

Multiple levels of allopatric divergence in the endemic Philippine fruit bat *Haplonycteris fischeri* (Pteropodidae)

TRINA E. ROBERTS*

Committee on Evolutionary Biology, University of Chicago, Chicago, IL 60637 USA
Field Museum of Natural History, 1400 S. Lake Shore Dr., Chicago, IL 60605, USA

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As part of a larger comparative phylogeographical study of Philippine fruit bats, I used fragments of the mitochondrial genes cytochrome *b* and ND2 to investigate phylogeography and diversification in *Haplonycteris fischeri*, a pteropodid bat endemic to the Philippines but widespread within the archipelago. Genetic diversity in *H. fischeri* was extremely high in these commonly studied genes, with 101 unique haplotypes in 123 sequenced individuals, although small, continuously isolated islands had less diversity than had large island complexes. Seven monophyletic groups and one paraphyletic group were restricted to individual islands, groups of islands, or parts of islands. Each Pleistocene island complex had a single resident monophyletic lineage; these five groups were separated by approximately 6–8% sequence divergence and apparently have been diverging for 4–6 Myr. Within island groups, monophyletic lineages on some individual islands suggest that current ocean channels have also been barriers to gene flow; in some cases, multiple allopatric clades were present on single islands. Basal divergence dates were estimated to be in the early Pliocene, and most diversification was apparently connected to the ongoing geological evolution of the Philippines. Geological history and current geography interact with ecology to cause substantial genetic differentiation within this primary forest-specialist species. © 2006 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2006, 88, 329–349.

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INTRODUCTION

Biodiversity on oceanic islands and archipelagoes has intrigued generations of scientists. The important role of islands in the study of biogeography and evolution stems partly from the presence of isolated populations and historical geographical connections, which combine to form unique biotas whose histories can be reconstructed with more certainty than is possible in many continental situations. Theories of island biogeography on both ecological and evolutionary scales also allow predictions about diversity and distribution patterns on islands. Disentangling the roles of geography, history, and ecology illuminates evolutionary processes, helps distinguish dispersal from vicariance, and shows how biodiversity has arisen in areas of high

endemism. Because oceanic islands are commonly home to a remarkable number of endemic species, understanding evolutionary processes in these areas is also crucial to conservation. I investigated mitochondrial DNA (mtDNA) sequence variation in *Haplonycteris fischeri* (Lawrence, 1939), a small fruit bat endemic to the Philippines, in order to determine the relative roles of changing geography, geology, and ecology in driving evolution in this species.

The Philippines (Fig. 1) is ideally situated for investigating evolutionary processes (e.g. Heaney, 1986, 2000; Heaney, Walsh & Peterson, 2005). The archipelago is extremely high in both biodiversity and endemism; approximately 60% of the more than 1000 native terrestrial vertebrates are endemic, including roughly two-thirds of the terrestrial mammals (Heaney *et al.*, 1998). High levels of endemism can be explained in part by isolation and in part by geographical history within the Philippines. The origin and geological history of the archipelago, while complex, is

*Correspondence. Current address: University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775, USA.
E-mail: trina.roberts@uaf.edu

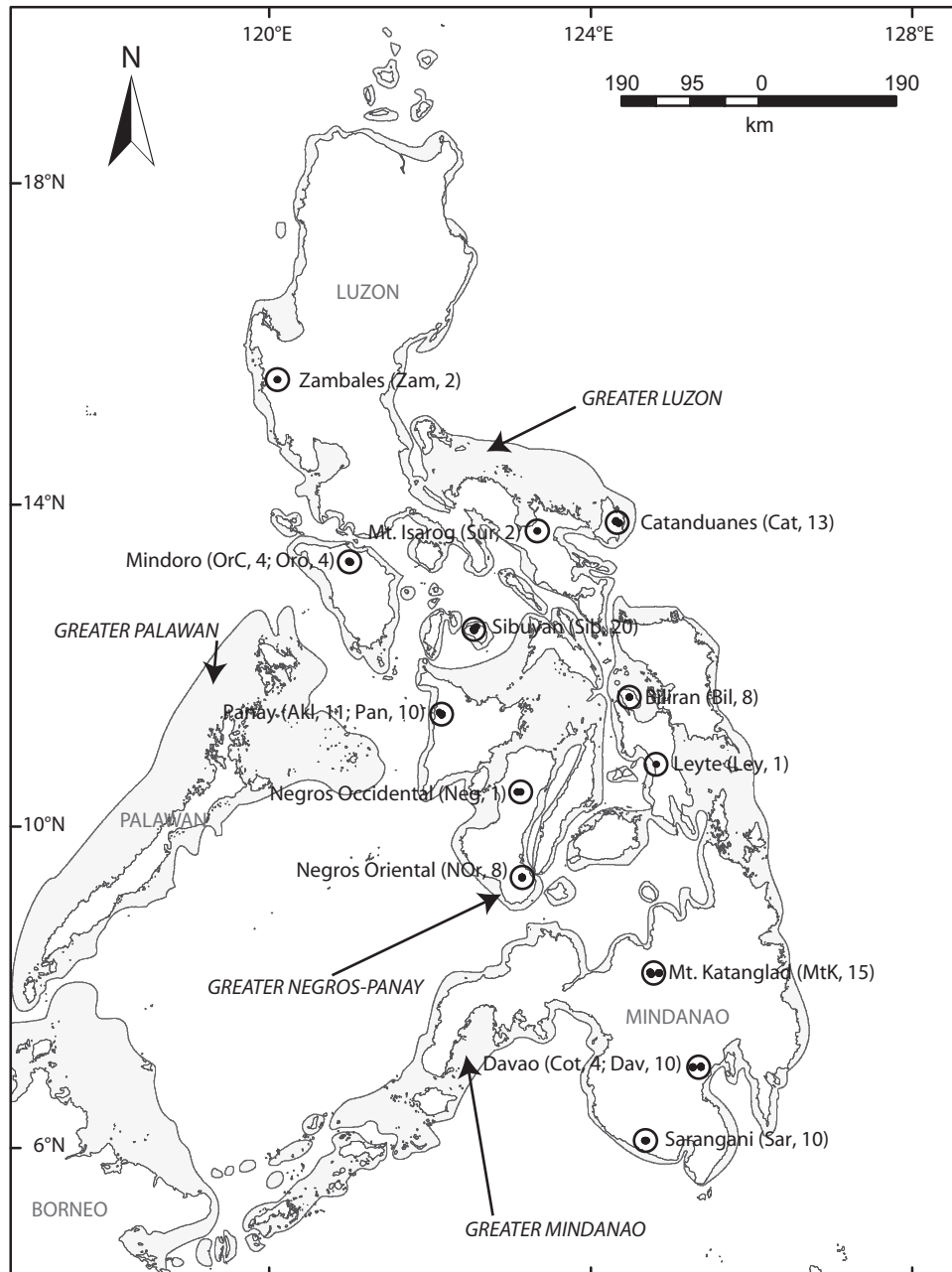


Figure 1. The Philippines, showing sampling locations and sample sizes for this study. Black dots show actual sampling localities, identified by three-letter codes. Circles enclosing them indicate the groups of locations used in the analyses, identified by name. Light grey areas indicate the extent of PAICs exposed by a 120 m drop in sea level.

well studied and has recently been described in detail (Hall, 1996, 1998, 2001, 2002). Because of the deep water surrounding the archipelago, most islands have never been connected to mainland Asia or the Sunda Shelf. The exception is the Palawan island group, which biogeographically is part of the Sunda Shelf region (Heaney, 1986; Esselstyn, Widmann & Heaney, 2004); for the purposes of this paper, 'the Philippines' refers to the oceanic region of the archipelago,

excluding the Palawan group. Relatively shallow water separates some islands in this region, and groups of current islands were repeatedly connected during Pleistocene episodes of low sea level (Heaney, 1986). During a 120 m drop in sea level, as during the late Pleistocene and other periods of glaciation, three large aggregate islands, each including multiple current islands, would have existed, while other more isolated islands would have remained independent

or formed smaller groups (Fig. 1). For consistency, I follow Brown & Diesmos (2002) and Evans *et al.* (2003) in calling these 'Pleistocene aggregate island complexes' (PAICs).

Each of the three large oceanic PAICs – Greater Luzon, Greater Negros-Panay, and Greater Mindanao – has been described as an area of endemism and a biogeographical region within the Philippines, as have several of the smaller, simpler, more isolated PAICs, including Sibuyan, Camiguin, and Mindoro (Heaney, 1986, 1991). Most mammal species endemic to the Philippines occur only on the islands of a single PAIC, suggesting that the Pleistocene coastlines have been an important factor in determining species ranges and biogeographical patterns (Heaney, 1986, 1999, 2000). Others, however, are endemic to single islands within a PAIC, particularly the large islands of Mindanao and Luzon, which contain the most diverse habitats and the greatest range of elevation (Heaney, 1986; Heaney *et al.*, 1998; Steppan, Zawadski & Heaney, 2003). In some cases, apparent diversification within the Philippines has resulted in endemic clades with representatives limited to one island or PAIC (e.g. Heaney & Rickart, 1990; Ruedi, 1996; Steppan *et al.*, 2003). It is clear that biogeographical patterns in the Philippines are linked to both past and present geographical relationships and connections among islands, and understanding the impact of these connections is crucial to understanding diversification and endemism in the archipelago. However, the role of these geographical factors at the intraspecific level has been addressed in only limited detail (e.g. Peterson & Heaney, 1993), and describing biogeographical patterns requires attention to variation within as well as among named species. Traditional species boundaries may fail to acknowledge cryptic or geographically structured variation, which are critical components of diversity. I tested the relative contributions of past and present island geography to phylogeographical structure and divergence in the Philippine dwarf fruit bat *H. fischeri* in order to determine the factors that have influenced evolution in this widespread, abundant, endemic species.

H. fischeri, the only member of its genus currently recognized, is a small frugivorous bat endemic to the oceanic Philippines but widespread within the archipelago. Its range includes islands of all three large PAICs, as well as the smaller PAICs of Mindoro and Sibuyan; it has not been found on some relatively young islands, such as Maripipi and Camiguin, despite the presence of apparently suitable habitat (Heaney, 1991). It is restricted to primary and other high-quality rainforest and is usually most common in upper lowland and submontane forest (600–1100 m), although it is also present as low as 150 m and in montane forest (Heaney *et al.*, 1989; Heideman & Heaney,

1989; Utzurrum, 1998). However, primary forest in the Philippines has been reduced by nearly 90% in the past century (Heaney & Regalado, 1998; Heaney *et al.*, 2000), and current geographical and elevational ranges may not reflect the majority of a species' evolutionary history. Although the species is abundant in appropriate habitat, it is rarely caught anywhere other than under the forest canopy (Heideman & Heaney, 1989). Morphological, genetic, and reproductive studies of *H. fischeri* have suggested some geographical subdivision among populations on the five PAICs on which it occurs (Heideman, 1988; Peterson & Heaney, 1993; Walsh, 1998; Heaney *et al.*, 2005). The substantial morphometric and allozyme differences between *H. fischeri* on Sibuyan and in the rest of the Philippines (Peterson & Heaney, 1993; Walsh, 1998; Heaney *et al.*, 2005) prompted its tentative listing as a separate (but as yet unnamed) species (Ingle & Heaney, 1992; Heaney *et al.*, 1998). I used mtDNA sequences to determine the degree of genetic structure in *H. fischeri* on a finer scale than that addressed by allozyme data, and to assess the contribution of current and historical geography to genetic patterns. Because *H. fischeri* is both endemic and ecologically restricted, and because of its reluctance to fly into open habitats, I expected it to have higher levels of mtDNA structure than other more mobile species; because it is widespread relative to most nonvolant endemics in the Philippines, I expected to find more gene flow than in other small mammals.

METHODS

SAMPLING

In order to investigate the roles of historical and current islands and coastlines in this species, I used a hierarchical geographical sampling scheme. All three of the large PAICs (Greater Luzon, Greater Mindanao, and Greater Negros-Panay; see Fig. 1) were represented. Within each of these, there were samples from at least two current islands; there were also at least two locations on at least one current island in each group, although sample sizes from some locations were small. I also included samples from two smaller PAICs, one small (Sibuyan) and one medium-sized (Mindoro). This allowed me to test the contribution of each of these hierarchical levels to the total geographical structure of the species. Sampling locations are shown in Figure 1; I included 1–20 samples per location (Table 1; Fig. 1), with a total of 123. The nine included islands ranged in size from 448 km² to 108 171 km² (Table 1). Locality information for each specimen was taken from field notes or museum collection catalogue information. Voucher specimens are deposited at the Field Museum, Cincin-

Table 1. Pleistocene aggregate island complexes (PAICs), islands, and locations in this study, with island areas and sample sizes

PAIC	Current island	Area (km ²)	Location (code(s))	<i>N</i>
Greater Mindanao	Mindanao	99 078	Sarangani (Sar)	10
			Mt. Katanglad (MtK)	15
			Davao City (Cot, Dav)	14
	Biliran	498	Biliran (Bil)	8
Greater Negros-Panay	Leyte	7 213	Leyte (Ley)	1
	Negros	13 670	Negros Oriental (NOr)	8
			Negros Occidental (Neg)	1
	Panay	12 300	Panay (Pan, Akl)	21
Greater Luzon	Luzon	108 171	Mt. Isarog (Sur)	2
			Zambales (Zam)	2
			Catanduanes (Cat)	13
Mindoro	Mindoro	9 735	Mindoro (OrC, Oro)	8
Sibuyan	Sibuyan	448	Sibuyan (Sib)	20

nati Museum Center, and the US National Museum of Natural History. Individual specimens are listed in the Appendix.

LABORATORY METHODS

I extracted DNA using the PureGene (Gentra Systems, Inc.) protocol for animal tissue. I amplified and sequenced fragments of the mitochondrial genes ND2 and cytochrome *b* using either primers ND2-3TX and ND2-2 (Olson, Goodman & Yoder, 2004), primers L14724 (Irwin, Kocher & Wilson, 1991) and RM102CTC (5'-TAGGCGAATAGGAAATATCACTC-3'), or primers designed for this species: HfisND2-F (5'-TATCTCCATTTCACTTCTGA-3'), HfisND2-R (5'-AACCTAAGTTCCCTTAYTCCA-3'), Hfiscytb-F (5'-TGACCAACATCCGAAAATC-3'), and Hfiscytb-R (5'-GGGGGAGTGTTTAGTGGGTT-3'). PCR reactions included 2.5 µL reaction buffer containing 1.5 mM MgCl₂, 2.0–3.0 µL mixed dNTPs, 1.0 µL each 10 mM primer, and either 0.2 µL Taq polymerase from Roche Pharmaceuticals or 1.0 µL Field Museum Taq polymerase (see Acknowledgements). Optimal PCR annealing temperatures were determined for each primer combination by testing multiple temperatures on a Dyad thermal gradient cyler (MJ Research Inc.). PCR products were purified either by band excision from a TALE agarose gel and agarose digestion with GelAse enzyme (Epicentre Technologies) or through vacuum filtration with a 96-well MultiScreen plate (Millipore). Both strands were sequenced using ABI BigDyes version 2.0, 3.0, or 3.1 on an ABI 3100 Genetic Analyser. Sequences were compiled, edited, and aligned in Sequencher 4.1 and checked by eye; no length variation was present in these two protein-coding genes and alignment was unambiguous.

Aligned sequences were trimmed to match the shortest sequence in the alignment, yielding 662 bp of ND2 and 712 bp of cytochrome *b*. All analyses used the combined sequence of 1374 bp. These sequences have been assigned GenBank accession numbers AY817758–AY818003 (Appendix).

ANALYTICAL METHODS

Statistical parsimony networks were made using TCS 1.13 (Clement, Posada & Crandall, 2000). Phylogenetic analysis was performed in PAUP* 4.0b10 (Swofford, 2002). Redundant individuals were merged in the phylogenetic data matrix, leaving 101 distinct haplotypes. The best model for maximum likelihood was determined by iterative testing. I used a neighbour-joining tree to estimate maximum-likelihood parameter sets under the models tested by Modeltest (Posada & Crandall, 1998), but also under the same models with site-specific (SS) rates for first, second, and third codon positions. For all models, site-specific rate variation was significantly better compared with variation modelled with a gamma distribution, invariant sites, or both, so from this point on I tested only site-specific models. I performed hierarchical likelihood-ratio tests of models from Jukes–Cantor (JC) + SS to General Time Reversible (GTR) + SS. The best model was used to estimate a maximum-likelihood tree, and the model estimation procedure was repeated on this tree. The model chosen from this second estimation, which was a constrained version of GTR + SS, was used to estimate the final topology. The final heuristic tree search used 100 random-addition sequence replicates. Support values were obtained from 100 bootstrap replicates.

I tested the applicability of a molecular clock by comparing the likelihoods of the maximum-likelihood

tree topology under the same model of nucleotide substitution with and without a clock constraint and performing a likelihood ratio test. Because the molecular clock model was not rejected, I assumed that rate variation among lineages within *H. fischeri* was negligible. I compared likelihood scores for clock-constrained trees with various roots to see which root was the most likely under the assumption of the clock. The maximum-likelihood rooting position and branch lengths with the clock constraint were used in estimating divergence times. However, I also calculated the age of the root using other plausible root positions to test the impact of this assumption.

Given a tree topology and branch lengths, the estimation of time from molecular phylogenetic data requires two steps: the estimation of the gene coalescence time and the estimation of the population divergence time from the coalescence time (Hillis, Mable & Moritz, 1996; Arbogast *et al.*, 2002; Graur & Martin, 2004). Coalescence time can be estimated from a single overall rate of nucleotide substitution on a particular tree topology and branch lengths. I used a point estimate of the rate of substitution, based on the pattern of variation observed in the data and on the apparent substitution rates in bats and other groups of mammals (Pesole *et al.*, 1999; Gissi *et al.*, 2000). Any absolute rate of evolution from a fossil calibration is not completely accurate (Graur & Martin, 2004) even for the clade containing the fossil, and extending the calibration to other clades may magnify the error, but the poor fossil record for bats made an external calibration necessary in this case. Nonsynonymous sites in bats appear to have substitution rates slightly slower than the reported average (Gissi *et al.*, 2000), but ND2 and cytochrome *b* evolve slightly faster than the average mtDNA protein-coding gene (Pesole *et al.*, 1999). The synonymous substitution rate in these two genes is also higher than average, and is less influenced by lineage-specific rate variation (Pesole *et al.*, 1999). I therefore used the mammalian average substitution rate of 1.8×10^{-3} subst/site/Myr for nonsynonymous changes and a conservatively elevated rate of 30×10^{-3} subst/site/Myr for synonymous changes (Pesole *et al.*, 1999). I estimated the number of synonymous and nonsynonymous sites in the data using DNAsp 4.0 (Rozas *et al.*, 2003), yielding frequencies of 0.2569 for synonymous sites and 0.7431 for nonsynonymous sites, and calculated the overall rate of substitution by weighting the nonsynonymous and synonymous substitution rates by these frequencies. This yielded an absolute substitution rate of 9.045×10^{-3} subst/site/Myr, corresponding to a pairwise divergence of approximately 1.8%/Myr.

Even if the average rate of evolution and branch lengths are known without error, the stochasticity of mutation means that time cannot be estimated exactly

from the number of mutations on a branch. I calculated 95% confidence intervals (CIs) for the age of coalescence at each node of interest using the assumption that DNA substitution in these genes is approximated by a Poisson process (Hillis *et al.*, 1996) with a known average rate of 9.045×10^{-3} subst/site/Myr, acting on a known topology and branch lengths. Under this assumption, confidence intervals can be calculated from a gamma distribution with shape parameter the number of estimated mutations and scale parameter the inverse of the assumed mean substitution rate. This provides, in essence, the best-case confidence interval, assuming that the rate calibration, topology, and branch lengths are all correct.

The observed coalescence of gene lineages is expected to predate a related divergence event by a time whose average depends on the ancestral population size (Nei, 1987; Avise, 2000; Arbogast *et al.*, 2002), but which cannot be determined exactly because of lack of knowledge about that ancestral population size and because of the stochastic nature of coalescence and lineage sorting. I used current genetic diversity as a proxy for ancestral diversity and corrected for ancestral intraspecific coalescence at each node by subtracting half of the average maximum likelihood distance within extant populations (Nei, 1987; Edwards, 1997). This reduced the estimated date of each node by 0.297 Myr.

Analyses of molecular variance (AMOVAS) were performed in Arlequin 2.001 (Schneider, Roessli & Excoffier, 2000), and tested variation within current islands, among current islands within a PAIC, and among PAICs. This hierarchy tested the contribution of current and historical coastlines to the partitioning of genetic variation; locations within any current island were combined. I also used AMOVAS within individual PAICs. Within Greater Mindanao, AMOVA levels were defined as within sampling locations, among sampling locations on a current island, and among current islands within the historical island group. In Greater Negros-Panay and Greater Luzon, where the number of samples available did not permit a three-level AMOVA, I tested only variance among current islands and among samples within a current island. Other population genetic statistics, such as nucleotide diversity and haplotype diversity, were calculated by hand, in Arlequin 2.001, or in DNAsp 4.0 (Rozas *et al.*, 2003).

RESULTS

PHYLOGENETIC ANALYSES

Statistical parsimony analysis (Templeton, Crandall & Sing, 1992) resulted in eight distinct haplotype networks that could not be connected by fewer than 16

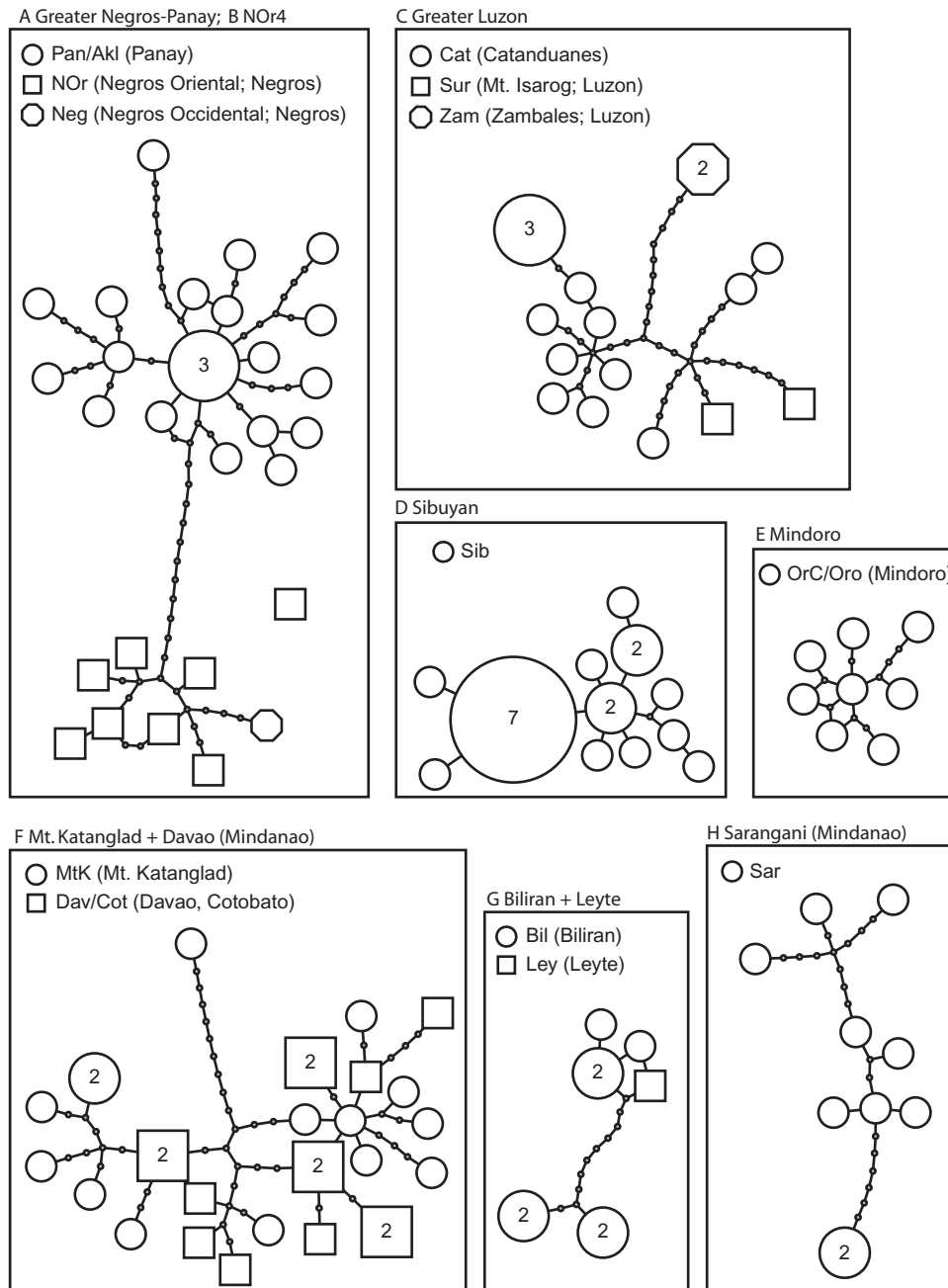


Figure 2. Statistical parsimony haplotype networks. In each network, different shapes (circle, square, or octagon) represent different locations. Size and labels of open shapes indicate the number of individuals sharing a haplotype; unlabelled haplotypes are singletons. Small closed circles represent unsampled haplotypes.

steps (Fig. 2). The two small PAICs, Sibuyan and Mindoro, and the large PAIC of Greater Luzon were each represented by exactly one network. Greater Mindanao haplotypes formed three networks that were themselves geographically structured, with one network for the Mt. Katanglad and Davao locations, one for the southern Sarangani samples, and one for

the samples from the two smaller islands, Biliran and Leyte. All but one of the haplotypes from Greater Negros-Panay formed a single network; a single individual from Negros was a second 'network'. In the former, samples from the current islands of Negros and Panay each clustered together and were separated from each other by 14 steps. In general, sampled

haplotypes were distantly spaced in the haplotype networks; they were connected in many cases by long strings of missing or unsampled haplotypes.

Phylogenetic analysis with maximum likelihood (Fig. 3) reconstructed the same clades as did statistical parsimony, but connected them into further-resolved geographical groups. The deepest divergence was among five major clades, each containing all and only the haplotypes from one PAIC. These five clades were connected by long branches in a star-like topology whose root was unresolved by outgroup analysis with either or both *Alionycteris* and *Otopteropus*, with which *Haplonycteris* has been suggested to form a monophyletic group (Rickart, Heaney & Rosenfeld, 1989; Walsh, 1998), or with the cynopterine fruit bats *Cynopterus brachyotis*, *Ptenochirus jagori*, *P. minor*, and *Megaerops wetmorei*. The topology of major clades in the ingroup was unaffected by the lack of a root. The groupings suggested by statistical parsimony were supported within the PAIC clades, with a single group for Panay within the Greater Negros-Panay clade, and monophyletic groups for Biliran/Leyte, Sarangani, and Mt. Katanglad/Davao within Greater Mindanao.

DIVERGENCE TIME ESTIMATION

A likelihood ratio test failed to reject the molecular clock for the entire *Haplonycteris* tree ($P = 0.30$), suggesting the variation in substitution rate among lineages to be negligible. The most likely root for the tree with the clock constraint was on the internal branch containing the midpoint of the maximum likelihood

tree, creating a basal split between (Greater Mindanao, Sibuyan) and (Mindoro, ((Greater Negros-Panay), (Greater Luzon))), and I used this root position in estimating divergence times. The estimated date of the base of the *Haplonycteris* tree was 6.01 Mya (95% CI, 4.70–7.49) (Fig. 4), and all five endemic PAIC lineages had diverged by 4.35 Mya (95% CI, 3.23–5.62). The Greater Mindanao clade diversified into the three current lineages from Biliran/Leyte, Sarangani, and Mt. Katanglad/Davao between 2.05 and 1.68 Mya (95% CI, 2.98–0.97). Divergence between Negros and Panay in the Greater Negros-Panay clade occurred between 0.80 and 0.64 Mya (95% CI, 1.46–0.18).

GENETIC DIVERSITY

Haplotype diversity in *H. fischeri* was extremely high. The 123 individuals sequenced had 101 unique haplotypes, a total haplotype diversity of 0.9948. Of these 101 haplotypes, 86 (85% of the haplotypes; 70% of the individuals) were singletons, 12 were shared by two individuals, two were shared by three individuals, and one was shared by seven individuals (Table 2). None of the 15 nonsingleton haplotypes was sampled from more than one location. The per-site nucleotide diversity, π (Nei, 1987), was 0.06034; the average uncorrected number of differences between sequences was 83.07 in the 1374 bases. Because of the high degree of geographical structure, however, statistics for the entire dataset do not indicate the diversity present in biological populations. Within the eight major clades suggested by phylogenetic analysis, haplotype and

Table 2. Distribution of singleton and shared haplotypes across populations

Location	N	h	Individuals sharing a haplotype				Total shared
			one	two	three	seven	
Catanduanes	13	11	10	0	1	0	1
Mt. Isarog	2	2	2	0	0	0	0
Zambales	2	1	0	1	0	0	1
Panay	21	19	18	0	1	0	1
Negros Occidental	1	1	1	0	0	0	0
Negros Oriental	8	8	8	0	0	0	0
Mt. Katanglad	15	14	13	1	0	0	1
Davao	14	10	6	4	0	0	4
Sarangani	10	9	8	1	0	0	1
Biliran	8	5	2	3	0	0	3
Leyte	1	1	1	0	0	0	0
Sibuyan	20	12	9	2	0	1	3
Mindoro	8	8	8	0	0	0	0
TOTAL	123	101	86	12	2	1	15

Locations are as in the map (Fig. 1).
h, number of haplotypes.

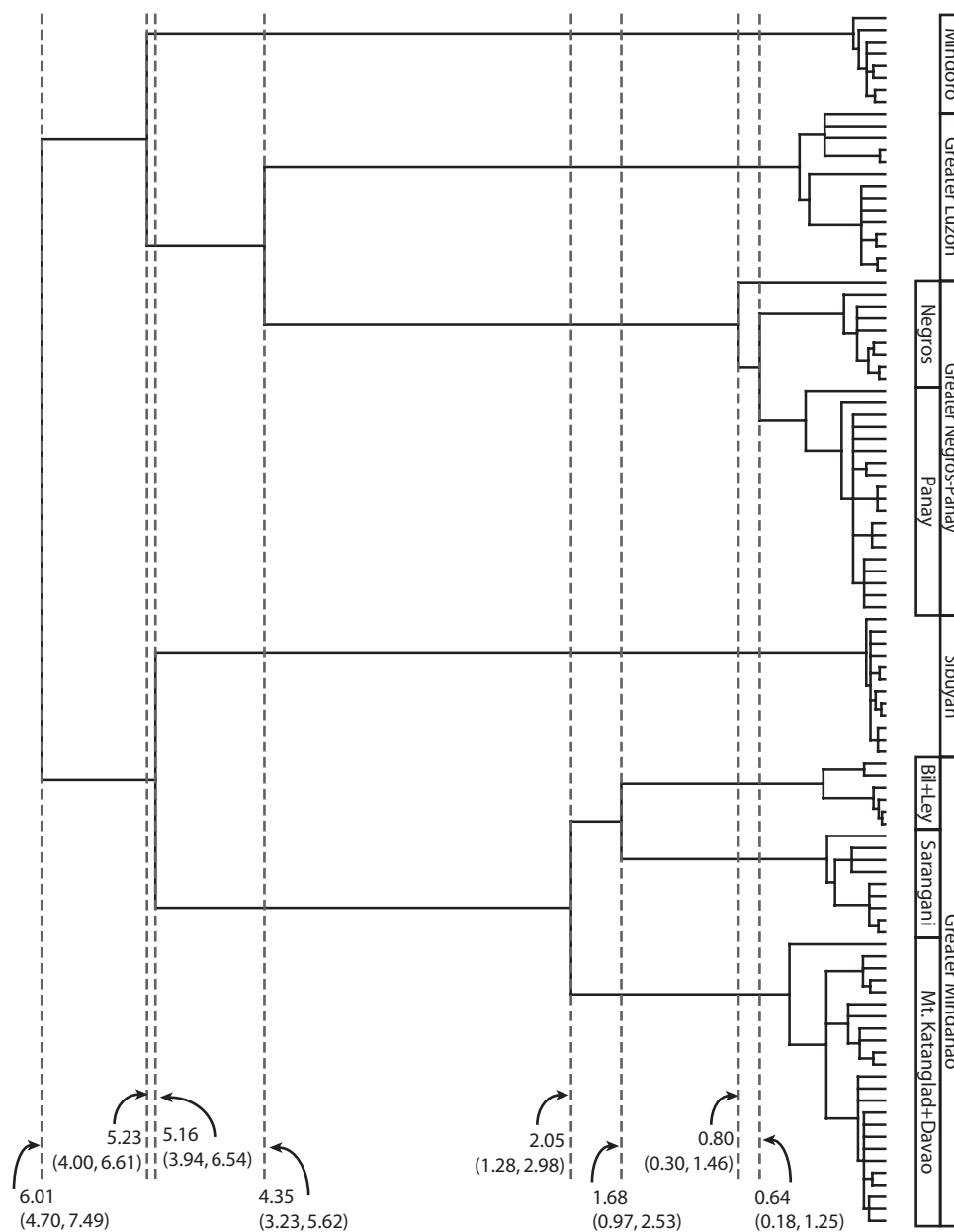


Figure 4. Rooted maximum likelihood tree with the molecular clock enforced, and inferred divergence times (Mya) for each major clade. Numbers in parentheses are the upper and lower bounds of the 95% stochastic confidence interval for the times.

nucleotide diversity were also high (Table 3), but nucleotide diversity on Sibuyan and Mindoro, the two continuously isolated islands, was low relative to other islands. The nucleotide diversity within the eight major clades ranged from 0.0015 to 0.0073 (Table 3). Sibuyan also showed the lowest haplotype diversity of any of the eight major groups. While the sample size for the whole species was large (123 individuals), in several cases single-island or single-location sample sizes were so small that sampling variances were quite high. The single divergent individual from

Negros caused the sampling variance for that population and island to be particularly high. The corrected pairwise genetic distance (D_a , Nei, 1987) between PAIC clades ranged from 0.0589 to 0.0763; the corrected maximum likelihood distance ($\text{TrN} + \Gamma$) ranged from 0.1046 to 0.1464 (Table 4).

ANALYSIS OF MOLECULAR VARIANCE

The AMOVA for the entire dataset indicated the variance among Pleistocene island groups to account

Table 3. Haplotype diversity (Hd) and per-site nucleotide diversity (π), with their standard errors, for the eight major clades and for those islands and PAICs that do not correspond to clades

	π	SE(π)	Hd	SE(Hd)
Clades				
Greater Luzon	0.007374	0.00097	0.971	0.0322
Negros	0.007035	0.00236	1.000	0.0523
Panay	0.004304	0.00067	0.986	0.0217
Mt. Katanglad/Davao	0.005869	0.00059	0.988	0.0118
Sarangani	0.004998	0.00059	0.978	0.0540
Biliran/Leyte	0.004933	0.00067	0.917	0.0725
Mindoro	0.002885	0.00044	1.000	0.0625
Sibuyan	0.001481	0.00022	0.879	0.0654
Other islands				
Mindanao	0.01683	0.00198	0.992	0.0077
Biliran	0.00504	0.00071	0.893	0.0858
Luzon	0.00946	0.00247	0.833	0.2224
Catanduanes	0.00537	0.00106	0.962	0.0496
Other PAICs				
Greater Mindanao	0.021839	0.00164	0.992	0.0055
Greater Negros-Panay	0.008959	0.00098	0.993	0.0118

Table 4. Genetic distances between clades

Clades	D_{xy}	D_a	ML
PAICs			
Greater Mindanao–Greater Luzon	0.0817	0.0671	0.1359
Greater Mindanao–Greater Negros–Panay	0.0777	0.0623	0.1261
Greater Luzon–Greater Negros–Panay	0.0671	0.0589	0.1046
Sibuyan–Greater Luzon	0.0808	0.0763	0.1464
Sibuyan–Greater Mindanao	0.0741	0.0624	0.1230
Sibuyan–Greater Negros–Panay	0.0770	0.0718	0.1390
Sibuyan–Mindoro	0.0723	0.0702	0.1317
Mindoro–Greater Luzon	0.0696	0.0645	0.1174
Mindoro–Greater Negros–Panay	0.0702	0.0644	0.1198
Mindoro–Greater Mindanao	0.0734	0.0610	0.1190
Islands: Negros–Panay	0.0145	0.0088	0.0099
Locations			
Davao/Mt. Katanglad–Sarangani	0.0443	0.0286	0.0387
Davao/Mt. Katanglad–Biliran/Leyte	0.0436	0.0290	0.0381
Sarangani–Biliran/Leyte	0.0422	0.0283	0.0390

Maximum likelihood (ML) distances were calculated using a TrN + Γ model of evolution with parameters estimated on the ML tree shown in Figure 3.

D_a , net pairwise distance between groups; D_{xy} , average pairwise distance between members of two groups.

for 78.16% of the structure in this species, compared with 12.38% among current islands and 9.46% within islands (Table 5). Like the phylogenetic analysis, AMOVA within individual PAICs suggested that current coastlines contribute a significant amount of variance (Table 6). In Greater

Mindanao, where sample sizes were sufficient to perform a three-level nested AMOVA within the island group, the variance associated with different locations within Mindanao was significantly large and the variance within locations was significantly small.

Table 5. Analysis of molecular variance (AMOVA) for the entire archipelago

Source of variation	Variance component	% of variation	<i>P</i>
Among PAICs	92.22072	78.16	0.00022
Among islands within a PAIC	14.61033	12.38	< 0.00001
Within an island	11.16507	9.46	< 0.00001

Islands are the lowest hierarchical level; locations within islands are combined.

Table 6. Analysis of molecular variance (AMOVA) for individual PAICs

PAIC	Source of variation	Variance component	% of variation	<i>P</i>
Greater Luzon	Between islands	2.12456	33.31	0.0040
	Within islands	4.25385	66.69	–
Greater Negros-Panay	Between islands	6.12677	63.69	< 0.0001
	Within islands	3.49320	36.31	–
Greater Mindanao	Among islands	7.81879	33.87	0.2011
	Among locations within islands	11.52915	49.95	< 0.0001
	Within locations	3.73538	16.18	< 0.0001

For Greater Luzon and Greater Negros-Panay, two levels were tested and locations within islands were combined; for Greater Mindanao, three levels were tested.

DISCUSSION

PATTERNS WITHIN THE ENTIRE ARCHIPELAGO

The majority of molecular variance (78.16%) was due to differences among the five PAICs of Greater Mindanao, Greater Luzon, Greater Negros-Panay, Sibuyan, and Mindoro. These five landmasses have never been connected to each other by dry land, and each has been described as a distinct biogeographical region and an important area of endemism in the Philippines (Heaney, 1986). Phylogenetic analysis showed the same deep genetic structure among these five areas, as samples from each PAIC formed a monophyletic clade. The 'star' phylogeny, with relatively long terminals and relatively short internal branches, suggests that all five major clades of *H. fischeri* arose within a relatively brief period of time, at least compared to the time since that divergence. Unfortunately, the tree shape contributes to the difficulty of rooting the tree convincingly with the current data.

Reciprocal monophyly implies that gene flow has been absent for long enough to result in coalescence of each lineage at a single location or on a single island or PAIC. Mitochondrial DNA, however, may represent only the history of female lineages, particularly if dispersal is male-biased, and further study of markers unlinked to the two mitochondrial genes presented here may be necessary to be sure that this is not true of *Haplonycteris*. However, the estimated movement distances of *H. fischeri* males and females in a mark-recapture study were not significantly different, and

on average, adult males moved only half as far as females (Heideman & Heaney, 1989). Also, the degree of mitochondrial geographical structure and the length of the branches separating the five major clades suggest a very long history with no gene flow, and are unlikely to be due to sex-biased dispersal.

While many islands and areas in the archipelago were not included in this analysis, the observed geographical structure is so substantial that data from additional locations are unlikely to change the overall pattern of strong geographical subdivision among the major clades. However, I predict that samples from additional islands and locations will in some cases reveal additional distinctive clades.

PATTERNS WITHIN LARGE PAICs

Within Greater Negros-Panay, the monophyly of samples from Panay and the near-monophyly of those from Negros suggest a lack of current gene flow between these two islands. In a two-level AMOVA comparing genetic variance within and between these two islands, 63.69% was attributable to variation between Negros and Panay (Table 6).

Within Greater Mindanao, samples from the three current islands of Mindanao, Leyte, and Biliran showed a more complex pattern. The single sample from Leyte was nested among those from Biliran. While it is impossible to determine the amount of gene flow between locations without multiple samples from each, the position of the Leyte sample, which is at an

internal node and appears to have direct haplotype descendents on Biliran, suggests current or recent gene flow between these two islands, which are separated by one of the narrowest and shallowest channels in the archipelago. Samples from the large island of Mindanao formed two clades, one comprising those from the southern province of Sarangani and one comprising those from the locations on Mt. Katanglad and near Davao City. In this case, the narrow channel between Biliran and Leyte seems not to bar gene flow, but the wider, deeper one between Leyte and Mindanao does. Other studies have found deep divergences between Mindanao and other islands in the PAIC in rodents (Steppan *et al.*, 2003), frogs (Evans *et al.*, 2003), and flying lizards (McGuire & Kiew, 2001). The divergence between the two clades from Mindanao was about the same as that between either of them and the Biliran/Leyte clade. The island of Mindanao is composed of multiple fragments that have become aggregated over the past 15 Myr (Hall, 1996, 1998) and contains several different mountain areas separated by large expanses of lower elevation. Recent gene flow seems to be lacking between populations in different mountain ranges, although some distant sites were part of the same clade. It is possible that dispersal through lowland forest habitat is rare for *Haplonycteris*, which is most abundant at middle elevations (Heaney *et al.*, 1989; Heideman & Heaney, 1989; Heaney, 1991), and that it is unable to disperse across parts of the large island of Mindanao.

The lack of sufficient sampling on Luzon, combined with high nucleotide diversity, makes it difficult to draw conclusions regarding the true shape of the gene tree on this island. The two samples from Zambales were identical, but the two from Mt. Isarog differed from each other by ten steps in the statistical parsimony network. Most samples from Catanduanes, a small island that is part of Greater Luzon and close to Mt. Isarog, were related closely to each other, but a few divergent haplotypes suggest that this island, too, may have been undersampled. Genetic differentiation within the Greater Luzon PAIC appears less than that for Greater Mindanao or Greater Negros-Panay, with 66.69% of genetic variance associated with variation among individuals within an island rather than differentiation between the islands of Luzon and Catanduanes (Table 6). Gene flow appears to be higher among islands in Greater Luzon than in other PAICs, but this can only be confirmed with increased sampling.

PATTERNS ON SMALL PAICs

Each of the two small PAICs, Sibuyan and Mindoro, had a distinct endemic clade. Nucleotide diversity on these two islands was lower than that on any of the

other islands or historical islands for which it could be calculated; the average number of mutations per site separating individuals was 0.0029 on Mindoro and 0.0015 on Sibuyan, compared with 0.0073–0.0218 for the large PAIC clades (Table 3). Haplotype diversity was lower on Sibuyan than it was on any other island or location studied, but the eight individuals from Mindoro all had unique haplotypes. Historical isolation and small area are presumably responsible for the lower levels of diversity on these two islands. Sibuyan is a small island and very isolated; mtDNA suggests a historically smaller population than on other islands, as well as a history of restricted gene flow between this island and other locations. In previous studies of allozymes and morphology in *H. fischeri*, the Sibuyan population was the most divergent of any location in the Philippines (Peterson & Heaney, 1993; Walsh, 1998; Heaney *et al.*, 2005). While the mitochondrial divergence between Sibuyan and the other PAIC clades is quite high, it is on the same scale as divergence among the other PAICs. This apparent discordance may be due in part to the reduced genetic diversity in the Sibuyan population; lack of intrapopulation diversity increases differentiation under statistics such as F_{ST} and D_a .

DETERMINANTS OF PHYLOGEOGRAPHIC STRUCTURE

Historical geography is the most important cause of genetic divergence in this species. The net sequence divergence (D_a) between PAICs ranged from 5.8906% to 7.6337% (Table 4), with each PAIC forming a distinct monophyletic group. Reciprocal monophyly, particularly with long intervening branches on the phylogenetic tree, implies that at least in mitochondrial lineages there is no gene flow among historical islands, and has not been for a long period of time. This was reflected by the entire-archipelago AMOVA, which showed 78.16% of genetic variance to be among PAICs. However, there was substantial divergence within island groups as well, evident both in the AMOVA and in the topology of the tree. Current coastlines thus also contribute to the observed genetic structure; there were allopatric clusters nested within the clades on two of the three large PAICs. Again, mitochondrial data suggest that there has been no recent gene flow between some pairs of current islands within a single PAIC. While current coastlines delimited less variation than did the PAICs, there seems to be very little effective gene flow across wide ocean channels. The temporal scale of divergence between current islands within a PAIC is less than that among the PAICs, since the monophyletic and paraphyletic groups corresponding to Negros, Panay, Biliran/Leyte, and Mindanao were nested within their respective PAIC clades. This indicates that gene flow among the

islands within a PAIC ceased more recently than did gene flow among the PAICs themselves. Within the large island of Mindanao, where my sampling was most extensive, there was substantial diversity and structured variation among locations. The presence of geographical structure resulting in reciprocal monophyly at multiple nested geographical levels suggests that gene flow in *H. fischeri* is extremely low or non-existent over water barriers and between some parts of existing islands. It is clear that the legacy of PAIC landmasses, which are strongly implicated in the distribution of species and higher taxa, is also important in determining differentiation among populations in this species. However, factors other than the lack of over-water dispersal among PAICs and islands must be invoked to explain the lower-level geographical structure within islands. The depth of the divergence between some lineages suggests that the geological history of the islands is responsible, but the distribution of forest and elevation may also be important.

In general, the combination of unexpectedly high nucleotide and haplotype diversity suggests large populations that have been stable over evolutionary time (Grant & Bowen, 1998; Avise, 2000). This pattern may not be unexpected in the tropics, where stability is one proposed explanation for diversity at the level of species and higher taxa (Fischer, 1960; Chown & Gaston, 2000) and more recently for genetic diversity (e.g. Crawford, 2003).

COMPARISON WITH PREVIOUS DATASETS

Allozyme variation in *H. fischeri* was examined by Peterson & Heaney (1993) and revisited recently by Heaney *et al.* (2005). Allozyme data show a high degree of differentiation between the small, isolated island of Sibuyan and every other population (Peterson & Heaney, 1993). This study also found lesser but substantial differentiation among the major Pleistocene islands, using samples from Luzon, Catanduanes, Leyte, Biliran, and Negros (Peterson & Heaney, 1993; Heaney *et al.*, 2005), with an overall F_{ST} of 0.606 and an estimate of the average number of migrants per generation (N_m) of 0.049. While most variance in allozyme frequency was found to be within islands, 30.7% was associated with variance among Pleistocene islands; only 0.8% was associated with differences among islands within a Pleistocene group. This study also found a positive correlation between genetic distance and distance between PAICs, and described *H. fischeri* as having high levels of genetic differentiation on distant Pleistocene islands, medium levels on adjacent Pleistocene islands, and nearly absent differentiation within single Pleistocene islands. In general, Peterson & Heaney (1993) and Heaney *et al.* (2005) interpreted the allozyme data as suggesting low levels

of current gene flow between Pleistocene islands, higher levels between current islands within a Pleistocene island, and very low levels between Sibuyan and any other island. My results are generally concordant with this hierarchical pattern, showing less genetic divergence between islands in a PAIC than among PAICs, but the pattern suggested by the mitochondrial sequence data is more extreme. The degree of mitochondrial separation implies that there is no current gene flow among PAICs or between the other islands and locations where haplotype clades have reached reciprocal monophyly. However, indirect estimates of gene flow from F_{ST} are based on a model whose assumptions are almost certainly violated in many natural populations (Bossart & Prowell, 1998; Whitlock & McCauley, 1999); they assume, for example, that populations are in migration-drift equilibrium and that migration is responsible for shared polymorphism, when shared ancestry may be an equally important cause (Slatkin, 1985; Slatkin & Maddison, 1989). Because the effective population size of nuclear allozymes is larger than that of mtDNA, and because the mutation rate of mitochondrial sequences is presumably faster than that of allozyme variants, it is not surprising to find more apparent differentiation in the mitochondrial dataset (Brown, George & Wilson, 1979; Moore, 1995; Avise, 2000). Overall, both datasets suggest that the oceanic PAICs have been isolated from each other for a long period of time, supporting the conclusion that PAIC coastlines are an important determinant of intraspecific geographical structure in *H. fischeri*.

Heideman (1988) found that reproductive timing in *H. fischeri* was synchronous on Luzon and Negros and on Biliran and Leyte, but was not synchronized between these pairs of islands. The mitochondrial data suggest that Greater Negros-Panay and Greater Luzon share a more recent common ancestor than either does with populations on Greater Mindanao, so an evolutionary divergence in reproductive timing may support the hypothesis of long-term isolation between these two groups.

TAXONOMIC IMPLICATIONS

Haplonycteris bats on the island of Sibuyan have proven to be distinct in morphology (Walsh, 1998), in allozyme variation (Peterson & Heaney, 1993; Heaney *et al.*, 2005), and in mtDNA (this study). I concur with previous authors (Ingle & Heaney, 1992; Heaney *et al.*, 1998) who have proposed elevating this population to species status. The other four mitochondrial PAIC lineages in the Philippines appear to have been isolated for as long as the population on Sibuyan, long enough for reciprocal monophyly to occur in all cases. The allopatric, monophyletic lineages within Greater Negros-

Panay and Greater Mindanao also appear to be isolated, although presumably not for as long. However, allozyme data have not refuted the possibility of gene flow among any of these populations, and I recognize that without nuclear evidence of genetic isolation their status is uncertain. Nonetheless, the degree of evolutionary independence indicated by mitochondrial monophyly suggests that they are isolated populations with little gene flow, if any; further evidence of isolation from other types of data would support their recognition as species.

THE TIMING OF DIVERGENCE EVENTS

Substantial divergence among allopatric lineages, as well as the relatively well-known recent geological and geographical history of the Philippines, makes it informative to determine the time at which divergence events occurred. While subject to error from several sources, the approximate point estimates of divergence time and stochastic confidence intervals presented here are the best possible with the available data. The estimated times are largely concordant with the geological history of the Philippine archipelago and suggest a pattern of Pliocene diversification during the post-Miocene uplift phase of the archipelago. The most recent geological reconstructions of the region (Hall, 1998, 2001, 2002) illustrate its complexity at this time, and it is important to remember that the presence, location, and size of many islands changed substantially during the Pliocene and Pleistocene. Between 10 and 5 Mya, the Philippines was moving as several dissociated island terranes, and many of the current islands had not yet emerged. Reconstructed land and sea distribution for the early Pliocene, 5 Mya (Hall, 1998), shows parts of Luzon and Mindanao, Mindoro, an area in the central Philippines, and an area that would later become part of western Mindanao and Negros as nearly the only major islands in the archipelago. Most of the current configuration of the Philippines has arisen in the past 5 Myr, during the Pliocene and Pleistocene, as a result of a complex of subduction and fault zones throughout the area. The Philippine and Negros trenches and their associated subduction zones originated about 5 Mya, and action in these areas and along the strike-slip fault running through the Philippines has been responsible for much of the continued geological activity in the past 5 Myr (Hall, 1998, 2001, 2002).

The divergences among the five PAIC clades, which were the deepest nodes in the tree, were dated to 6.01–4.35 Mya, in the late Miocene and early Pliocene; the basal coalescence in the tree was dated to approximately 6.31 Mya. The first set of diversification events in *H. fischeri* thus appears to be correlated with a period of major geological change, in which many

islands were being formed and the Philippines in general was beginning to approach its current configuration. However, it is worth noting that Sibuyan, the smallest of the PAICs in this study, has been considered much younger than the lineage that inhabits it (Steppan *et al.*, 2003). No close relative of the Sibuyan mitochondrial lineage has been found elsewhere in the archipelago, but I cannot exclude the possibility that a closely related lineage exists in an area that was not sampled for this study, and colonized Sibuyan recently, or that relatively recent colonization from another island was followed by extinction of the ancestor population, leaving a relict endemic lineage on Sibuyan. The genetic data, however, strongly suggest that the endemic Sibuyan lineage is as old as the other PAIC lineages and therefore that it has persisted on the island for millions of years. Parts of all of the other PAICs included in this study were above water by the early Pliocene (Hall, 1998). Mindoro, the larger of the two small PAICs, existed in the Miocene, although even 5 Mya it was considerably north and west of its current location, quite close to old parts of Luzon and Palawan but not to the central Philippines. In the most likely rooting scenarios for the phylogenetic tree, Mindoro was associated with the Greater Luzon and Greater Negros-Panay clades, perhaps suggesting early dispersal events between Luzon and Mindoro within what was then the northern part of the archipelago.

Between approximately 4 and 2 Mya, during a period of continued geological evolution including the emergence of many islands, there was apparently no further diversification in *Haplonycteris*. In contrast, many lineages of the endemic rodent genus *Apomys* arose during this interval, possibly in connection with the emergence of new islands (Steppan *et al.*, 2003). The next period of diversification in *H. fischeri* occurred between approximately 2.05 and 1.68 Mya, with the divergence of three lineages within what is now the Greater Mindanao PAIC. The island of Mindanao, which is composed of multiple fragments that have aggregated at multiple times, has two endemic lineages. While the central region of the island, including Mt. Katanglad and Davao, first existed between 15 and 10 Mya (Hall, 2001), south-western Mindanao, containing the Sarangani lineage, arose within the past 5 Myr. Fragments of Leyte may have existed by the late Miocene, but most arose during the Pliocene (Hall, 1998, 2002); because of the complexity of the fault and subduction systems in this region, more detailed reconstructions are not currently available. With the limited data available, the diversification within Greater Mindanao around 2 Mya appears to be consistent with colonization of newly emerged islands and parts of islands. The order of divergence among these three lineages should be interpreted with cau-

tion; since the two coalescence events appear to have occurred within approximately 370 000 years, a relatively brief period compared to the time necessary for intrapopulation coalescence, their order may reflect the idiosyncratic history of mtDNA rather than the true population history. Because of the stochasticity inherent in lineage sorting, the observed order of coalescence events is likely to be consistent with any of the possible divergence scenarios and relationships among these three groups. Further information from other molecular markers may help to clarify the order of divergence events, although genetic data may not have the power to resolve multiple rapid divergences completely.

The most recent divergence between allopatric lineages occurred between Negros and Panay between 400 000 and 560 000 years ago. These islands were connected as part of Greater Negros-Panay during Pleistocene periods of reduced sea level. The apparent timing of the split between them might be consistent with Pleistocene divergence as a result of sea-level change and the formation of land bridges. However, the most recent geological reconstructions of the Philippines also suggest that Negros and Panay only reached their current close proximity during the Pleistocene, and were separated by deep water as recently as 1 Mya (Hall, 2002); Negros may only have emerged 1–4 Mya (Heaney, 1991). Thus, the relatively late Pleistocene divergence between populations on these islands may be the result of over-water colonization of one island from the other as they emerged and came into proximity, not due to gene flow across late Pleistocene land bridges. The paraphyly of mitochondrial haplotypes from Negros suggests that the time since this divergence has been insufficient for complete lineage sorting to occur. Because lineages on Greater Negros-Panay diverged within approximately 160 000 years, a short period of time compared with that which may be necessary for lineage sorting to occur, the order of coalescence events should not be interpreted as necessarily indicating the order of divergence events.

In general, the evolution of *H. fischeri* in the Philippines involves deep divergences in the Pliocene or early Pleistocene. While many scenarios of speciation posed in other parts of the world have focused on the effects of such recent events as Pleistocene glaciation, diversification in this case is due to older events and is correlated with tectonic events in the region and the origin of the Philippine islands themselves. The same pattern is apparent for rodents of the endemic genus *Apomys*, in which the ages of species and lineages are significantly related to the ages of the islands they inhabit (Steppan *et al.*, 2003). Genetic data for *Apomys* also reject a Pleistocene sea level-related model of speciation in favour of Pliocene diversification (Steppan *et al.*, 2003). Sea-level change may have

been important in divergence within some PAICs, such as between Negros and Panay, but other such divergences, as in Greater Mindanao, are much older, and the divergence among PAIC lineages is likely to be due to deeper geological events associated with the tectonic history of the Philippine archipelago.

The estimation of divergence dates depends on the assumption that the true tree topology and branch lengths are known. In this case, the branching order of major clades within each PAIC was robust, as was the unrooted topology among the PAICs, but the root of the tree was not. Data from different molecular markers, perhaps slower-evolving nuclear genes or introns, may be necessary to solidify the basal relationships among *H. fischeri* clades. Expanding or changing the outgroup taxa in the phylogenetic analysis may also be necessary, as the systematic relationships among pteropodid bats remain uncertain. However, the lack of a robust root makes very little difference in the estimated age of the deepest node. Rooting the tree at any of the seven probable locations, on the five PAIC clade branches and the two internal branches, yielded a range of estimates for the root divergence of 5.99–6.52 Mya with the minimum and maximum confidence intervals ranging from 4.71 to 8.01.

ISLAND BIOGEOGRAPHY IN THE PHILIPPINES

Ecological theories of island biogeography have often focused on turnover as an important component of the determination of community composition. *Haplonycteris* does not seem to be experiencing rapid turnover among island populations. On the islands where it occurs, divergence from other island populations shows that it has been present for an extremely long time. Over-water colonization must be responsible for the presence of *H. fischeri* on most of the islands in its range, since they have been isolated throughout their history; the exception is populations on islands within a PAIC, which could have been colonized across Pleistocene land bridges. However, no recent colonization or recolonization among PAICs was indicated by the mitochondrial data. Along with the uniformly high levels of genetic diversity, with 101 haplotypes in 123 sampled individuals, this strongly suggests a high degree of stability in the population distribution of this species. It is impossible to know with certainty whether the current absence of *H. fischeri* on certain islands, such as Camiguin and Maripipi, is due to local extinction or lack of colonization, but it is clear that in either case there has been no recent successful colonization or recolonization. Since these are relatively young islands, formed volcanically during the Pleistocene, it is possible that there has not been sufficient time for a successful colonization to occur. In general, the population dynamics of *Haplonycteris* appear to be

driven by a pattern involving over-water dispersal and subsequent divergence, not continuous gene flow. This bat seems to colonize new areas over water but does not maintain genetic contact among populations over long periods of time. Occasional successful colonization events, rather than continuous gene flow, probably explain the dominant pattern of diversification in allopatry.

Population genetics and genetic theories of island biogeography suggest that equilibrium genetic variation should be lower on small islands than it is on large ones (Wright, 1969; Frankham, 1997). Others have suggested that large islands can become arenas for in situ diversification (e.g. Heaney, 2000), which may result in the presence of divergent lineages on a single island. Both of these theories are supported in *H. fischeri*. The small PAIC of Sibuyan had less haplotype and nucleotide diversity than any other island, and the slightly larger PAIC of Mindoro had less nucleotide diversity than did any single landbridge island in the three large PAICs. In the Philippines, the legacy of sea-level change, which causes repeated aggregation and fragmentation of islands, means that diversity levels may not be correlated with current island size and configuration, but may instead reflect the larger islands that have frequently existed during less fragmented periods of history. The lingering effect of past island size may explain why the two continuously isolated islands showed less genetic diversity than did the small landbridge islands of Biliran and Catanduanes.

CONCLUSIONS

H. fischeri, a primary forest bat endemic to the oceanic Philippines, has a deep history that has been driven by Pliocene dispersal and diversification in allopatry during periods of geological evolution. The legacy of Pleistocene land bridges as dispersal routes connecting recent populations may be important within PAICs, but most divergence among lineages greatly predates the Pleistocene. Monophyletic clades on each of five Pleistocene island complexes, as well as within two of the larger PAICs, imply that *H. fischeri* has diverged in allopatry on multiple spatial and temporal scales as a result of colonization of new habitat. The high genetic diversity within populations likewise suggests that they have experienced long periods of demographic stability, but both island size and isolation also seem to affect genetic diversity. Current taxonomy fails to recognize multiple allopatric lineages that are quite old, and deserves revision in the context of genetic data. As has been shown many times for other Philippine taxa, the biogeographical history of this bat has little to do with recent dispersal or dynamics on an ecological time scale; it is instead closely tied to the geographical and

geological history of the archipelago over long periods of evolutionary time.

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APPENDIX

Museum voucher, location, and GenBank (GB) information for the 123 specimens in this study. Institutions are CMC: Cincinnati Museum Center; USNM: US National Museum of Natural History (Smithsonian); FMNH: Field Museum of Natural History.

Location code	Sample ID	Voucher	GB ND2	GB cyt b
Akl: Panay, Aklan Province				
	HfisAkl1	CMC 3015	AY817758	AY817881
	HfisAkl2	CMC 3011	AY817761	AY817884
	HfisAkl3	CMC 3008	AY817762	AY817885
	HfisAkl4	CMC 3009	AY817763	AY817886
	HfisAkl5	CMC 3010	AY817764	AY817887
	HfisAkl6	CMC 3005	AY817765	AY817888
	HfisAkl7	CMC 3007	AY817766	AY817889
	HfisAkl8	CMC 3014	AY817767	AY817890
	HfisAkl9	CMC 3006	AY817768	AY817891
	HfisAkl10	CMC 3012	AY817759	AY817782
	HfisAkl11	CMC 3013	AY817760	AY817783
Bil: Biliran				
	HfisBil1	USNM 458170	AY817769	AY817892
	HfisBil2	USNM 458171	AY817770	AY817893
	HfisBil3	USNM 458172	AY817771	AY817894
	HfisBil4	USNM 458173	AY817772	AY817895
	HfisBil5	USNM 458174	AY817773	AY817896
	HfisBil7	USNM 458181	AY817774	AY817897
	HfisBil8	USNM 458182	AY817775	AY817898
	HfisBil9	USNM 458183	AY817776	AY817899

APPENDIX *Continued*

Location code	Sample ID	Voucher	GB ND2	GB cyt b
Cat: Catanduanes				
	HfisCat1	FMNH 142333	AY817777	AY817900
	HfisCat2	FMNH 142334	AY817782	AY817905
	HfisCat3	FMNH 142335	AY817783	AY817906
	HfisCat4	FMNH 142336	AY817784	AY817907
	HfisCat5	FMNH 142330	AY817785	AY817908
	HfisCat6	USNM 573216	AY817786	AY817909
	HfisCat7	USNM 573217	AY817787	AY817910
	HfisCat8	USNM 573218	AY817788	AY817911
	HfisCat9	USNM 573219	AY817789	AY817912
	HfisCat10	USNM 573220	AY817778	AY817901
	HfisCat11	FMNH 142331	AY817779	AY817902
	HfisCat12	USNM 573141	AY817780	AY817903
	HfisCat13	USNM 573228	AY817781	AY817904
Cot: Mindanao, Cotobato Province				
	HfisCot1	CMC 3170	AY817790	AY817913
	HfisCot2	CMC 3169	AY817791	AY817914
	HfisCot3	CMC 3173	AY817792	AY817915
	HfisCot4	CMC 3172	AY817793	AY817916
Dav: Mindanao, Davao City				
	HfisDav1	CMC 3158	AY817794	AY817917
	HfisDav2	CMC 3154	AY817796	AY817919
	HfisDav3	CMC 3153	AY817797	AY817920
	HfisDav4	CMC 3156	AY817798	AY817921
	HfisDav5	CMC 3155	AY817799	AY817922
	HfisDav6	CMC 3149	AY817800	AY817923
	HfisDav7	CMC 3150	AY817801	AY817924
	HfisDav8	CMC 3152	AY817802	AY817925
	HfisDav9	CMC 3592	AY817803	AY817926
	HfisDav10	CMC 3151	AY817795	AY817918
Ley: Leyte				
	HfisLey1	USNM 458188	AY817804	AY817927
MtK: Mindanao, Bukidnon Province, Mt. Katanglad				
	HfisMtK1	FMNH 146628	AY817805	AY817928
	HfisMtK2	FMNH 146629	AY817812	AY817935
	HfisMtK3	FMNH 146630	AY817813	AY817936
	HfisMtK4	FMNH 146631	AY817814	AY817937
	HfisMtK5	FMNH 147834	AY817815	AY817938
	HfisMtK6	FMNH 148105	AY817816	AY817939
	HfisMtK7	FMNH 148106	AY817817	AY817940
	HfisMtK8	FMNH 148107	AY817818	AY817941
	HfisMtK9	FMNH 148108	AY817819	AY817942
	HfisMtK10	FMNH 148109	AY817806	AY817929
	HfisMtK11	FMNH 146979	AY817807	AY817930
	HfisMtK12	FMNH 146618	AY817808	AY817931
	HfisMtK13	FMNH 146619	AY817809	AY817932
	HfisMtK14	FMNH 146620	AY817810	AY817933
	HfisMtK15	FMNH 146621	AY817811	AY817934
Neg: Negros, Negros Occidental Province				
	HfisNeg4	CMC 3368	AY817820	AY817943

APPENDIX *Continued*

Location code	Sample ID	Voucher	GB ND2	GB cyt <i>b</i>
NOr: Negros, Negros Oriental Province				
	HfisNOr1	USNM 458199	AY817821	AY817944
	HfisNOr2	USNM 459092	AY817822	AY817945
	HfisNOr3	USNM 458201	AY817823	AY817946
	HfisNOr4	USNM 458202	AY817824	AY817947
	HfisNOr5	USNM 458204	AY817825	AY817948
	HfisNOr6	USNM 458206	AY817826	AY817949
	HfisNOr7	USNM 458207	AY817827	AY817950
	HfisNOr8	USNM 458208	AY817828	AY817951
OrC: Mindoro, Mindoro Oriental Province				
	HfisOrC1	CMC 3121	AY817829	AY817952
	HfisOrC2	CMC 3119	AY817830	AY817953
	HfisOrC3	CMC 3123	AY817831	AY817954
	HfisOrC4	CMC 3120	AY817832	AY817955
Oro: Mindoro, Mindoro Oriental Province				
	HfisOro1	FMNH 150785	AY817833	AY817956
	HfisOro2	FMNH 150786	AY817834	AY817957
	HfisOro3	FMNH 150787	AY817835	AY817958
	HfisOro4	FMNH 150788	AY817836	AY817959
Pan: Panay, Antique Province				
	HfisPan1	CMC 2963	AY817837	AY817960
	HfisPan2	CMC 3698	AY817839	AY817962
	HfisPan3	CMC 2961	AY817840	AY817963
	HfisPan4	CMC 2962	AY817841	AY817964
	HfisPan5	CMC 2964	AY817842	AY817965
	HfisPan6	CMC 2966	AY817843	AY817966
	HfisPan7	CMC 2967	AY817844	AY817967
	HfisPan8	CMC 2968	AY817845	AY817968
	HfisPan9	CMC 2970	AY817846	AY817969
	HfisPan10	CMC 2971	AY817838	AY817961
Sar: Mindanao, Sarangani Province				
	HfisSar1	CMC 3196	AY817847	AY817970
	HfisSar2	CMC 3197	AY817849	AY817972
	HfisSar3	CMC 3207	AY817850	AY817973
	HfisSar4	CMC 3208	AY817851	AY817974
	HfisSar5	CMC 3198	AY817852	AY817975
	HfisSar6	CMC 3201	AY817853	AY817976
	HfisSar7	CMC 3203	AY817854	AY817977
	HfisSar8	CMC 3213	AY817855	AY817978
	HfisSar9	CMC 3214	AY817856	AY817979
	HfisSar10	CMC 3215	AY817848	AY817971
Sib: Sibuyan				
	HfisSib1	FMNH 145616	AY817857	AY817980
	HfisSib2	FMNH 145617	AY817868	AY817991
	HfisSib3	FMNH 145618	AY817870	AY817993
	HfisSib4	FMNH 145619	AY817871	AY817994
	HfisSib5	FMNH 145621	AY817872	AY817995
	HfisSib6	FMNH 136977	AY817873	AY817996
	HfisSib7	FMNH 136978	AY817874	AY817997
	HfisSib8	FMNH 135660	AY817875	AY817998
	HfisSib9	FMNH 135661	AY817876	AY817999
	HfisSib10	FMNH 135662	AY817858	AY817981
	HfisSib11	FMNH 135663	AY817859	AY817982

APPENDIX *Continued*

Location code	Sample ID	Voucher	GB ND2	GB cyt b
	HfisSib12	FMNH 136979	AY817860	AY817983
	HfisSib13	FMNH 145614	AY817861	AY817984
	HfisSib14	FMNH 145615	AY817862	AY817985
	HfisSib15	FMNH 145622	AY817863	AY817986
	HfisSib16	FMNH 136975	AY817864	AY817987
	HfisSib17	FMNH 136976	AY817865	AY817988
	HfisSib18	FMNH 135657	AY817866	AY817989
	HfisSib19	FMNH 135658	AY817867	AY817990
	HfisSib20	FMNH 135659	AY817869	AY817992
Sur: Luzon, Camarines Sur Province, Mt. Isarog				
	HfisSur1	USNM 573656	AY817877	AY818000
	HfisSur2	USNM 573418	AY817878	AY818001
Zam: Luzon, Zambales Province				
	HfisZam1	CMC 2938	AY817879	AY818002
	HfisZam2	CMC 2878	AY817880	AY818003