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ABSTRACT

Marine species with ranges that span the Indo-Australian Archipelago (IAA) exhibit a range of phylogeographic patterns, most of which are interpreted in the context of vicariance between Indian and Pacific Ocean populations during Pliocene and Pleistocene low sea level stands. However, patterns often vary among ecologically similar taxa, sometimes even within genera. This study compares phylogeographic patterns in two species of highly dispersive Neritid gastropod, *Nerita albicilla* and *Nerita plicata*, with nearly sympatric ranges that span the Indo-Pacific. Mitochondrial COI sequences from > 1000 individuals from 97 sites reveal similar phylogenies in both species (two divergent clades differing by 3.2% and 2.3%, for *N. albicilla* and *N. plicata* respectively). However, despite ecological similarity and congeneric status, the two species exhibit phylogeographic discordance, with *N. albicilla* maintaining reciprocal monophyly of Indian and Pacific Ocean populations, while *N. plicata* is panmictic between oceans, but displays a genetic cline in the Central Pacific. Although this difference might be explained by qualitatively different demographic histories, parameter estimates from three coalescent models indicate that both species have high levels of gene flow between demes ($2N_e m > 75$), and share a common history of population expansion that is likely associated with cyclical flooding of continental shelves and island lagoons following low sea level stands. Results indicate that ecologically similar co-distributed species may respond very differently to shared environmental processes, suggesting that relatively minor differences in traits such as pelagic larval duration or microhabitat association may profoundly impact phylogeographic structure.
INTRODUCTION

Pliocene and Pleistocene glaciations have strongly influenced patterns of species and population level diversity on a global level. In the temperate zones, glacial cycles resulted in species range contractions into lower latitudes followed by range expansions during interglacial periods (Hewitt 1996; Hewitt 2000). In many terrestrial and freshwater species, these fluctuations resulted in a characteristic pattern of reduced genetic diversity within higher latitude populations and reciprocal monophyly or narrow zones of secondary contact among formerly allopatric populations (Avise 1992; Bernatchez & Wilson 1998; Hewitt 1996). Within this general pattern, qualitatively different demographic histories have led to a variety of distinct sub-patterns that are unique to geographic regions (reviewed in Avise 2000; Hewitt 2000).

In the ocean, many temperate marine species also experienced latitudinal range contractions as a result of Plio-Pleistocene glaciations (see Wares 2002 for review). However, for tropical marine species, one of the primary impacts was the effects of sea levels dropping to 130m below present during glacial maxima (Porter 1989; Voris 2000). These impacts were particularly strong in tropical areas like the Indo-Australian Archipelago (IAA), which are characterized by broad, shallow continental shelves that become exposed during low sea level stands. In the IAA Pleistocene sea level fluctuations closed the Torres, Sunda and Malacca straits more than ten times over the past million years (Pillans et al. 1998), and on six different occasions during the past 250,000 years alone, amounting to about half of this time period (Voris 2000).

Many marine species possess highly dispersive planktonic larvae (Thorson 1950) that should enhance genetic connectivity at large spatial scales (Lester & Ruttenberg 2005). However, the restrictions to the seaways of the IAA resulting from lowered sea levels combined with freshwater
discharge from large river drainages (Voris 2000) and cold-water upwelling (Fleminger 1986) may have resulted in allopatric barriers, promoting regional lineage diversification. Many pairs of geminate species with ranges that abut at the IAA (Randall 1998; Springer & Williams 1990), along with intraspecific phylogeographic studies (below), uniformly implicate Pleistocene vicariance in promoting regional divergence in taxa that span the IAA.

Marine species vary broadly in the nature and magnitude of their apparent genetic response to increased isolation across the IAA during low sea levels, but three qualitative patterns emerge (cf. categories I, II and IV of Avise 2000). First, despite contemporary sea levels and physical oceanography that should promote mixing between the Pacific and Indian Ocean, many taxa consist of two or more reciprocally monophyletic clades corresponding to these formerly allopatric ocean basins, with only a small amount of admixture due to recent gene flow or hybridization. Examples include butterflyfish of the Chaetodon punctatofasciatus and rhombochaetodon species complexes (McMillan & Palumbi 1995), black tiger prawns Penaeus monodon (Benzie et al. 2002; Duda & Palumbi 1999b), the urchin Diadema paucispinum (Lessios et al. 2001), stomatopods Gonodactylellus viridis, Haplosquilla pulchella, and H. glytocercus (Barber et al. 2006), seahorses Hippocampus kuda and H. trimaculatus (Lourie et al. 2005), and the gastropod Echinolittorina trochoides A (Reid et al. 2006). Although there is little concordance in the exact location of phylogeographic breaks and divergence dates range from the late Pleistocene to early Miocene (Barber & Bellwood 2005; Kirkendale & Meyer 2004; Lourie et al. 2005), most of these studies implicate vicariance during glacial cycles as a force in creating the observed patterns.

Second, many taxa retain two divergent clades that occur in sympatry. While the degree of admixture differs among species, differences in the relative frequencies of the two lineages usually
result in moderate genetic structuring between ocean basins. Proposed examples include the seastar *Linckia laevigata* (Williams & Benzie 1998), the snapping shrimp *Alpheus lottini* (Williams et al. 2002), the tasselfish *Polynemus sheridani* (Chenoweth & Hughes 2003), the barramundi *Lates calcarifer* (Chenoweth et al. 1998), and the pelagic scad *Decapterus russelli* (Perrin & Borsa 2001).

The depth of divergence among these clades suggests that they arose from historical allopatry, particularly due to Pleistocene sea-level fluctuations.

Finally, despite the profound effects of sea level fluctuations, some taxa show no phylogeographic evidence of historical isolation between ocean basins. Examples include the urchins *Eucidaris metularia* (Lessios et al. 1999), *Diadema savignyi* (Lessios et al. 2001) and *Tripneustes gratilla + depressus* (Lessios et al. 2003), the trumpetfish *Aulostomus chinensis* (Bowen et al. 2001), and the gastropod *Echinolittorina reticulata* (Reid et al. 2006). Regional genetic structure in these taxa is usually correspondingly low (but not always, see Lessios et al. 2003). The absence of phylogeographic structure in these taxa could result from continued dispersal through the IAA during low sea level stands as the waterways of the IAA were restricted, but not closed (Voris 2000).

Alternatively, lack of observed divergence could result if one of the two divergent lineages were lost due to local extinction or a selective sweep (Grant & Bowen 1998) or if the ranges of these species did not span the IAA during Plio-Pleistocene sea level fluctuations.

The diversity and complexity of phylogeographic patterns exhibited by species whose ranges presently span the IAA suggest that taxa respond differently to sea-level fluctuations at the IAA. Moreover, discordances among congeneric taxa (e.g. *Diadema spp.* and *Echinolittorina spp.* cited above) imply that potentially subtle taxon-specific variation in characteristics such as dispersal ability, biotic interactions or habitat restrictions along with historical differences in distribution, gene flow, or
effective population size may contribute to phylogeographic patterns across the IAA. It is difficult, however, to untangle the effects of taxon-specific traits from contingency in historical demography.

*Nerita albicilla* and *N. plicata* (Neritimorpha: Neritidae) are intertidal gastropods with long-lived planktotrophic larvae (Kano 2006) and largely sympatric ranges that span the IAA. While both species extend to the shores of East Africa in the west, *N. albicilla* reaches its eastern boundary at the Cook Islands, while *N. plicata* extends all the way to Easter Island. Pelagic larval duration of *Nerita* species may extend up to six months (Underwood 1975b); however, there are no precise estimates of pelagic larval duration for either *N. albicilla* or *N. plicata*. Nevertheless, the observed differences in range size between these species suggest that the larvae of *N. plicata* may spend a longer period in the plankton, since Indo-Pacific range sizes may be correlated with estimates of pelagic larval duration in both fish and gastropod taxa (Lester & Ruttenberg 2005; Paulay & Meyer 2006). The two species are broadly similar in their adult ecology, but differ subtly in their habitat associations. While *N. plicata* lives in the high intertidal, generally on any unbroken rocky substrate above the waterline, *N. albicilla* primarily inhabits rubble and cobble fields with low wave energy that are often associated with inner-reef flats (Hughes 1971; Vermeij 1971).

Given their similarities in adult ecology and broadly sympatric geographic distributions that span the IAA, we predict that these congeneric taxa should have been similarly impacted by the numerous Plio-Pleistocene sea level fluctuations that periodically increased isolation of Pacific and Indian Ocean populations. To test this hypothesis, we characterize phylogeographic patterns in these two congeners using cytochrome oxidase I (COI) mtDNA sequences comprehensively sampled from populations throughout the ranges of both species. We then reconstruct the demographic history of
each species using several different coalescent models to determine whether qualitative differences in
demographic history or species-specific traits may impact the observed phylogeographic patterns.

MATERIALS AND METHODS

Sampling and Sequencing

We collected *Nerita albicilla* (n=529, 60 localities) and *N. plicata* (n=653, 73 localities) from a
total of 97 localities throughout the Indo-Pacific region from 2000 until 2005 (Figure 1) and preserved
specimens in 95% ethanol or 20% DMSO.

DNA amplification and sequencing was conducted at Boston University and the University of
California, Davis. We amplified a 658-bp region of the mitochondrial cytochrome oxidase subunit-I
gene (COI) using primers HC0-2198 and LCO-1490 (Folmer et al. 1994). At Boston University, DNA
was extracted in a 10% Chelex™ (Biorad) solution (Walsh et al. 1991). PCR occurred in 25 µl
reactions with 2.5 µl of 10x buffer, 2 µl MgCl₂ (25 mM), 2.5 µl DNTPs (8 mM), 1.25 µl of each 10
mM primer, 1 µl of template, and 0.625 units of Amplitaq™ (Applied Biosystems Inc.). At UC Davis,
DNA was extracted with a Puregene™ kit (Gentra) and PCR-amplified in 50 µl volumes, with 5 µl of
10x buffer, 5 µl MgCl₂ (25 mM), 5 µl DNTPs (1 mM), 0.5 µl of Bovine Serum Albumin, 2.5 µl of each
10 mM primer, 2 µl of template, and 1 unit of Amplitaq. Thermocycling conditions were: initial
denaturation 94°C (15s), main cycle 94°C (30s), 50°C (30s) and 72°C (30-40s) for 35-39 cycles, then a
final extension of 72°C (3-10min).

Following ethidium-bromide visualization on a 1% agarose gel, we cleaned PCR products by
adding 0.5 units of Shrimp Alkaline Phosphatase (Biotech Pharmacon) and 5 units of Exonuclease I
(GE Healthcare) to 5µl of PCR product and incubating at 37°C for 30 minutes and 80°C for 15 minutes.
Sequencing reactions were performed for both forward and reverse strands using Big Dye™ (Applied Biosystems Inc.) terminator chemistry, and run on an ABI 377 or ABI 3730 (Applied Biosystems Inc.) automated DNA sequencer. Complementary strands for each sample were proofread and aligned in Sequencher™, and translations confirmed using MacClade 4.05 (Maddison & Maddison 2002).

Phylogeographic Analyses

We characterized phylogeographic patterns iteratively. First, we calculated pairwise \( \phi_{st} \) values among sampling localities with Arlequin 2.0 (Schneider et al. 2000), and tested significance of these values with 10,000 random replicates and applied a standard Bonferroni correction for multiple tests. To improve statistical power, we then pooled localities that showed no pairwise structure (i.e., those localities with a pairwise \( \phi_{st} \) that did not significantly differ from 0) into demes defined by country and region, and recalculated pairwise \( \phi_{st} \) as well as pairwise Wright’s \( F_{st} \). We excluded demes with fewer than five individuals from all population-level analyses due to lack of statistical power. Second, we used Analysis of Molecular Variance (AMOVA), implemented in Arlequin 2.0, to characterize regional patterns of genetic differentiation and to calculate haplotype and nucleotide diversity indices. Initially we defined \textit{a priori} regional groupings comprised of Pacific and Indian Ocean demes; however for \textit{N. plicata}, we also ran analyses with Rarotonga, Rangiroa, and Society Island localities in one group and all other localities in the other, corresponding to the observed phylogeographic structure. For \textit{N. albicilla}, we included Krakatau and Pulau Seribu with Pacific Ocean demes, due to their extremely high proportion of Pacific clade haplotypes. For \textit{N. plicata}, in which distinct mitochondrial clades were geographically mixed, we analyzed the data with highly divergent clades (those differing by more than 10 steps) included and excluded so that results were not driven by admixture.
Minimum-spanning trees were calculated in Arlequin based on pairwise sequence differences and hand-drawn with Adobe Illustrator™. Alternative connections were evaluated for any possible significant changes in topology. The frequencies of clades exceeding 10 steps divergence were summarized in pie diagrams and plotted onto a map of the Indo-Pacific region. For each tip clade that qualitatively resembled a star polytomy, we calculated the mean and variance of the mutational distance to the central haplotype as an unbiased indicator of the age of the clade relative to others, assuming the correct topology and a panmictic population (Saillard et al. 2000).

Coalescent Models of Demographic History

Phylogeographic analyses suggested strong departures from neutrality and gene flow-drift equilibrium, which can be caused either by selection or changes in effective population size. We addressed this issue with analyses using four different coalescent approaches. The first of these uses summary statistics to identify departures from neutrality, while the other three use Markov-Chain Monte Carlo (MCMC) methods to estimate demographic parameters of three different but complementary models.

We first tested the data against a neutral Wright-Fisher model using Fu’s $F_s$ (Fu 1997) which aims to identify an excess of recent substitution events caused by population growth, genetic hitchhiking, or background selection. To further evaluate the relative contributions of these processes to genetic patterns apparent in the data, we also calculated Fu and Li’s $D^*$ (1993), which considers the difference between two estimates of $\Theta$, one of which incorporates only substitutions on the external branches. Fu (1997) found that $F_s$ has more power to detect population growth and genetic hitchhiking than $D^*$, while the opposite is true for background selection. Thus, the two statistics can be used in
conjunction to evaluate the relative importance of background selection versus hitchhiking or population growth (Fu 1997). We performed all of these tests in DnaSP 4.10.7 (Rozas et al. 2003).

Significance of $F_s$ was determined by 1,000 coalescent simulations of the neutral model, while that of $D^*$ was determined by the critical values presented in Fu and Li (1993), where $p$ must be less than 0.02 to be significant due to the non-normal distribution of the statistic.

Second, because departures from neutrality are often caused by changes in effective population size, we used a Bayesian skyline plot to fit demographic models to the data from each species for comparative purposes. The Bayesian skyline plot (Drummond et al. 2005) is an extension of the generalized skyline plot (Strimmer & Pybus 2001), which constructs a model of demographic history based on how the number of coalescent events over a given interval differs from that expected under a neutral model for a panmictic population, where optimal intervals are chosen using an Akaike criterion.

While the generalized skyline plot uses a single genealogy, the Bayesian method, as implemented in BEAST v. 1.4.1 (Drummond 2003; Drummond et al. 2002) summarizes over all possible genealogies and provides confidence intervals for all parameters in the model. Because the model assumes a single panmictic population we analyzed the two clades of both species separately, using randomized subsets of 100 sequences for each clade. To check for convergence, we ran each subset twice for 50 million steps under an HKY+G model of mutation (chosen using hLRTs in Modeltest 3.7, Posada & Crandall 1998) with parameters estimated from the data, a constant skyline model with five groups, and uniform priors. We checked replicate runs from each subset against each other in the adjunct program, Tracer.

We combined the log files from both runs using LogCombiner to check effective sample size values, but report results from only one of the replicate runs due to computer memory constraints. We also
used BEAST to estimate the time to most recent common ancestor (T_{MRCA}) for each clade as well as for the combined dataset.

Third, because AMOVA analyses revealed genetic breaks between geographic regions in both species, we used the program IM to fit a coalescent model of isolation with migration (Hey & Nielsen 2004) to both of these breaks. This model allows us to estimate the coalescent parameter, \Theta, for two divergent populations and their common ancestor, as well as the migration rate between them, m, and the time, t, since they split. For *N. albicilla* the dataset included 200 randomly selected sequences composed of 100 sequences from each of the Indian and Pacific Ocean demes, as defined above. We created a similar dataset for *N. plicata* with sequences drawn from Rarotonga, the Society Islands and Rangiroa vs. the remaining Indo-Pacific. Following several initial runs of ~1 million steps, the maximum values for priors were set for *N. albicilla* as \Theta_{12} = 2500, \Theta_A = 500, t = 5, and m_{12} = 5 and for *N. plicata* as \Theta_{12} = 20,000, \Theta_A = 2000, t = 5, and m_{12} = 20. To check for convergence, the *N. albicilla* dataset was run three times for more than 30 million steps (and a burn-in of 150,000 steps), while the *N. plicata* dataset was run once with 20 million steps, and then 3 more times with a heated burn-in, and between 4-7 heated metropolis-coupled chains that were each run for between 3 and 7 million steps.

Fourth, to compare dispersal potential in both species, we estimated gene flow in the Pacific under a structured model of the coalescent available in the Bayesian implementation of LAMARC 2.0.2 (Kuhner 2006; Kuhner et al. 2005). Gene flow was estimated between demes on New Caledonia, Fiji, American Samoa and Rarotonga under a non-equilibrium island model (\Theta, m and growth rate (g) all constrained to be equal between demes). These demes are separated by an average of ~2000 km of deep water that requires long distance dispersal of larvae for successful gene flow to occur. We ran the
analyses under an F84 mutation model with the observed ti:tv ratios of 10.29 and 20.56, and gamma shape parameters of 1.26 and 1.51 for *N. albicilla* and *N. plicata* respectively, as determined by PAUP* 4.0b10 (Swofford 2002) on a neighbor-joining tree. Uniform, linear priors for migration and growth rate were from -500 to 4000 and 1 to 2000 respectively, and a logarithmic prior for \( \Theta \) ranged from 0.001 to 100. We ran three replicate runs for each species, one of four million steps and two of two million steps each, with a sampling increment of 20, and 200,000 steps discarded for the burn-in. Because we found a sharp cline in clade frequency in this region for *N. plicata*, we also ran 3 replicate runs on a dataset with clade B removed. Ancestral \( \Theta \) was calculated using the formula \( \Theta_A = \Theta e^{-\mu t} \) where \( t \) is any time in the past divided by a three-year generation time. We chose \( t \) equal to the mean T_MRCA estimated by BEAST for clade B in *N. albicilla* and clade A in *N. plicata* (Table 6, Figure 4). Because the latter three models give parameters that are scaled by a mutation rate, we converted the estimates to demographic units assuming a generation time of 3 years (Underwood 1975b, Y. Kano, pers. comm.) and a per-site lineage mutation rate of 0.5% per million years to make all estimates comparable to each other and intuitive with respect to the history of the region. This lineage mutation rate is equal to a 1%/myr divergence rate, which falls in the middle of a range of previously calculated fossil-calibrated divergence rates based on Molluscan COI (Marko 2002, 0.7% - 1.2% per million years).

**Results**

We sequenced a total of 658 bp of mitochondrial COI from 653 individual *N. plicata* and 529 *N. albicilla*, yielding 467 and 353 unique haplotypes, respectively (Genbank Accession # XXXX-XXXX). All sequences aligned easily and translated without stop codons. Six different non-
synonymous substitutions occurred in single individuals in *N. plicata*, whereas all substitutions were synonymous in *N. albicilla*.

Haplotype diversity was extremely high in all demes for both species (h > 0.94 in *N. albicilla* and h > 0.99 in *N. plicata*). Nucleotide diversity ranged from 0.006 to 0.016 in *N. albicilla* and 0.009 to 0.023 in *N. plicata* (Tables 1 and 2). In *N. albicilla*, 49 (13.9%) haplotypes were shared between more than one individual, with 40 shared among demes and nine private to a single deme. Similarly, in *N. plicata* 76 (16.2%) haplotypes occurred multiple times, with 64 shared with at least one other deme, and the remainder being private.

Phylogeographic Analyses

Minimum-spanning trees (MSTs) for both species exhibit similar phylogenetic patterns, but differ markedly in their phylogeographic structure (Figures 2a, 3a). Both trees have two clades (A and B) separated by a large genetic break of 21 steps (3.2% sequence divergence, 4.1% average divergence, 3.4% net divergence) for *N. albicilla* and 15 steps (2.3% sequence divergence, 3.4% average divergence, 2.6% net divergence) for *N. plicata*. In *N. albicilla* the two clades largely correspond to the Indian Ocean (clade A) and Pacific Ocean (clade B), with minor mixing occurring in Singapore, Krakatau, Pulau Seribu, New Caledonia, and Rarotonga (Figure 2b). Additionally, a single divergent individual from South Africa fell out 18 steps from the Pacific clade. This individual is monophyletic with *Nerita albicilla* when placed on a larger phylogeny of *Nerita* (M. Frey, unpublished data), but was removed from coalescent analyses. In contrast to the regional concordance in *N. albicilla*, both clades of *N. plicata* are distributed across the entire range of the species (Figure 3b). However, clade B is much less frequent than clade A, representing 10% of total haplotypes, and has a proportionally larger
representation of Central Pacific haplotypes (63% come from the Society Islands, Rarotonga, Rangiroa, Marquesas, American Samoa, and Fiji).

The general topology of both MSTs consists of multiple “star” polytomies, with central high frequency haplotypes separated by one or two base differences from multiple singleton haplotypes, that lack discernible geographic clustering. For example, in *N. plicata* the most common haplotype appeared 28 times in 17 out of 27 demes, from South Africa to Easter Island. Of the rarer haplotypes, three co-occur in the Marquesas and South Africa, two appear in the Society Islands and Tanzania, and two are shared between both Easter Island and Kenyan samples. In *N. albicilla* there is a similar lack of geographic association for haplotypes within both the Indian and Pacific Ocean clades, but only one haplotype is shared between oceans. The mean mutational distance to the central haplotype in star-like tip clades ranged from 0.58 to 1.83 in *N. albicilla*, corresponding to 177,000 to 557,000 years before present. In *N. plicata* this distance ranged from 1.08 to 2.08, or 330,000 to 633,000 years before present.

AMOVA showed strong genetic structure between regions dominated by each clade, and weak or nonexistent structure within regions (Table 3). In *N. albicilla*, the partition between the Indian and Pacific Oceans explains 77% of observed nucleotide variation (*f*<sub>st</sub> = 0.77, *p* < 0.0001) and structure within regions was small but significant (*f*<sub>sc</sub> = 0.011, *p* < 0.0001). In contrast, *N. plicata* showed no evidence of a split between the Indian and Pacific Oceans (*f*<sub>st</sub> = 0.010, *p* = 0.15). However, the partition between the Society Islands, Rarotonga, Rangiroa, and the rest of the Indo-Pacific explained 20.7% of the variance (*f*<sub>st</sub> = 0.207; *p* < 0.00066); the remaining variance was distributed within demes and there was no detectable intraregional structure (*f*<sub>sc</sub> = 0.0; *p* = 0). When clade B was removed from the analysis, *f*<sub>st</sub> was not significant across the entire range of *N. plicata*. After Bonferroni correction,
the only significant pairwise $\phi_{st}$ or $F_{st}$ values in either species were between demes on either side of the breaks identified by AMOVA, with one exception: a pairwise $F_{st}$ of 0.005 between American Samoa and Queensland found in *N. plicata*.

Coalescent Models of Demographic History

The two tests of neutrality yielded contrasting results. Fu’s $F_s$ rejected neutrality ($p < 0.05$) for all demes in both species with $n > 10$. However, Fu and Li’s $D^*$ was significant ($p < 0.02$) for only 10 out of 23 regional demes tested in *N. plicata* and 7 out of 26 demes tested in *N. albicilla* (Tables 1 and 2). Both tests were significant when samples were grouped by ocean basin.

Bayesian skyline plots generated from BEAST reveal concurrent histories of exponential population growth between clades in both species (Figure 4). Based on a conservative heuristic mutation rate of 0.5%/my, growth commenced at approximately 500,000 years before the present in *N. albicilla* and 650,000 years before the present in *N. plicata*. These dates correspond to the earliest dates inferred from mutational depths of the tip clades in each species. Figure 4 shows estimates of current effective population size of each clade, with 95% confidence intervals. Ancestral population size was estimated from the last interval of the skyline plot and is presented as the sum of the population sizes from both clades, since this interval falls within the confidence interval for the $T_{MRCA}$ of each clade (Table 4). Replicate runs from two random subsets of each clade produced highly similar parameter estimates in all cases, and all combined effective sample sizes were greater than 200.

Parameter estimates for an isolation with migration (IM) model for *N. albicilla* (Table 5) were equal or very similar across all three replicates. All effective sample size values were greater than 100 and plots of parameter trends indicated sufficient mixing among chains. Both descendant populations
(Pacific and Indian) have effective population sizes more than an order of magnitude larger than the ancestral population. The heuristic estimate for $t$, the time of population splitting, is 634,000 years ago (95% CI 530,000 – 798,000 years before present), corresponding to a time just prior to the period of population expansion estimated by BEAST for *N. albicilla*, and approximately the same time as the population expansion in *N. plicata*. Estimates of gene flow are low ($2N_e m = 0.79$ into the Indian Ocean and 0.36 into the Pacific); and, since the tails of the posterior distributions were not complete, these estimates are not significantly greater than 0 (Nielsen & Wakeley 2001). The estimates for current and ancestral effective population size are generally lower than those produced by BEAST, but fall within BEAST’s 95% confidence intervals (Figure 4).

The IM model did not similarly converge for the genetic break in the Central Pacific in *N. plicata*. For example, replicate estimates for effective population size for the Central Pacific island demes ranged from 29 million to 700 million and population migration rates ($2N_e m$) ranged from 350 to 17,000. None of the effective sample size values rose above 50, and parameter plots showed multiple peaks – further evidence that the chains were not mixing. Three different two-step heating schemes did not improve on this result. The only parameter that did not vary across runs was the $T_{MRCA}$, which remained stable at about 3.5 million years ago (95% CI 2.0 – 4.5 million years before present).

Results from the non-equilibrium island model of the structured coalescent examined in LAMARC indicate that while *N. albicilla* has a higher migration rate ($m/\mu$) than *N. plicata* in the Central Pacific, it also had a smaller $\Theta$, making the population migration rate ($2N_e m$) about equal in the two species (Table 6). *N. albicilla* had a faster growth rate ($g$) than *N. plicata*, which is consistent with the Bayesian skyline plots. Estimates of current and ancestral $N_e$ are in rough agreement with what was found by BEAST and IM (Figure 4). A similar analysis that was run on an *N. plicata* dataset with clade
B removed gave the same the same values for $2N_e m$, although migration rate was slightly higher, and
$\Theta$ was slightly lower (Table 6). Multiple runs converged on the same posterior distributions.

**DISCUSSION**

Despite having similar ecologies and broadly sympatric distributions spanning the Indian and Pacific Oceans, the congeneric taxa *Nerita albicilla* and *N. plicata* have responded uniquely to the dynamic environments of the IAA. Although both species have two reciprocally monophyletic mtDNA lineages and experienced demographic expansions that date broadly to the Pleistocene, their phylogeographic patterns differ sharply. In *N. albicilla* the two lineages have allopatric distributions in the Pacific and Indian Oceans, corresponding to a classic pattern of Indo-Pacific vicariance reported in many marine species (Barber *et al.* 2000; Duda & Palumbi 1999b; Lavery *et al.* 1996). In contrast, *N. plicata* has a genetic break between demes in the Central Pacific and Pacific/Indian Ocean populations lying to the west.

The disparity in phylogeographic patterns contrasts with the strong similarity in phylogenetic and demographic patterns. In previous marine studies at the IAA and elsewhere, such disparities between congeners have been attributed to ecological differences that allowed qualitatively different histories in which one species was able to use refugial habitat that was unavailable to others (Hickerson & Cunningham 2005; Marko 2004; Reid *et al.* 2006). While the similarity of intraspecific phylogenies and historical demography suggests similar evolutionary histories in both species, subtle differences in ecology or dispersal ability may still have played a role in creating phylogeographic discordance.

A Shared History of Population Expansion
The minimum spanning trees recover two clades of similar divergence in both species (Figures 2 and 3). Both trees have multiple star-like radiations that strongly suggest non-equilibrium dynamics (Grosberg & Cunningham 2001). This latter result is supported by the strongly negative regional $F_s$ and $D^*$ values (Tables 1 and 2) that could indicate a demographic expansion, selective sweep, or background selection. Because $D^*$ was not significant within many demes, and because it is more sensitive to background selection than $F_s$, background selection is unlikely to fully explain the lack of neutrality in these demes. It is also possible that positive selection at a linked mitochondrial locus could have produced an advantageous variant (e.g. Rawson & Burton 2006) which then swept to fixation (Gillespie 2001). However, such an event would diminish haplotype diversity, leaving a single central haplotype rather than the high diversity and multiple stars and nearly simultaneous expansions observed in both species. It seems unlikely that two taxa would experience contemporaneous selective sweeps in two separate ocean basins.

A perhaps more parsimonious alternative explanation of non-equilibrium dynamics comes from three separate coalescent models that strongly suggest exponential population expansion in both species. Although the demographic estimates may be upwardly biased with respect to absolute time and effective population size (due to the conservative mutation rate used in the conversion; Ho et al. 2005), Bayesian skyline plots suggest a broadly contemporaneous demographic expansion that occurred just after the population split reckoned by IM for *N. albicilla* (Figure 4). This result, combined with the relative agreement across the different coalescent models employed in BEAST, IM and LAMARC implies a shared history of demographic expansion during the Pleistocene in both species.
Demographic expansion in these two species is expected given the geologic history of the Indo-
Pacific region. During low sea level stands, the Sunda Shelf was frequently terrestrial (Voris 2000), as
were the lagoons of many oceanic islands (Ladd 1960; Paulay 1990). This loss of habitat would have
caused cyclical local extinction of many demes, followed by re-colonization as sea levels rose. The
cyclical nature of range expansion and contraction caused by sea level change is hinted at by an older
population expansion detected in the skyline plot of *N. plicata* clade A (Figure 4), as well as the
differing average mutational depths of the star polytomies in all four clades (Figures 2a and 3a). The
multiple star polytomies in the minimum spanning trees may have been formed across a chronological
sequence of range contractions followed by expansion events that stochastically favored only a few
haplotypes (and form the characteristic star-shape; Slatkin & Hudson 1991) during any given event.
Similar patterns in other Pacific species have been attributed to range expansions into the Central
Pacific islands (Thacker 2004; Thompson *et al.* 2005) and the IAA (Klanten *et al.* 2007).

Population Divergence and the Maintenance of Reciprocal Monophyly in *N. albicilla*

A distinct genetic break between Indian and Pacific Ocean clades (clades A and B, respectively)
occurs along the west coast of the Malay Peninsula in *N. albicilla*. This roughly matches the location
of strong genetic breaks exhibited by a number of other reciprocally monophyletic taxa (Benzie 1999b;
Duke *et al.* 1998; Reid *et al.* 2006; Williams & Benzie 1998), and corresponds broadly to the periodic
barrier between the Indian and Pacific oceans at the Malacca strait (Figure 1).

A neutral estimate of divergence time based on the net divergence between clades \( d_X \); Nei & Li
1979) and a divergence rate of 1%/myr would result in a population divergence time estimate, \( t \), of 3.4
million years. However, this equilibrium estimate is likely inflated because of the large ancestral
effective population size ($N_e \approx 500,000$) that would harbor a substantial amount of genetic polymorphism that would only sort once populations became allopatric (Arbogast et al. 2002; Edwards & Beerli 2000). Even the more recent estimate of $t \approx 634,000$ years obtained in IM may be inflated given that it comes from a single locus that is almost reciprocally monophyletic (Arbogast et al. 2002; Carstens & Knowles 2007). Nevertheless, even with a 95% confidence interval of about 250,000 years and using the slowest rate published for molluscan CO1 (Marko 2002), this estimate still falls squarely in the Pleistocene, and immediately precedes exponential population expansion calculated independently by BEAST. Since many marine species in the Indo-Pacific may have large $N_e$, failure to take ancestral $N_e$ and population growth into account could lead to large overestimates of divergence time (Edwards & Beerli 2000) such as the fivefold error calculated for net divergence above.

The persistence of regionally concordant, nearly reciprocally monophyletic clades in *N. albicilla* could occur if larval dispersal and gene flow among regions is rare ($2N_em \leq 1$), such that gene flow is swamped by the standing populations (Cunningham & Collins 1998). However, high estimates of gene flow from LAMARC ($2N_em > 90$) across thousands of kilometers of ocean where intermediate habitats don’t exist suggest that dispersal and gene flow are not intrinsically limited. These high estimates of gene flow from elsewhere suggest that while larval dispersal between oceans may be common, successful gene flow is rare due to pre or post-zygotic barriers between the two clades (Benzie 1999a; Edmands & Burton 1999), or due to Allee effects. While the number of effective female migrants per generation ($2N_em$) estimated by LAMARC is high in evolutionary terms, it is a tiny proportion ($m$) of the total effective population size. Thus, if reproductive barriers do exist between clades, then migrants from one clade that cross to the other ocean are very unlikely to encounter members of their own clade and reproduce successfully, simply due to an Allee effect.
Waters et al. (2005) came to a similar conclusion for a temperate congener, *Nerita atramentosa*, that shows reciprocally monophyletic clades corresponding to two different opercular colors across a historical barrier at Wilson’s Promontory in Australia. They examined >7000 snails within a few hundred km of the former barrier, using opercular color as a proxy for mitochondrial clade, and found a cline with the frequency of each opposite clade decreasing with distance on each side of Wilson’s Promontory. In the present study we observed a similar pattern, with a low frequency of clade A haplotypes in demes that are dominated by clade B, especially along the boundary of the Indian and Pacific Oceans. Given the high gene flow estimates obtained across large distances of open ocean, post-dispersal processes may provide a better explanation for the maintenance of the phylogeographic break than rare dispersal.

The origin of genetic structure in *Nerita plicata*

Significant $\phi_{ct}$ values separated Rarotonga, the Society Islands and Rangiroa from demes to the west. However, no structure was detected within individual clades (Table 3). This absence of detectable genetic structure ($\phi_{st}$ and $\phi_{ct}$) across more than 22,000 km of ocean (from South Africa to the Marquesas) is surprising, even for a species with high dispersal potential such as *N. plicata*. This result suggests that the regional structure above likely results from admixture of clades A and B and not from restricted gene flow and population divergence in the Central Pacific. This hypothesis is supported by the failure of IM to converge for this dataset, as well as high estimates of gene flow in the Western and Central Pacific made by LAMARC for datasets that both included and excluded clade B (Table 6).

There are several hypotheses that could explain the pattern of deep divergence in *Nerita plicata*. First, the two lineages may be cryptic species. However, since both clades occur sympatriically
throughout the entire Indo-Pacific region, the hypothesis of cryptic sister species seems unlikely in the absence of any evidence for ecological speciation. Without nuclear markers to delineate species boundaries, this hypothesis cannot yet be falsified.

A second possibility is that the two lineages arose sympatrically and stochastically, and represent the two deepest coalescing branches in a panmictic population with large $N_e$ (Donnelly & Tavare 1995). The persistence of multiple divergent clades is very likely given the large effective population sizes and high migration rates estimated under the coalescent models. However, the clear phylogeographic pattern with higher frequencies of clade B in the Central Pacific is unlikely to evolve stochastically under panmixia, but would instead require either significant limits to gene flow or allopatric divergence across the waters of the Central Pacific that would allow differential lineage sorting in the two geographic regions.

A third possibility, therefore, is that the two lineages of *N. plicata* sorted in allopatry in the Central Pacific where the genetic break presently occurs. Phylogeographic divergence has been observed across this region, but only in species characterized by high genetic structure that show even deeper divergence at the IAA (Bernardi *et al.* 2001; McCafferty *et al.* 2002). In contrast, *N. plicata* appears to be panmictic across 22,000 km, but only shows a genetic break in the Central Pacific. There were no major changes in the geostrophic flow of the South Equatorial Current during the last glacial maximum (Thunell *et al.* 1994) that would have separated or reunited lineages spanning this putative barrier. The absence of a clear physical filter to dispersal combined with relatively high coalescent estimates of gene flow in the region (Table 6) argue against allopatric divergence in the Central Pacific. However, it is still possible that the divergence has allopatric origins, perhaps in another geographic location.
In the absence of a plausible dispersal barrier in the Central Pacific, there are limited possibilities for the location of a putative allopatric dispersal barrier. Divergence across the Eastern Pacific Barrier or in Hawaii is possible, but unlikely, given that our small sample from Easter Island reflects the same clade B frequencies (~25%) as in other Central Pacific demes, and *N. plicata* is absent from South America and rare in Hawaii (S. Godwin, pers. comm.). The most logical location for allopatric divergence is at the Indo-Australian Archipelago. The phylogeographic and demographic patterns in *N. plicata* could be explained by historical allopatry at the IAA, followed by the rapid expansion of clade A into the Pacific Ocean (perhaps mediated by selection). While this hypothesis is the most consistent with the remarkably similar phylogenetic and demographic histories inferred for both clades in both *Nerita* species, it is also presently untestable. Statistical methods for establishing concordance in comparative phylogeography are in their infancy (e.g. Hickerson *et al.* 2006). The development of new spatially explicit models linking genetics and dispersal in marine environments (e.g. Galindo *et al.* 2006) may provide a framework for exploring the potential outcomes of vicariance followed by range expansion.

Reconciling Phylogeography with Demographic History

*Nerita albicilla* and *N. plicata* share a history of demographic expansion and high levels of gene flow, yet show markedly discordant phylogeographic patterns across the same geographic regions. *N. plicata* has maintained or re-established gene flow across the IAA, while *N. albicilla* has not. Although the ultimate explanation for this pattern remains to be resolved, species-specific differences in pelagic larval duration could contribute to the observed patterns. Although LAMARC revealed no interspecific differences in gene flow across large pelagic distances, *N. plicata*’s larger range size indicates that it
may have a more leptokurtic dispersal kernel (Kinlan et al. 2005; Paulay & Meyer 2006). Thus, *N. plicata* may have sustained gene flow across the IAA during periods of lowered sea level, or re-established gene flow more quickly than *N. albicilla* once sea levels rose again.

Alternatively, the phylogeographic differences may stem from subtle, species-specific disparities in habitat requirements that have large impacts on genetic structure. Paulay (1990) found that uplifted islands such as Niue lack bivalve taxa that require inner-reef habitats. These islands approximate the condition during glacial cycles when lowered sea levels would have stranded reef flats, destroying prime habitat for *N. albicilla*. In contrast, the steep reef slope exposed during sea level regressions could have continued to support *N. plicata*, allowing it to maintain intermediate stepping-stone populations and thus higher levels of gene flow. This would also explain the faster rate of population growth detected by both BEAST and LAMARC for *N. albicilla*, as this species would have gained more habitat area as sea levels rose again. The hypothesis that pre-existing differences in habitat requirements explain differences in response to vicariance at the IAA is similar to that of Reid et al. (2006), who ascribed different congeneric responses to species with “oceanic” and “continental” habitat requirements. Dissimilar responses to climate change within a genus have also been attributed to habitat specificity in other tropical marine species (Bird et al. 2007; Fauvelot et al. 2003; Thacker 2004), as well as temperate marine (Hickerson & Cunningham 2005; Marko 2004) and terrestrial habitats (Hewitt 2000). Indeed, habitat specificity may be as important as the physical dispersal of larvae in contributing to contemporary genetic patterns.

Conclusions
Despite their different purposes and assumptions, three coalescent models of demography yielded remarkably similar estimates of growth from a smaller ancestral $N_e$ to an exponentially larger $N_e$ for both species (Figure 4; Tables 4-6). Although methods for demonstrating statistical concordance between species and among models are still being developed (e.g. Hickerson et al. 2006), the range expansion inferred here for both species lies well within the climatic oscillations of the Pleistocene, even when using a relatively slow molecular clock (Figure 4). While the effects of specific glaciations cannot be detected with the current dataset, both species have likely shared a history of recurring population fragmentation and expansion due to sea level fluctuations.

Despite this similar demographic history, the discordant patterns of phylogeography in the two Nerita species indicate that relatively closely related taxa can respond very differently to the cyclical fluctuations in sea level at the IAA. Subtle differences in dispersal ability, and/or habitat requirements may allow higher rates of gene flow and thus maintenance or resumption of genetic connectivity. Our analyses, in addition to similar studies in this region, suggest that the IAA may represent an unusually large suture zone (Benzie 1999a; Hewitt 2000; Remington 1968), in which the genetic signature of past demography is retained by large effective population sizes and the boundaries between highly divergent Indian and Pacific Ocean clades are defined by the rate at which they expand into newly submerged habitat following sea level rise, and their degree of admixture. Additional nuclear and mitochondrial genetic data from Indo-Pacific species will be instrumental in understanding the impacts of Pleistocene vicariance in shaping phylogeographic and demographic patterns of species spanning this dynamic region.

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18 nanum* (Lamarck) and *Cellana tramoserica* (Sowerby) (Gastropods: Prosobranchia) in S.E. 


Author Information:

Eric Crandall is a Ph.D. Candidate at BU. His dissertation research uses comparative phylogeography to address questions about the ecology and evolution of marine species in the Indo-Pacific. Melissa Frey, a Ph.D. Candidate at UC Davis, studies the evolutionary and historical processes that influence diversification in marine organisms. Research in the Grosberg lab emphasizes the interplay among life histories, genetic structure, biogeography, and evolutionary diversification of behavioral, physiological, and other adaptively significant traits, mostly in freshwater and marine invertebrates. Paul Barber’s lab focuses on understanding processes generating biodiversity in marine environments and using this information to guide conservation policy.
Figure 1. Map of the Indo-Pacific showing localities sampled. Dark grey shading delineates the approximate 100m continental depth contour for continental regions (Smith & Sandwell 1997; Voris 2000). Sea level was at or below this depth for ~25% of the last 250 kya, closing most of the major seaways between the Indian and Pacific Oceans. Black circles mark localities where only *N. albicilla* was sampled, white circles mark localities where only *N. plicata* was sampled and grey circles mark localities where both were sampled. Sampling of only one species does not necessarily mean that the other species was absent from this locality.

Figure 2. Phylogeography of *N. albicilla*: (a) Unrooted minimum-spanning tree showing the relationships between 467 unique CO1 haplotypes. White haplotypes were found in Indian Ocean localities and grey haplotypes were found in Pacific Ocean localities. Circles are sized proportionally to the frequency of occurrence, ranging from 1 to 30. All haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks or number. For tip clades, the mean mutational distance to the central haplotype is given with a standard deviation. (b) Map of the Indo-Pacific with pie diagrams showing the relative frequency of each clade at each deme listed in Table 1. Clade A haplotypes are colored white and Clade B haplotypes are grey. The size of each pie diagram is proportional to the number of individuals sampled from each deme, ranging from 2 in Madagascar to 65 in Tanzania.
Figure 3. Phylogeography of *N. plicata*: (a) Unrooted minimum-spanning tree showing the relationships between 353 unique CO1 haplotypes. White haplotypes were found in Indian Ocean localities and grey haplotypes were found in Pacific Ocean localities, except for black haplotypes which highlight haplotypes found in Rarotonga, Rangiroa, the Society Islands and Easter Island. Circles are sized proportionally to the frequency of occurrence, ranging from 1 to 28. All haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks or a number. For tip clades, the mean mutational distance to the central haplotype is given with a standard deviation. (b) Map of the Indo-Pacific with pie diagrams showing the relative frequency of each clade at each deme listed in Table 2. Clade A haplotypes are colored white and Clade B haplotypes are grey. The size of each pie diagram is proportional to the number of individuals sampled from each deme, ranging from 2 in Madagascar and elsewhere to 59 in Tanzania. Six non-synonymous mutations are marked with shaded rectangles.

Figure 4. A comparison of effective population size (*N_e*) and time estimates made under the three coalescent models of demography employed in this paper. Estimates have been converted from mutational units using a heuristic divergence rate of 1% per million years, and a generation time of three years (see Materials and Methods). Solid lines depict results from *N. albicilla* while dotted lines depict results from *N. plicata*. Curves represent Bayesian skyline plots for *N_e* running from the present to their mean Time to Most Recent Common Ancestor (*T_{MRCA})*, with 95% confidence intervals plotted at both time points. The shaded box in the center of the figure represents 95% confidence intervals (C.I.) for the time of population splitting estimated for Pacific and Indian populations of *N. albicilla*. Shaded and open ovals represent 95% C.I. for ancestral and current *N_e* respectively, estimated for four
Pacific demes in both species by LAMARC. Boxes represent 95% C.I. for $N_e$ estimated by IM for $N.$ 

*albicilla* only, with the Pacific population in white, the Indian population in black and the ancestral population in grey.
Table 1. Summary statistics and neutrality test statistics for *Nerita albicilla*. Haplotype diversity (h) and nucleotide diversity (π) calculated in Arlequin (Schneider *et al.* 2000). F_s and D*, measure departure of the data from neutrality (Fu, 1997) and were calculated in DNAsp (Rozas *et al.* 2003). Indian Ocean demes are in bold type.

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>n</th>
<th>h</th>
<th>π</th>
<th>F_s</th>
<th>D*</th>
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<td>0.989</td>
<td>0.021</td>
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Table 2. Summary statistics and neutrality test statistics for *Nerita plicata*. Haplotype diversity (h) and nucleotide diversity (π) calculated in Arlequin (Schneider *et al.* 2000). F<sub>s</sub> and D* measure departure of the data from neutrality (Fu 1997) and were calculated in DNAsp (Rozas *et al.* 2003). Indian Ocean demes are in bold type.

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<th>π</th>
<th>Clade B %</th>
<th>F&lt;sub&gt;s&lt;/sub&gt;</th>
<th>D*</th>
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<td>25.00%</td>
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<td>10.00%</td>
<td>-34.83*</td>
<td>-2.24 (ns)</td>
</tr>
<tr>
<td>Guam</td>
<td>25</td>
<td>0.99</td>
<td>0.020</td>
<td>4.00%</td>
<td>-12.27*</td>
<td>-3.03*</td>
</tr>
<tr>
<td>Indonesia - West Papua</td>
<td>19</td>
<td>0.99</td>
<td>0.012</td>
<td>5.26%</td>
<td>-7.98*</td>
<td>-2.43*</td>
</tr>
<tr>
<td>Indonesia - Krakatau</td>
<td>17</td>
<td>0.99</td>
<td>0.015</td>
<td>5.88%</td>
<td>-6.88*</td>
<td>-2.47*</td>
</tr>
<tr>
<td>Indonesia - North Sulawesi</td>
<td>16</td>
<td>1</td>
<td>0.011</td>
<td>0.00%</td>
<td>-10.45*</td>
<td>-2.15 (ns)</td>
</tr>
<tr>
<td>Indonesia - Pulau Seribu</td>
<td>19</td>
<td>1</td>
<td>0.013</td>
<td>0.00%</td>
<td>-12.56*</td>
<td>-2.07 (ns)</td>
</tr>
<tr>
<td>Japan - Okinawa</td>
<td>30</td>
<td>1</td>
<td>0.010</td>
<td>0.00%</td>
<td>-31.19*</td>
<td>-3.79*</td>
</tr>
<tr>
<td>Kenya</td>
<td>50</td>
<td>0.99</td>
<td>0.011</td>
<td>2.00%</td>
<td>-33.80*</td>
<td>-3.77*</td>
</tr>
<tr>
<td>Madagascar</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>0.00%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malaysia - Borneo</td>
<td>10</td>
<td>1</td>
<td>0.017</td>
<td>10.00%</td>
<td>-3.16*</td>
<td>-1.50 (ns)</td>
</tr>
<tr>
<td>Marquesas – Nuku Hiva</td>
<td>20</td>
<td>0.99</td>
<td>0.017</td>
<td>15.00%</td>
<td>-8.61*</td>
<td>0.81 (ns)</td>
</tr>
<tr>
<td>Marshall Islands - Bikini</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>0.00%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mauritius</td>
<td>6</td>
<td>1</td>
<td>0.009</td>
<td>0.00%</td>
<td>-1.91(ns)</td>
<td>-0.87 (ns)</td>
</tr>
<tr>
<td>New Caledonia</td>
<td>40</td>
<td>0.99</td>
<td>0.012</td>
<td>2.50%</td>
<td>-33.51*</td>
<td>-2.76965*</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>27</td>
<td>1</td>
<td>0.015</td>
<td>7.41%</td>
<td>-32.60*</td>
<td>-2.06 (ns)</td>
</tr>
<tr>
<td>Phillipines</td>
<td>32</td>
<td>0.99</td>
<td>0.013</td>
<td>6.25%</td>
<td>-20.96*</td>
<td>-1.68 (ns)</td>
</tr>
<tr>
<td>Rangiroa</td>
<td>18</td>
<td>0.99</td>
<td>0.022</td>
<td>44.44%</td>
<td>-23.17*</td>
<td>-0.43 (ns)</td>
</tr>
<tr>
<td>Rarotonga</td>
<td>40</td>
<td>0.99</td>
<td>0.022</td>
<td>35.00%</td>
<td>-5.55*</td>
<td>-2.24 (ns)</td>
</tr>
<tr>
<td>Reunion</td>
<td>8</td>
<td>1</td>
<td>0.010</td>
<td>0.00%</td>
<td>-18.85*</td>
<td>-1.37 (ns)</td>
</tr>
<tr>
<td>Society Islands</td>
<td>48</td>
<td>0.99</td>
<td>0.023</td>
<td>43.75%</td>
<td>-3.21*</td>
<td>-2.53*</td>
</tr>
<tr>
<td>South Africa</td>
<td>40</td>
<td>0.99</td>
<td>0.012</td>
<td>0.00%</td>
<td>-20.56*</td>
<td>-3.11*</td>
</tr>
<tr>
<td>Tanzania</td>
<td>59</td>
<td>0.99</td>
<td>0.012</td>
<td>3.39%</td>
<td>-28.12*</td>
<td>-2.68*</td>
</tr>
<tr>
<td>Micronesia - Truk</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>0.00%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Clade A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clade A</td>
<td>586</td>
<td>0.995</td>
<td>0.010</td>
<td>0.00%</td>
<td>-33.75*</td>
<td>-3.09*</td>
</tr>
<tr>
<td><strong>Clade B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clade B</td>
<td>67</td>
<td>0.979</td>
<td>0.007</td>
<td>100.00%</td>
<td>-47.34*</td>
<td>-3.11*</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td>653</td>
<td>0.995</td>
<td>0.01396</td>
<td>10.26%</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. AMOVA results for both species. *N. plicata* analyses were performed for each clade individually as well as combined. Significant values (*) indicate a p < 0.0001 after 10,000 random permutations of the data.

<table>
<thead>
<tr>
<th>Definition of Regions</th>
<th>Indian Ocean vs. Pacific Ocean</th>
<th>Societies, Rangiroa, Rarotonga vs. All other demes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall $\phi_{ct}$ (between regions)</td>
<td>0.772*</td>
<td>0.205*</td>
</tr>
<tr>
<td>Overall $\phi_{sc}$ (within regions)</td>
<td>0.011*</td>
<td>0.0 (ns)</td>
</tr>
</tbody>
</table>

% Variation:
- Among Regions: 77.03% 20.68% -0.17% 0.84%
- Among demes within regions: 0.26% 0% 0.24% 4.62%
- Within demes: 22.72% 79.32% 99.93% 94.54%
Table 4. Mean and 95% Highest Posterior Density Intervals for effective population size and time to most recent common ancestor for a panmictic population modeled in BEAST. $T_{\text{MRCA}}$ was calculated for each clade individually, and for the entire mitochondrial dataset in each species. Time estimates are scaled by the reciprocal of the per-site mutation rate $1/\mu$.

<table>
<thead>
<tr>
<th></th>
<th>Nerita albicilla</th>
<th>Nerita plicata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Theta$ (per site)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clade A</td>
<td>Clade B</td>
</tr>
<tr>
<td>Mean</td>
<td>0.459</td>
<td>0.499</td>
</tr>
<tr>
<td>95% Low</td>
<td>0.113</td>
<td>0.102</td>
</tr>
<tr>
<td>95% High</td>
<td>1.581</td>
<td>1.460</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Time in years to Most Recent Common Ancestor ($T_{\text{MRCA}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clade A</td>
</tr>
<tr>
<td>Mean</td>
<td>0.0052</td>
</tr>
<tr>
<td>95% Low</td>
<td>0.0030</td>
</tr>
<tr>
<td>95% High</td>
<td>0.0077</td>
</tr>
</tbody>
</table>
Table 5. Mode and 95% confidence intervals for the parameters of an isolation-with-migration model estimated by IM for Indian and Pacific Ocean populations of *N. albicilla*. Time estimates are scaled by the reciprocal of the per-site mutation rate, $1/\mu$, and estimates of $\Theta$ are all per site.

<table>
<thead>
<tr>
<th></th>
<th>$\Theta$</th>
<th>$\Theta$</th>
<th>$\Theta$</th>
<th>$t$</th>
<th>$T_{MRCA}$</th>
<th>$2N_m$ into</th>
<th>$2N_m$ into</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indian</td>
<td>Pacific</td>
<td>Ancestral</td>
<td></td>
<td></td>
<td>Indian$^a$</td>
<td>Pacific$^a$</td>
</tr>
<tr>
<td>Mode</td>
<td>0.239</td>
<td>0.111</td>
<td>0.008</td>
<td>0.0032</td>
<td>0.0204</td>
<td>0.79$^b$</td>
<td>0.36$^b$</td>
</tr>
<tr>
<td>95% Low</td>
<td>0.155</td>
<td>0.080</td>
<td>0.005</td>
<td>0.0027</td>
<td>0.0134</td>
<td>0.51</td>
<td>0.26</td>
</tr>
<tr>
<td>95% High</td>
<td>0.489</td>
<td>0.185</td>
<td>0.018</td>
<td>0.0040</td>
<td>0.0301</td>
<td>30.55</td>
<td>17.63</td>
</tr>
</tbody>
</table>

$^a$ Effective migrants per generation

$^b$ Not significantly different from 0
Table 6. Mode and 95% confidence intervals for the parameters of an non-equilibrium island model of the structured coalescent estimated by LAMARC for Pacific Ocean demes of both species at New Caledonia, Fiji, American Samoa and Rarotonga. All estimates of $\Theta$ are per site.

<table>
<thead>
<tr>
<th></th>
<th>$\Theta$ - Current</th>
<th>Growth Parameter (g)</th>
<th>$\Theta$ - Ancestral</th>
<th>$2N_e m^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nerita albicilla</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode</td>
<td>0.193</td>
<td>1,994</td>
<td>0.023$^b$</td>
<td>164</td>
</tr>
<tr>
<td>95% Low</td>
<td>0.082</td>
<td>1,308</td>
<td>0.002$^b$</td>
<td>93</td>
</tr>
<tr>
<td>95% High</td>
<td>1.010</td>
<td>3,429</td>
<td>0.254$^b$</td>
<td>519</td>
</tr>
<tr>
<td><strong>Nerita plicata – Both Clades</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode</td>
<td>0.546</td>
<td>1,187</td>
<td>0.156$^c$</td>
<td>118</td>
</tr>
<tr>
<td>95% Low</td>
<td>0.241</td>
<td>849</td>
<td>0.043$^c$</td>
<td>77</td>
</tr>
<tr>
<td>95% High</td>
<td>1.488</td>
<td>1,636</td>
<td>0.607$^c$</td>
<td>168</td>
</tr>
<tr>
<td><strong>Nerita plicata – Clade A only</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode</td>
<td>0.396</td>
<td>1,087</td>
<td>0.126$^c$</td>
<td>121</td>
</tr>
<tr>
<td>95% Low</td>
<td>0.202</td>
<td>800</td>
<td>0.038$^c$</td>
<td>78</td>
</tr>
<tr>
<td>95% High</td>
<td>1.349</td>
<td>1,575</td>
<td>0.580$^c$</td>
<td>163</td>
</tr>
</tbody>
</table>

$^a$ Effective female migrants per generation
$^b$ Estimated for $N. albicilla$ clade B $T_{MRCA} = 0.0074/\mu$ years before present.
$^c$ Estimated for $N. plicata$ clade A $T_{MRCA} = 0.0075/\mu$ years before present.
Figure 1.
Figure 2.
Figure 3.
Figure 4