 individuals) were amplified from individuals of *M. vivesi* from eleven islands in the Gulf of California (Floyd *et al*., 2010). Currently, most coalescent methods assume closed populations and no internal substructure (Wakeley and Aliacar, 2001). To exclude confounding from the above mentioned effects on the inferred demographic history, two alternatives have been proposed: the use of currently well-defined and discrete populations as entities in the analysis (splitting approach) or the treatment of samples from a wide geographical distribution as a single population (lumping approach; Heller *et al*., 2008). The splitting approach could violate the assumption of closed population, whereas the lumping approach could violate the assumption of no internal structure. For purposes of this work, we conducted tests to reconstruct the demographic history of *M. vivesi* using the lumping approach. We based our choice on previous work with microsatellites markers that suggest a very weak population structure for *M. vivesi* (Floyd *et al*., 2010). Additionally, low available sample sizes for all *M. vivesi* populations (with the exception of Partida Norte island (n = 59)) could bias the results of coalescent based approaches (Beaumont, 1999) and prevent the use of the splitting approach.

mtDNA Control Region

The mismatch distribution for the pure demographic (including the parameters q_0 and q_1) and spatial expansion (including the parameters q, t and *M*) models were estimated in Arlequin 3.5 (Excoffier *et al*., 2005). Additionally, the age of the expansion was estimated as described by Rogers and Harpending (1992) using the substitution rates proposed by Petit *et al.* (1999). The F_s (Fu, 1997) and R_2 (Ramos-Onsins and Rozas, 2002) neutrality tests were estimated in DnaSP 5.1 (Librado and Rozas, 2009), and the significance values were assessed with 10,000 coalescent simulations. The F_s test uses information for the haplotype distribution and is expected to have high negative values under scenarios of population expansion or genetic hitchhiking. On the other hand, the R_2 test relies on the rationale that the expected number of singletons on a genealogy branch is equal to k/2 after a recent population expansion; consequently, low values of R_2 are expected if a recent population expansion occurred. A haplotype network was built with the Median Joining algorithm as implemented in the Network 4.6 program (Bandelt *et al.*, 1999). Finally, Bayesian skyline plots (BSP) were used to detect changes in *Ne* as implemented in Beast 1.5.1 (Drummond *et al.*, 2005). In the absence of a specific substitution rate for the HVII region in *M. vivesi*, we used the substitution rates proposed by Petit *et al.* (1999) for the same region in *Nyctalus noctula* (6.5% and 25.2%) that could be considered representative for other bat species (Petit *et al*., 1999; Bilgin *et al*., 2009). Three independent Markov chains assuming a strict molecular clock and a coalescent Bayesian skyline model were run for 50×10^6 generations and sampled every 10,000 steps to ensure that all ESS estimators were higher than 200 (Drummond *et al*., 2002). The results of the three independent chains were combined in LogCombiner 1.5.4 (Drummond and Rambaut, 2007) and the BSP were generated in Tracer 1.5 (Rambaut and Drummond, 2009).

Microsatellite Markers

Three different approaches were used to reconstruct the demographic history of *M. vivesi* from nuclear markers. In order to detect the possibility of recent bottlenecks we used the software Bottleneck 1.2 (Cornuet and Luikart, 1996) and the M-ratio test (Garza and Williamson, 2001). The software Bottleneck assumes that an excess of heterozygosity would be expected in several loci in a population that has experienced a recent bottleneck. On the other hand, the M-ratio test assumes that populations that have experienced a bottleneck will have low M values compared with equilibrium populations. For the bottleneck program we used the two-phase model (TPM) with 95% single step mutations and a variance of 12 for the multi-step mutations (Piry *et al.*, 1999). Meanwhile, for the Garza-Williamson test the M value was computed in Arlequin 3.5 (Excoffier *et al*., 2005) and compared with the critical value obtained using the software CRITICAL_M.EXE (Williamson, 2007). Finally, in order to detect past population changes we used a Bayesian coalescent approach as implemented in the program msvar 1.3 (Storz and Beaumont, 2002). The parameters of interest from msvar were: N_0 (current effective population size), N_1 (ancestral population size at the time of demographic change) and T (the time since this change). Based on the juvenile population size estimated on a previous census for Partida Norte island (Flores-Martínez *et al.*, 2005), we estimated the *Ne* of this island assuming that each juvenile is equivalent to one reproductive female and one reproductive male (*Ne* = 5 adults/m^2). This assumption might overestimate the number of reproductive males in case of polygyny, however little is known about the mating system of *M. vivesi*. We estimated the total number of reproductive adults in Partida Norte island (*Ne* = 46,480) considering only the portion of the island that is used by the bat to roost (0.79%). Because there are no population censuses in any of the other islands included in our study, we estimated their *Ne* using the same density value and portion of usable habitat as estimated for Partida Norte island. The global *Ne* derived from this process for the eleven islands was ≈ 160,000. Due to the high uncertainly of this estimator, we used a large variance for current and ancient effective population sizes (20,000). In the absence of a specific generation time value for *M. vivesi*, we used an average of the generation times estimated for other *Myotis* species. Carstens and Dewey (2010) suggest a generation time of three to five years for *M. lucifugus*, meanwhile You *et al.* (2010) suggest a generation time of two years for *M. davidii*; therefore, we used a generation time of 3.5 years. Finally, the time elapsed since the last demographic change was estimated from the date expansion events occurred in other lineages in the Gulf of California (Pfeiler *et al*., 2005, 2008; Hurtado *et al*., 2007). In order to obtain unbiased results, we performed exploratory runs with 25,000 thinned updates and a thinning interval of 20,000 under three demographic scenarios: a stable population ($N_0 = N_1$), an expanding population ($N_0 > N_1$), and a bottleneck population ($N_0 < N_1$) with different population sizes (20,000 and 160,000), generation times (2, 3.5 and 5 yrs) and times since the demographic change (25,000 and 150,000 yrs). All exploratory runs always led to a drastic decrease in *Ne*. After selecting the run with the narrower distribution (Heller *et al.*, 2008), we performed five independent chains with 50,000 thinned updates

and a thinning interval of 20,000 steps, leading to a total number of 10^9 updates. We assumed that $\log_{10}N_0 = \log_{10}N_1$ (5.2, 4.3), that the time since the demographic change $\log_{10}T$ was (5.00, 4.69), and that the mean mutation rate was ($\log_{10}$) of (-3.3, 0.25), as suggested by Storz *et al*. (2002) for this analysis. In order to test for convergence of the MCMC chains, the Brooks, Gelman and Rubin convergence diagnostic statistic (Gelman and Rubin, 1992; Brooks and Gelman, 1998) was applied on data sets from the five chains, with the first 10% discarded as burn-in. Then, the last half of each chain was used to make a combined consensus chain which was assumed to summarize the posterior distribution of N_0, N_1 and T (Storz and Beaumont, 2002). All output files from msvar were analyzed using the BOA program (Smith, 2005) in R package version 2.11.1 (R Development Core Team, 2010).

Results

mtDNA control region

The mismatch distribution for the pure spatial expansion model was trimodal, with the highest mode at zero, followed by three and five pairwise differences (Fig. 1). In the pure demographic expansion model, the mismatch distribution was identical, with q_1 (1708.2578) higher than q_0 (0), although in this case the program failed to generate the confidence intervals for these parameters. On the other hand, for the spatial expansion model, the theta (0.0007, lower bound 0.0003, upper bound 0.9592) and M (1.9917, lower bound 0.3386, upper bound 7.0465) values were low with a time since the expansion value t = 4.0936 (lower bound 0.8782, upper bound 7.0233). $q_1 > q_0$ indicates that *M. vivesi* experienced a population size expansion, whereas the mode centered around the expansion time (t), the low mean, and the highest mode at zero suggest a recent spatial expansion due to recent coalescent events. For the low substitution rate (6.3%), the mean time of the expansion in years was 230,440 (lower bound 17,259 yrs, upper bound 468,814 yrs) and for the high substitution rate (25.2%) the mean time since the expansion was 57,610 years (lower bound 4,320 yrs, upper bound 117,204 yrs). Both neutrality tests suggest that *M. vivesi* experienced a recent population expansion: F_s = -27.7123 ($P < 0.001$) and $R_2 = 0.086$ ($P < 0.001$). The haplotype network shows that the three most frequent haplotypes differed by only two mutations, where haplotype 1 was the most frequent (present in 77 of the 134 sampled individuals) and widely distributed (present in all islands) (Fig. 2). The BSP revealed a similar demographic scenario (Fig. 3). For the low substitution rate (Fig. 3A), the BSP suggests that *M. vivesi* experienced a demographic expansion between 200,000 to 50,000 years before present, followed by a slight demographic decrease between 30,000 to 5,000 years ago. Finally, although the BSP graph suggests a demographic expansion in the last 5,000 years, the HPD intervals did not support it. On the other hand, for the high substitution rate,

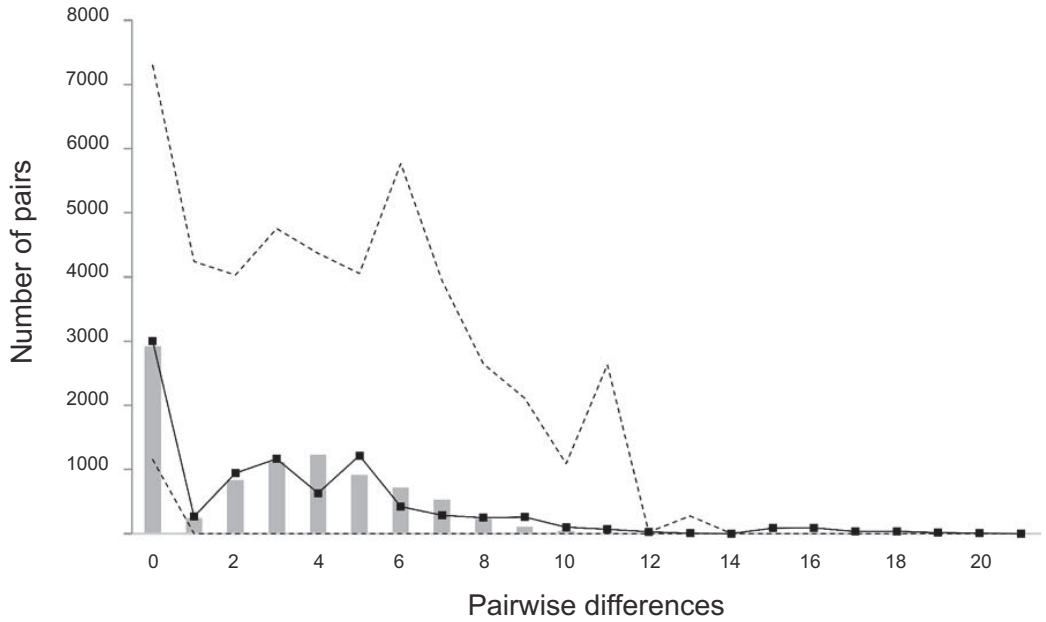

FIG. 1. Mismatch distribution for the spatial expansion model of *M. vivesi* in the Gulf of California based on a 282 bp fragment of the mtDNA control region. Gray bars represent the expected distribution, solid line represents the observed distribution, dotted line represents the 95% confidence interval

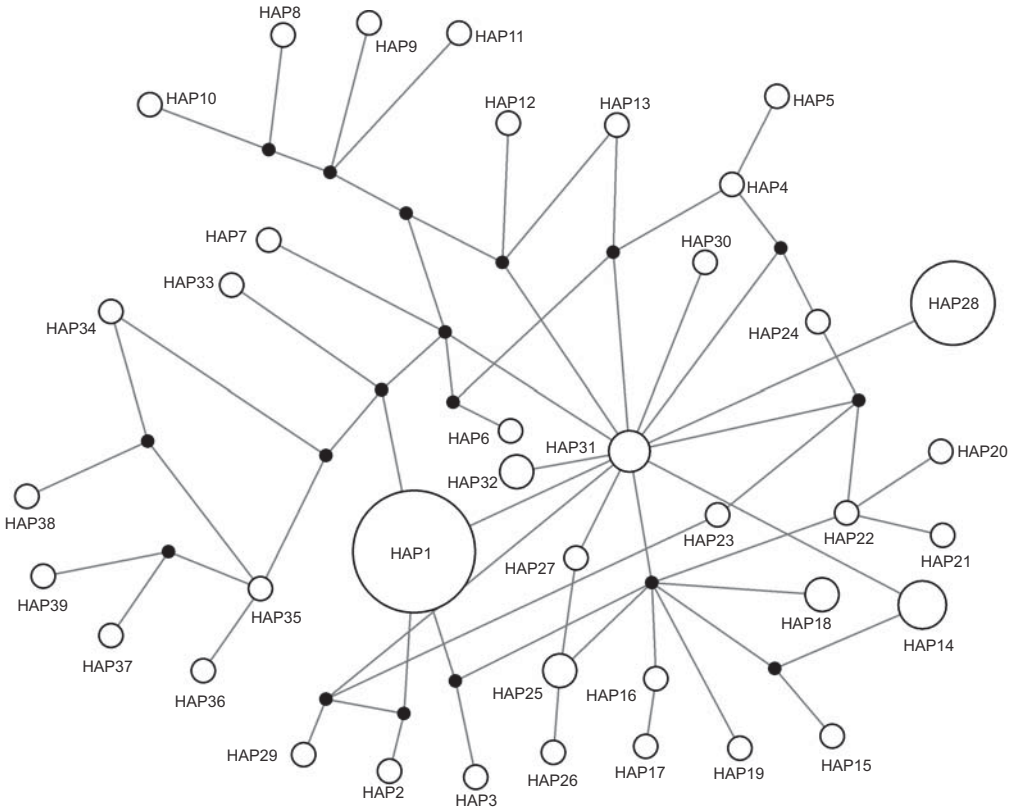

FIG. 2. Median joining network among the 39 haplotypes of *M. vivesi* in the Gulf of California. Size of the nodes are proportional to the frequency of the haplotype. The small solid circles represent missing intermediates haplotypes

the BSP tracked the demographic history only for the last 140,000 years (Fig. 3B). The BSP indicates that the population remained stable from 140,000 to 30,000 years ago, with a slight demographic decrease between 3,000 to 5,000 years ago. Similarly to the low substitution rate, the BSP graph shows a recent demographic expansion that it is not supported by the HPD intervals.

Microsatellite Markers

The TPM model did not detect any evidence of heterozygosity excess (Wilcoxon test $P = 0.72$) that could suggest a recent bottleneck. The same result was found with the Garza-Williamson test where the M value ($M = 1.016$) was higher than the critical value ($M_C = 0.9206$). Contrary to the previous results, the Bayesian coalescent approach implemented in msvar suggests a drastic reduction in *Ne*. The Brooks-Gelman-Rubin statistic was lower than the threshold value for the three parameters of interest: N_0 (1.1694), N_1 (1.0048) and T (1.1655). The current effective size (N_0) was very low ($\bar{x} = 108$, $q_{0.025} = 10$, $q_{0.975} = 1{,}150$) and it is equivalent only to 0.04% of the ancient population size (N_1) ($\bar{x} = 267{,}465$, $q_{0.025} = 60{,}446$, $q_{0.975} = 1{,}152{,}455$). The time when the population started to decrease could be traced to 3,498 years before present ($q_{0.025} = 354$, $q_{0.975} = 32{,}002$).

DISCUSSION

The results found in this work suggest that, after going through population expansion in the Pleistocene, *M. vivesi* experienced a reduction in population size followed by a spatial expansion during the Late Pleistocene-Holocene. The Gulf of California is a long, narrow marginal sea surrounded by arid lands and mountains to the east, north and west, and open to the Pacific Ocean to the south. Its geographic position at the edge of the tropical region results in a monsoon climate of varying winds with hot-wet and cold-dry seasons clearly defined (Douglas *et al.*, 2007). In general, the gulf is a highly productive basin due to its hydrographic features, but the interannual variability in the water circulation pattern induced by the El Niño-Southern Oscillation (ENSO) results in

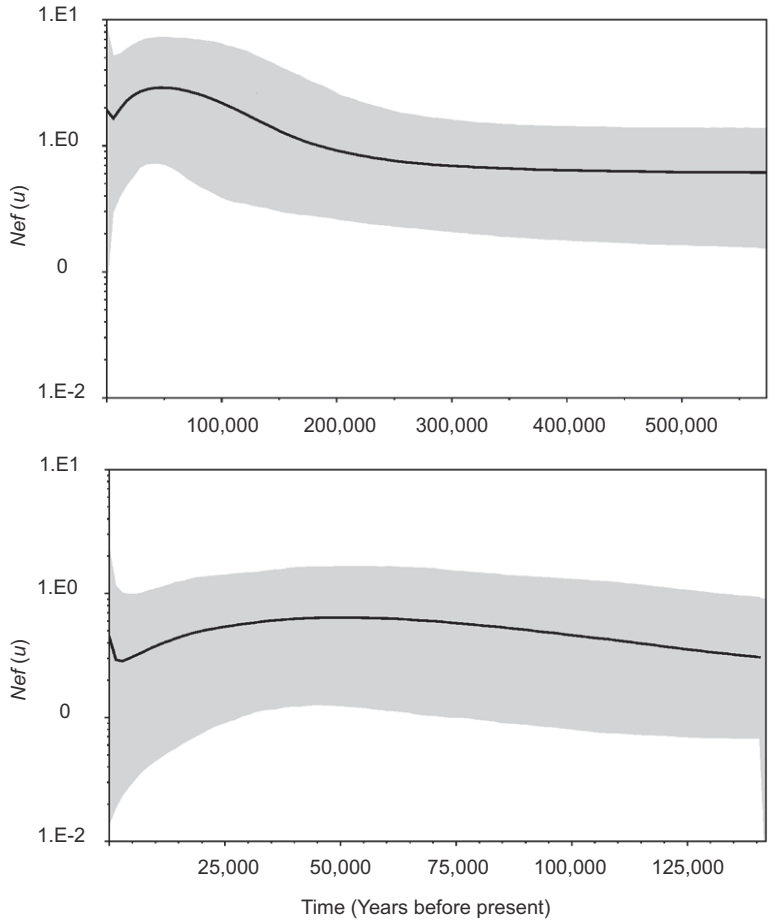

FIG. 3. Bayesian skyline plots of the *M. vivesi* population in the Gulf of California including the 95% highest probability density (HPD) interval. A — Low substitution rate (6.3%); B — High substitution rate (25.2%). Time is plotted linearly; *Nef* (*u*) is presented on a logarithmic scale

lower productivity in the years when ENSO occurs (Álvarez *et al*., 2010). Pfeiler *et al*. (2008) have proposed that the Pleistocene environmental changes could be linked with demographic expansion of some lineages in the Gulf of California. The time estimated for the demographic expansion in *M. vivesi* is similar to patterns found in other lineages in this region (Pfeiler *et al*., 2005, 2008; Hurtado *et al*., 2007).

Paleoecological reconstructions have allowed scientists to model the paleoclimatic conditions in the Gulf of California for the last 17,000 years (Barron *et al*., 2005; Dean, 2006; Douglas *et al*., 2007; Álvarez *et al*., 2010). These studies have revealed that during the Younger-Dryas period (12,900 to 11,600 yrs before present) and the Hipsithermal period (6,000 to 5,700 yrs before present) the conditions in the Gulf of California were similar to those observed during ENSO. There is no evidence of the possible effects of ENSO on *M. vivesi* population, but other studies have demonstrated that it can affect the trophic network. For example, the reproductive success (breeding success and mean number of fledglings) of the Herman Gull (*Larus heermanni*) in one island of the Gulf of California collapsed near to zero during two ENSO years (Velarde *et al*., 2004). Similar patterns have been observed in other sea birds, such as Brandt's cormorans (*Phalacrocorax penicillatus*), double-crested cormorans (*P. auritus*), yellow-footed gulls (*Larus livens*), blue-footed boobies (*Sula nebouxii*), brown boobies (*S. leucogaster*), and brown pelicans (*Pelecanus occidentalis*) (see references in Velarde and Ezcurra, 2002).

Both the neutrality tests and the mismatch distribution observed in this study confirm that *M. vivesi* experienced a population expansion. Neutrality tests attempt to detect an excess of low or high frequency mutants in the distribution of the polymorphism, where an excess of low frequency mutants is usually interpreted as an evidence of population expansion. However, an excess of low frequency mutants can also be consequence of selective sweeps

(Tajima, 1989; Hahn *et al.*, 2002), spatial expansion (Ray *et al.*, 2003; Excoffier, 2004) or metapopulation structures (Wakeley and Alicar, 2001). Although it is accepted that the HVII region of the mtDNA control region in mammals is a non-coding region (Wilkinson and Chapman, 1991) and that large and highly significant values of the F_s test could rule out the possibility of genetic hitchhiking (Okello *et al.*, 2005), we cannot discard a selective sweep (i.e., the replacement of mtDNAs through the entire population with a phenotypically advantageous allele) followed by an accumulation of neutral variants (Maruyama and Birky, 1991). All expansion models described herein assumed that deme sizes and migration rates have been constant over time. However, as stated by Wegmann *et al*. (2006), many species are actually subdivided into locally breeding demes of different size showing metapopulation dynamics (extinction and recolonization). The mismatch distribution found in this study (Fig. 1) is similar to that obtained by Wegmann *et al.* (2006) under a spatially and temporally variable environment and suggests a recent spatial expansion of *M. vivesi.* According to paleoecological reconstructions, the cold and dry conditions that promote higher primary production have dominated the Gulf of California during the last 5,000 years (Barron *et al.*, 2005; Douglas *et al*., 2007), a situation that could favor an increase in the geographical range and a demographic expansion of *M. vivesi.*

The *Ne* obtained in this study from the msvar analysis suggests a severe bottleneck in *M. vivesi*. The current *Ne* obtained with the msvar analysis for *M. vivesi* (*Ne* = 108) was very different from the *Ne* estimated by a census (see above). Similar results have been found in other works where the effective population size calculated by msvar is smaller than that obtained from census data (Johnson *et al*., 2009; Milton *et al*., 2009). Although the Brooks-Gelman-Rubin statistic confirms that the three estimated parameters obtained from msvar achieve convergence, we cannot ruled out the possibility of poor informative priors (Beaumont, 1999; Storz *et al*., 2002) or the existence of population fluctuations or metapopulation structure.

The discrepancies found in this study, where mtDNA suggests a demographic expansion and the microsatellites markers suggest a bottleneck, are also found in other studies. A possible explanation is that rapidly evolving markers such as microsatellites could track very recent demographic events (Koskinen *et al.*, 2002, Saillant *et al.*, 2004), meanwhile the mtDNA tracks more ancient events. However, some authors have recently suggested that the rates of substitution in population studies are much higher than those used in phylogenetic studies, thus precluding the use of a standard substitution rate or even the use of a substitution rate estimated from other lineages (Ho *et al*., 2005; Ho and Larson, 2006). In the present work we used two substitution rates (6.5% and 25.2%) for the mtDNA control region that have been used in studies of other bats. The only available substitution rate for mtDNA of *M. vivesi* comes from the phylogeny of the New World *Myotis* (3.5% for the cytochrome *b* gene — Stadelmann *et al*., 2007). Studies on human mtDNA have estimated that the substitution rate of the control region is 10 times higher than the substitution rate of coding regions (Santos *et al*., 2008) and that the instantaneous rate of mutation is 5 to 10 times higher than the rate of substitution (Howell *et al*., 2003). The substitution rates used in this study were 1.85 to 7.2 higher than the substitution rate estimated for cytochrome *b*. However, the rate could be 50 to 100 times higher. Regardless, the BSP of both substitution rates gave similar patterns and dates (Fig. 3) (only differing in the *Nef* (*u*) values) and the timing for the population decline almost fit the range estimated for the microsatellites markers in msvar. While the exact time of a demographic event is difficult to achieve, the results found in this study support the hypothesis that current genetic patterns of *M. vivesi* are influenced by historical events and suggest that its conservation can be compromised by the climatic events that affect sea productivity.

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