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ORIGINAL ARTICLE

High gene flow due to pelagic larval dispersal among South Pacific archipelagos in two amphidromous gastropods (Neritomorpha: Neritidae)

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The freshwater stream fauna of tropical oceanic islands is dominated by amphidromous species, whose larvae are transported to the ocean and develop in the plankton before recruiting back to freshwater habitat as juveniles. Because stream habitat is relatively scarce and unstable on oceanic islands, this life history would seem to favor either the retention of larvae to their natal streams, or the ability to delay metamorphosis until new habitat is encountered. To distinguish between these hypotheses, we used population genetic methods to estimate larval dispersal among five South Pacific archipelagos in two amphidromous species of Neritid gastropod (*Neritina canalis* and *Neripteron dilatatus*). Sequence data from mitochondrial cytochrome oxidase I (COI) revealed that neither species is genetically structured throughout the Western Pacific, suggesting that their larvae have a pelagic larval duration (PLD) of at least 8 weeks, longer than many marine species. In addition, the two species have recently colonized isolated Central Pacific archipelagos in three independent events. Since colonization, there has been little or no gene flow between the Western and Central Pacific archipelagos in *N. canalis*, and high levels of gene flow across the same region in *N. dilatatus*. Both species show departures from neutrality and recent dates for colonization of the Central Pacific archipelagos, which is consistent with frequent extinction and recolonization of stream populations in this area. Similar results from other amphidromous species suggest that unstable freshwater habitats promote long-distance dispersal capabilities.
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Introduction

The life histories of marine and freshwater animals are generally very different: marine animals often have planktonic larvae that are potentially dispersive (Thorson, 1950), whereas freshwater animals typically develop in benthic or brooded egg capsules, probably to reduce dispersal and downstream loss from adult habitat (Holthuis, 1995; Bohonak and Jenkins, 2003). Notable exceptions to this ontogenetic trend can be found in diadromous species, which may reproduce in fresh water before recruiting to marine habitats (anadromy), or reproduce in the ocean before recruiting to freshwater habitats (catadromy). Amphidromy is a lesser-known type of diadromy that has evolved independently in several families of decapod crustaceans, gastropod mollusks and teleost fishes (Myers, 1949; Holthuis, 1995; McDowall, 2004). Although the adults of amphidromous species live and reproduce in streams, rivers or estuaries, their planktotrophic larvae are released downstream to the ocean, where marine salinities are required for their successful development (Anger *et al*., 1990; Diesel and Schuh, 1998; Crandall, 1999; Diele and Simith, 2006). After metamorphosis and recruitment to river mouths, juveniles migrate upstream to freshwater habitats (Schneider and Frost, 1986; Blanco and Scatena, 2005; Torres *et al*., 2006).

Amphidromous species dominate the fish, decapod and gastropod stream fauna on tropical oceanic islands, most likely because they are the only lotic species capable of regularly colonizing these habitats (Resh and de Szalay, 1995; McDowall, 2004). However, although a community ecologist would view them as freshwater animals (for example, Bandel and Riedel, 1998; Smith *et al*., 2003), their population ecology may be more similar to that of a marine species, because of their pelagically dispersing larvae. Relatively long pelagic larval durations (PLDs) have been estimated from laboratory cultures of amphidromous gastropod veligers (40–98 days, Holthuis, 1995; Kano, 2006) and the otoliths of amphidromous Galaxiid fishes and gobies (63–266 days, Radtke *et al*., 1988, 2001; Mcdowall *et al*., 1994; Hoareau *et al*., 2007b). These PLDs fall at or above the high end of the range found in the planktotrophic larvae of marine invertebrates (7–293 days, Shanks *et al*., 2003) and fish (~20–90 days, Brothers *et al*., 1983).

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Consistent with this high dispersal potential, genetic structure within high-island archipelagos is low or non-existent in amphidromous Neritid and Neritiliid snails (Hodges and Allendorf, 1998; Myers *et al*., 2000; Kano and Kase, 2004), as well as in Galaxiid and Sicydiine fishes (Chubb *et al*., 1998; Waters *et al*., 2000; Berrebi *et al*., 2005; Hoareau *et al*., 2007a), suggesting that populations of amphidromous species are genetically structured at scales similar to fully marine species. In contrast, fully lotic species are frequently genetically structured within watersheds or even within reaches (Bunn and Hughes, 1997; Marten *et al*., 2006).

Lotic habitats are rare in the South Pacific, occurring only on volcanic islands that are tall enough to generate their own adiabatic rainfall. Given the high levels of larval mortality and the effects of diffusion (Cowen *et al*., 2000), it seems unlikely that significant numbers of larvae that drifted away from their natal archipelago would be able to find suitable freshwater habitat for settlement. Therefore, local selection for traits that favor self-recruitment could be particularly strong for amphidromous species (Sponaugle *et al*., 2002; Strathmann *et al*., 2002). Consistent with this prediction, Sorensen and Hobson (2005) found that newly recruited amphidromous gobies had stable isotope signatures that were similar to inshore plankton rather than offshore plankton, suggesting that larvae prefer to stay in coastal waters. Similar homing behaviors have been suggested for the larvae of amphidromous shrimp and snails (Benstead *et al*., 2000; Haynes, 2000). Such larval retention could result in limited realized dispersal and pronounced genetic structure among archipelagos.

However, in addition to their rarity, riverine habitats on oceanic islands are inherently unstable. They are characterized by short overall lengths (generally <5 km), with small catchments, and extremely variable flows (Resh and de Szalay, 1995; Craig, 2003). Climactic fluctuations over the past several million years (Hope, 1996) and the rapid erosion and eventual subsidence of individual islands (Whittaker *et al*., 2008) ensure that populations in oceanic island streams will be subject to local extinction and re-colonization over evolutionary timescales (Covich, 2006). These processes can be expected to leave a molecular signature in the form of shallow, star-like genealogies (Slatkin and Hudson, 1991), and estimates for colonization events that greatly post-date the formation of each archipelago (Price and Clague, 2002).

Chaotic population dynamics have also been shown to promote the evolution of long-distance dispersal ability (Johnson and Gaines, 1990; Holt and Mcpeek, 1996). As the planktotrophic larvae of amphidromous species must settle in a rare, unstable habitat, they could be selected for the ability to delay metamorphosis and extend their planktonic life indefinitely ('death before dishonor' hypothesis, Bishop *et al*., 2006, see Elkin and Marshall, 2007 for a numerical model). Such a strategy could result in extremely long-distance dispersal, limiting genetic differentiation among archipelagos.

In this study, we assess mitochondrial genetic variation in two amphidromous snail species from the family Neritidae (Gastropoda: Neritopsina). *Neritina canalis* (Sowerby, 1825) and *Neripteron dilatatus* (Lesson, 1830) have planktotrophic larvae, as indicated by the 'D'-shaped initial region of their opercula (Kano, 2006) and probably share an amphidromous common ancestor (Holthuis, 1995). *Neritina canalis* is found under stones in riffles within 1–2 km of the sea, and has a range that extends from the Philippines to the Marquesas (Haynes, 2001). *Neripteron dilatatus* is able to tolerate relatively high salinities (Liu and Resh, 1997), but has only been collected from rocky substrate in the estuaries of running streams ranging from the Philippines to the Society Islands (Pointier and Marquet, 1990; Haynes, 2001). If the larvae of these species have developed behaviors for retention in coastal waters, then we would expect to see genetic structure between or even within archipelagos. Conversely, if the larvae are passively dispersed, but have adapted to extend their pelagic duration until they can recruit to freshwater habitat, then we would expect to see little genetic structure across the South Pacific, with relatively frequent long-distance dispersal and gene flow occurring in the direction of the prevailing currents.

Materials and methods

Sampling and sequencing

We collected *N. canalis* ($n=202$) and *N. dilatatus* ($n=151$) from two or more islands in the West Pacific archipelagos of Vanuatu Fiji, and Samoa, as well as from the Society and Marquesan archipelagos in the Central Pacific (Figure 1 and Table 1). We fixed whole specimens in 95% ethanol, with the opercula propped open to allow proper preservation. *N. dilatatus* is not known to be present in the Marquesas (Pointier and Marquet, 1990,

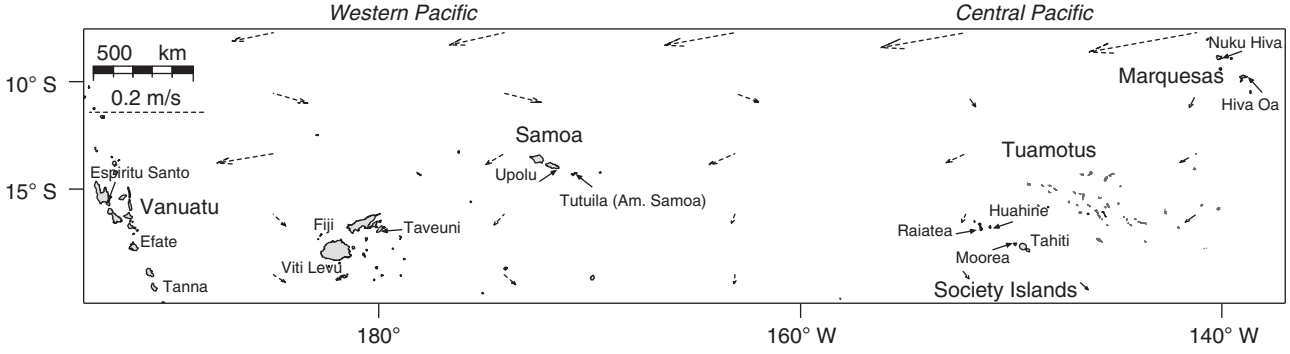

Figure 1 Map of the South Pacific, showing localities sampled in the five archipelagos of high islands. Islands in the Tuamotu archipelago are atolls devoid of running freshwater habitats. The dotted vectors depict a 16-year mean surface currents (Bonjean and Lagerloef, 2002) for the period between October and December, when most Neritid egg cases are hatched (Resh and de Szalay, 1995).

Table 1 Summary statistics and neutrality test statistics for each island deme shown in Figure

Region	*Archipelago*	*Island*	Neritina canalis					Neripteron dilatatus				
			n	*No. of haps*	h	π	F_S	n	*No. of haps*	h	π	F_S
West Pacific	*Vanuatu*	Espiritu Santo						19	12	0.871	0.009	−3.13
		Efate						23	18	0.941	0.007	**−12.76**
		Tanna	6	6	1.000	0.004	**−2.86**					
	Fiji	Viti Levu	17	15	0.985	0.008	**−9.95**	2	2	1.000	0.012	NA
		Taveuni	13	10	0.970	0.005	**−5.05**	23	20	0.976	0.013	**−11.60**
	Samoa	Upolu	22	17	0.935	0.005	**−14.85**	23	17	0.937	0.007	**−11.02**
		Tutuila	24	19	0.968	0.005	**−19.76**	19	11	0.889	0.008	−2.52
Central Pacific	*Society Islands*	Raiatea	17	11	0.912	0.005	**−6.11**	24	16	0.960	0.008	**−7.16**
		Huahine	18	14	0.967	0.007	**−8.73**					
		Moorea	25	20	0.970	0.005	**−21.20**	18	15	0.978	0.011	**−7.70**
		Tahiti	18	15	0.961	0.006	**−11.98**					
	Marquesas	Nuku Hiva	23	13	0.921	0.006	**−5.07**					
		Hiva Oa	19	12	0.924	0.006	**−5.53**					

Abbreviation: NA, not applicable.
Haplotype diversity (h), nucleotide diversity (π) and F_s (Fu, 1997) calculated using Arlequin 3.1 (Excoffier *et al*., 2005). Significant values of F_s ($P<0.02$) are shown in bold.

T Eichhorst, personal communication), and we did not find it there. We extracted genomic DNA from the foot muscle tissue in a 10% Chelex solution (Walsh *et al*., 1991). We initially PCR amplified a 658-bp region of mitochondrial cytochrome oxidase I (COI) with standard invertebrate primers, HC0-2198 and LCO-1490 (Folmer *et al*., 1994). Because these primers amplified with a low success rate (<50%), we designed an internal forward primer, NerL (5′-ATGTAATTGTRACTGCTCATGC-3′) that amplifies a 520-bp region of the gene, in conjunction with HCO-2198. Reactions occurred in 25 μl volumes with 2.5 μl of 10 × buffer, 2 μl of magnesium chloride (25 mM), 2.5 μl of deoxynucleotide triphosphate (8 mM), 1.25 μl of each 10 mM primer, 1 μl of template and 0.625 units of Amplitaq (Applied Biosystems Inc., Foster City, CA, USA). Thermocycling conditions were: initial denaturation at 94 °C (15 s), main cycle at 94 °C (30 s), 50 °C (30 s) and 72 °C (30–40 s) for 35–39 cycles, and then a final extension of 72 °C (3–10 min). A total of 5 μl of successful PCR products were cleaned by adding 0.5 units of shrimp alkaline phosphatase (Biotech Pharmacon, TromsØ, Norway) and five units of Exonuclease I (GE Healthcare, Piscataway, NJ, USA), and incubating at 37 °C for 30 min and 80 °C for 15 min. We sequenced forward and reverse directions of double-stranded PCR products with Big Dye 3.1 terminator chemistry (Applied Biosystems) on an ABI 377 sequencer, and proofread the resulting chromatograms using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA). Proper translation using the invertebrate mitochondrial code was confirmed using MacClade 4.05 (Maddison and Maddison, 2002).

Data analysis

We used Arlequin 3.1 (Excoffier *et al*., 2005) to calculate standard measures of genetic diversity (h, π) for each island deme, and also used Fu's F_s (Fu, 1997), which tests the data for an excess of recent mutations that are indicative of non-neutral processes, such as positive selection or population growth. We then visualized the genetic relationships among haplotypes with a minimum-spanning tree (MST) calculated in Arlequin for each species and then re-drawn by hand using Adobe Illustrator. Each alternative connection identified by the program was evaluated by eye to determine whether it would significantly alter the topology of the MST.

We evaluated hierarchical genetic structure among archipelagos using analysis of molecular variance as implemented in Arlequin 3.1. The data were partitioned into two separate regions: (1) the Western Pacific (Vanuatu, Fiji and Samoa archipelagos) and (2) the Central Pacific archipelagos (Society Islands and Marquesas). We evaluated significance with 10 000 random replicates. Pairwise ϕ_{st} among demes was also calculated with 10 000 random replicates, and the significance of each value was established after a Bonferroni correction.

Low levels of genetic structure and haplotypes that are shared between distant populations can be explained either by ongoing gene flow over a relatively long period of time or by incomplete lineage sorting after a relatively recent colonization event (Nielsen and Wakeley, 2001). To differentiate between these alternative hypotheses, we used the isolation with migration (IM) program (Hey and Nielsen, 2004) to fit the IM model with the genetic data from the Western and Central Pacific populations of both species. The program uses a Markov chain Monte Carlo methodology to simulate millions of coalescent genealogies while varying the model parameters, comprising time of population splitting (t), migration rates after the populations split (m/μ), current θ and ancestral θ_A ($=2N_e\mu$). The parameter values that are visited most frequently by the program have the highest probability and can be taken as parameter estimates with confidence intervals including 95% of all values visited by the program. These estimates allow comparison of the model parameters between the two species, assuming that they experience a similar substitution rate.

The migration rate (N_em) is the product of the parameters m/μ and θ divided by two. This value summarizes the effective number of migrants per generation that move between the populations after their time of splitting, but does not distinguish between constant migration and a few massive dispersal events. Therefore, because IM explicitly estimates genealogies with migration events between populations, we also used it to produce a histogram of the number of independent migration events inserted during each iteration of the Markov chain (Won and Hey, 2005). To get a heuristic estimate of the maximum amount of time since population divergence, we converted the divergence time, t, to years using a relatively slow divergence rate of 1% per million years (based on fossil-calibrated Molluscan rates, Marko, 2002). Finally, we evaluated differences in population size as the proportion of genealogies for which the θ value for one population was larger than the other, expressed as a P-value.

For both species, we constructed IM data sets that were partitioned between the West Pacific archipelagos (Vanuatu, Fiji and Samoa combined into a single population), and the Society Islands. For *N. canalis,* we constructed a second data set to compare populations from the West Pacific and the Marquesas (*N. dilatatus* was not present in the Marquesas). After several exploratory runs, we set priors with maximums at $\theta = 5000$, $\theta_A = 500$, $t = 5$ and $m = 10$, with migration rate in either direction constrained to be equal. We chose an Hasegawa–Kishino–Yano model of mutation over the alternative infinite sites model because several sites included more than one type of substitution. We ran the Markov chains for a minimum of 78 million steps without heating, and a burn-in period of 200 000 steps. We determined whether these runs were adequate using effective sample size, which the authors of IM recommend to be >50. We replicated runs for each data set at least thrice.

Results

Mitochondrial COI sequences from 202 *N. canalis* contained 117 unique haplotypes (GenBank accession number GU001171–GU001372), whereas 85 unique haplotypes were found in 151 COI sequences from *N. dilatatus* (GenBank accession number GU001373–GU001523). All sequences aligned properly and translated without stop codons, as expected for a coding gene. Three non-synonymous changes were found in *N. canalis*, all of them singletons, and one singleton amino-acid change was found in *N. dilatatus*. Haplotype diversity (h) was high in all demes, with the lowest value for *N. canalis* being 0.912 at Raiatea and the lowest for *N. dilatatus* being 0.871 at Espiritu Santo. Nucleotide diversity was relatively low, ranging from 0.004 to 0.008 in *N. canalis* and from 0.008 to 0.0013 in *N. dilatatus*. Fu's F_s values were strongly and significantly negative, indicating departures from the neutral expectations for a demographically stable population for all demes in both species except for two *N. dilatatus* demes: Espiritu Santo and Tutuila. The results are summarized in Table 1.

Minimum-spanning trees for both species contain star polytomies that are also indicative of processes that cause departures from the neutral model, such as selection or population growth (Slatkin and Hudson, 1991). However, the geographic distribution of this variation differs between species (Figures 2a and b). *N. canalis* shows significant population structure. The central star polytomy contains representatives from all five archipelagos (although haplotypes from the Society Islands and the Marquesas occur at a relatively low frequency), a second polytomy contained only haplotypes from the Society Islands, whereas a third polytomy is almost entirely made up of Marquesan haplotypes (with the exception of one individual from the Societies). Both of these polytomies were rooted at the central polytomy, and none of the 27 alternative connections suggested that they are more closely related to one another. In contrast, the MST topology for *N. dilatatus* shows no evidence of regional structure. The large star topology is dominated by a single central haplotype that was found in 36 snails from all four sampled archipelagos. A few less-frequent haplotypes that are between 2 and 8 bp differences away from the central haplotype are at the center of smaller polytomies.

We qualitatively detected different patterns of genetic structure in the two snail species. Congruent with the patterns observed in the MSTs, we found strong regional structuring between the Western and Central Pacific populations of *N. canalis*, which explained 11.0% of the genetic variation at the COI locus (Table 2). Regional pairwise ϕ_{ST} values in *N. canalis* revealed no significant structure among the Western Pacific archipelagos of Samoa, Fiji and Vanuatu (ϕ_{ST} ranging from 0 to 0.02, no values significant). However, significant structure was detected in pairwise comparisons between these Western Pacific demes and those from the Marquesas and Societies, respectively (ϕ_{st} ranging from 0.21 to 0.37, $P < 0.0001$, Table 3). In contrast to *N. canalis,* genetic structure in *N. dilatatus* was uniformly non-existent among all archipelagos (global $\phi_{st} = 0.005$, pairwise ϕ_{st} ranging from 0 to 0.04, no values significant), as suggested by the high degree of haplotype sharing.

Parameter estimates from three replicate IM runs converged to the same or very similar values for data sets from both species (Table 4). Effective sample sizes were all >75, and generally >100. Trend lines for each parameter indicated that the chain was well mixed. For all three replicate runs of the *N. dilatatus* data set, the posterior distribution for θ in the Western Pacific archipelagos was not complete before it reached the maximum value of the prior distribution, probably because of high levels of gene flow with unsampled populations to the west (Beerli, 2004).

The isolation with migration model inferred differing rates of gene flow between the Central and Western Pacific in the two species. In *N. canalis,* the 95% confidence interval (CI) for the migration rates (N_em, Table 4) between the West Pacific archipelagos and the Society Islands included the lowest assayed value, and the number of independent migration events reached a modal value at zero, indicating that gene flow in this region is not significantly different from zero in this species. There was a similar result for migration events between the Marquesas and the West Pacific, except that westerly migration events reached their modal value at 2 (95% CI 0–13), hinting at a small amount of post-colonization gene flow in this direction. In contrast, the migration rate was significantly >0 for *N. dilatatus*, and migration events had a modal value of 13 (95% CI 4–238)

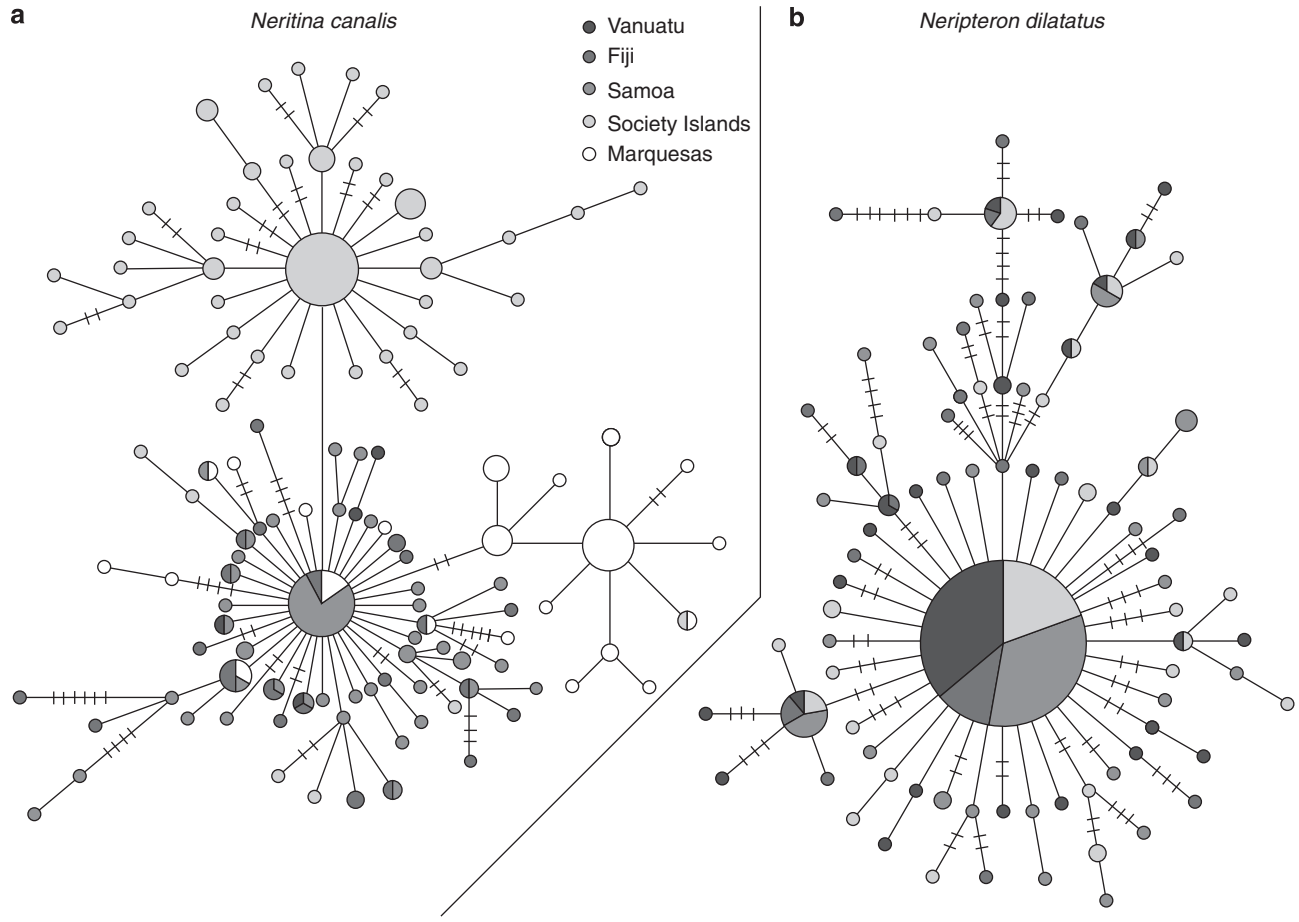

Figure 2 Minimum-spanning trees (MSTs) for (**a**) *Neritina canalis* and (**b**) *Neripteron dilatatus*. The circles are sized proportionally to the frequency of occurrence, ranging from 1 to 15 in *N. canalis* and from 1 to 36 in *N. dilatatus*. All haplotypes are separated by one mutational step unless denoted by a higher number of hatch marks.

Table 2 AMOVA results for comparisons among West Pacific and Central Pacific regions in both species

	Neritina canalis	Neripteron dilatatus
Overall ϕ_{ct} (between regions)	**0.110**	0.001 (NS)
Overall ϕ_{sc} (within regions)	**0.208**	0.004 (NS)
% Variation		
Among regions	10.96%	0.13%
Among demes within regions	18.52%	0.38%
Within demes	70.52%	99.48%

Abbreviations: AMOVA, analysis of molecular variance; NS, not significant.
The West Pacific region includes Vanuatu, Fiji and Samoan archipelagos. The Central Pacific region includes the Marquesas and Society archipelagos. Significant values in bold indicate a $P<0.05$ after 10 000 random permutations of the data.

from the Societies to the West Pacific and 3 (95% CI 1–191) in the opposite direction (see Supplementary Figure 1).

The Markov chain Monte Carlo simulations suggest that the Western Pacific populations of both species have higher effective population sizes than those in the Central Pacific, as indicated by their consistently larger θ values ($P<0.02$ in all cases). In addition, θ values for both contemporary populations of *N. dilatatus* were consistently $>\theta$ for the ancestral population ($P<0.001$), indicating a significant increase in effective population size in both populations. This was not the case for *N. canalis*, in which θ values for contemporary populations in both Central Pacific archipelagos were not significantly larger than the ancestral population. Divergence time estimates with a heuristic rate of 1% per million years indicate that Western Pacific and Society Archipelago populations of *N. canalis* diverged approximately 0.6 million years ago (95% CI 0.52–1.76 mya), whereas *N. dilatatus* populations diverged significantly later, approximately 0.3 million years ago (95% CI 0.22–0.42 mya). The Western Pacific and the Marquesas populations of *N. canalis* diverged approximately 0.4 million years ago (95% CI 0.28–1.82 mya).

Discussion

Long-distance dispersal among Western Pacific archipelagos

Although the adults of both *N. canalis* and *N. dilatatus* occur only in freshwater streams or their estuaries, neither species showed any evidence of genetic structure within or among the Western Pacific archipelagos of Vanuatu, Fiji and Samoa. The simplest explanations for

Table 3 Pairwise ϕ_{st} values for island demes in *Neritina canalis*

Locality	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
1. Tanna	0										
2. Viti Levu	0.007	0									
3. Taveuni	0.019	0.022	0								
4. Upolu	0.010	0.017	0.018	0							
5. Tutuila	0.006	0.010	0.003	0.000	0						
6. Raiatea	**0.323**	**0.251**	**0.296**	**0.294**	**0.300**	0					
7. Huahine	**0.244**	**0.204**	**0.249**	**0.253**	**0.254**	0.018	0				
8. Moorea	**0.314**	**0.261**	**0.303**	**0.293**	**0.291**	0.000	0.004	0			
9. Tahiti	**0.254**	**0.210**	**0.252**	**0.253**	**0.251**	0.000	0.000	0.000	0		
10. Nuku Hiva	0.256	**0.225**	**0.264**	**0.269**	**0.269**	**0.418**	**0.351**	**0.420**	**0.381**	0	
11. Hiva Oa	**0.363**	**0.295**	**0.361**	**0.363**	**0.366**	**0.492**	**0.418**	**0.490**	**0.450**	0.024	0

Values in bold were significant after Bonferroni correction for multiple tests (individual $P<0.0009$).

Table 4 Mode and 95% confidence intervals for the parameters of an isolation-with-migration model estimated using IM for Western Pacific (WP) populations (Vanuatu, Fiji and Samoa) and Central Pacific (CP) populations of both species

	θ *West Pacific*	θ *Central Pacific*	θ *Ancestral*	t_{split}	N_em *(CP→WP)*	*Westward migration events*	*Eastward migration events*
			Neritina canalis—*Society Islands and Western Pacific*				
Mode	0.375	0.187	0.117	0.0033	0.49	0	0
95% low	0.252	0.129	0.075	0.0026	0.49	0	0
95% high	0.967	0.310	0.456	0.0088	5.36	2	1
			N. canalis—*Marquesas and Western Pacific*				
Mode	0.725	0.060	0.122	0.0020	8.48	**2**	0
95% low	0.429	0.029	0.079	0.0014	0.94	0	0
95% high	2.121	0.125	0.381	0.0091	40.5	13	5
			Neripteron dilatatus—*Society Islands and Western Pacific*				
Mode	2.582	0.322	0.071	0.0015	104	13	3
95% low	1.341	0.168	0.041	0.0011	23.5	4	1
95% high	9.361	1.659	0.130	0.0021	3830	238	191

Confidence intervals for westerly N_em are given for the parameter m, conditional on the modal value of θ for the West Pacific. Time estimates are scaled by the reciprocal of the per-site mutation rate, $1/\mu$, and estimates of θ are per site.

the absence of genetic structure observed in the Western Pacific are that either both species have maintained high equilibrium levels of gene flow across the Western Pacific ($N_em>10$ migrants per generation) for a long period of time, or that they have undergone a recent range expansion through the region, colonizing each archipelago over a relatively short amount of time. Although our present data are unable to distinguish between these two scenarios, long-distance larval dispersal (that is, dispersal far beyond the mean dispersal distance for these species) must have occurred under both of them.

The velocity of the South Equatorial Current is highly variable, but it generally moves southwest through the study region at average speeds not $>0.07\,m\,s^{-1}$ (11-year average from Ocean Surface Current Analyses—Real time, http://www.oscar.noaa.gov, Bonjean and Lagerloef, 2002). However, climactic fluctuations can periodically produce much faster current velocities. For example, during the 1999 La Niña event, current velocities in the north of the region reached nearly $0.2\,m\,s^{-1}$. A veliger larva that is released into the South Equatorial Current during such an event would take about 50 days to cross the $\sim$850 km of open ocean that separate Samoa from Fiji or Fiji from Vanuatu, if it were to travel in a straight line. The larvae of both species must therefore be able to delay metamorphosis for at least this long to create or maintain the panmixia observed across this region. Similarly, the amphidromous goby *Sicyopterus lagocephalus*, which is known to have a very long PLD (133–266 days), was found to be panmictic between the Comoros and Mascarene archipelagoes in the Indian Ocean (Hoareau *et al*., 2007a).

Multiple colonizations of the Central Pacific

Although both species were genetically homogenous in the Western Pacific, *N. canalis* showed pronounced structure in the Central Pacific. Significantly smaller θ values in the relatively young Central Pacific archipelagos (Table 4) suggest that existing populations of *N. canalis* in the Society Islands and the Marquesas are probably the result of two independent, eastward colonization events from the older archipelagos of the Western Pacific. Analysis under the IM model found that, since colonization, no significant gene flow has occurred between the Society Islands population of *N. canalis* and the Western Pacific population, whereas perhaps only a small westward trickle has occurred between the Marquesas and the West Pacific. The haplotypes shared between Central Pacific and Western Pacific populations are thus most likely the result of incomplete sorting of lineages after colonization, and not

ongoing gene flow. Genetic structure or divergence across the large expanses of open water (~2000 km) that lie between archipelagos in the Western and Central Pacific, such as what we found in *N. canalis*, is commonly observed in marine species (Palumbi *et al*., 1997; Bernardi *et al*., 2001; Lessios *et al*., 2001; Crandall *et al*., 2008) as well as in an amphidromous goby (Keith *et al*., 2005).

It is therefore remarkable that *N. dilatatus* shows no evidence of genetic structure across this span. There are two possible explanations for this pattern: either one population was recently founded by a massive colonization event from the other population, or else there has been ongoing gene flow between the two populations after colonization. Distinguishing between these two models is a classic problem in population genetics that can be addressed with the IM model (Nielsen and Wakeley, 2001; Hey and Nielsen, 2004). Our IM analyses indicate that the Society Islands population of *N. dilatatus* was probably founded by an eastward colonization event, as indicated by its significantly smaller value for θ. This event occurred no more than 420 000 years ago, which is significantly younger than the colonization of the Society Islands by *N. canalis*. However, because IM was unable to reject a model with migration, it is possible that *N. dilatatus* has maintained at least intermittent gene flow across the intervening ~2000 km of ocean between the Society Islands and Western Pacific populations ($N_e m > 23.5$ migrants per generation) after this colonization event.

It is interesting to note that IM reckoned a higher number of westerly migration events than easterly events. This indicates that after the inferred eastward colonization event, most of the gene flow in *N. dilatatus* has run westward in the direction of the South Equatorial Current (Table 4). Even at the high speeds estimated for La Niña events (0.2 m s^{-1}, see above), it would take 115 days for the South Equatorial Current to transport a larva from the Society Islands to the Samoan archipelago. The possible difference in gene flow across an area that completely lacks freshwater habitats implies that *N. dilatatus* larvae may be able to delay metamorphosis for longer than the larvae of *N. canalis*, and indeed, most marine species.

Local extinction and recolonization

Coalescent estimates of population splitting indicate that both species colonized the Central Pacific archipelagos starting no more than 1.82 million years ago, in three independent events. These dates are relatively recent when compared with the geologic age of the oldest island in each archipelago (6 million years in the Marquesas and 10 million years in the Society Islands; Craig *et al*., 2001). In addition, shallow star polytomies in both MSTs and strongly negative values of F_s in all but two island demes indicate non-equilibrium population dynamics, such as recent population expansions because of colonization (Slatkin and Hudson, 1991; Fu, 1997). Together, these data support a history of local extinctions followed by re-colonization, as has been suggested for other amphidromous species (Cook *et al*., 2008).

These recent dates of colonization could possibly be explained by an absence of suitable habitat in the Central Pacific archipelagos until about a million years ago, or a low probability of eastward colonization (Paulay and Meyer, 2002) because of the prevailing westerly currents of the South Equatorial Current. However, decadal current reversals during El Niño events provide a mechanism for occasional colonization events to occur (Bonjean and Lagerloef, 2002; Lessios and Robertson, 2006). Moreover, as islands in these hotspot archipelagos are formed sequentially, they have a large range of ages and therefore offer a wide array of habitats at any one time (Paulay, 1994; Craig *et al*., 2001). We therefore find it more likely that each of these species has re-colonized the Central Pacific archipelagos after local extinction.

Consistent with this inference, freshwater stream habitats on oceanic islands are known to be inherently unstable at multiple temporal scales. On a decadal scale, individual streams may dry up because of drought, or be scoured by massive floods, causing local extinction of stream populations (Maciolek and Ford, 1987; Resh and de Szalay, 1995, personal observation). Plio-Pleistocene glacial periods resulted in extended periods of decreased rainfall on the Pacific islands, likely drying up streams throughout the region (Hope, 1996). Fluctuating sea levels during this period would have also alternately created and destroyed riverine habitats (Dickinson, 2004). On still deeper timescales, freshwater habitats on oceanic islands undergo substantial change as erosion modifies steeply profiled streambeds into more mature pool and riffle habitats with relatively broad alluvial estuaries. Ultimately, island subsidence reduces adiabatic rainfall to a point where continuous flow cannot be sustained, and riverine habitats are lost (Craig *et al*., 2001; Craig, 2003).

The evolution of dispersal in amphidromous species

Species with frequent extinction and recolonization of local populations face two conflicting selective pressures on their dispersal ability (Olivieri and Gouyon, 1997). On one hand, lotic freshwater habitats are rare in the Pacific Ocean, and a larva that recruits to its natal stream should be favored over one that disperses away, as it is unlikely that the dispersive larva will find another stream in which to settle (Strathmann *et al*., 2002). In contrast, frequent local extinction of stream populations should favor the evolution of larvae that have the ability to delay metamorphosis long enough to find new streams (Holt and Mcpeek, 1996; Elkin and Marshall, 2007). These pressures might not necessarily act in direct opposition. Whereas the former might select for behaviors that favor retention (for example, using chemical cues to stay near natal habitat, Gerlach *et al*., 2007), the latter might select for developmental and physiological changes that allow for delayed metamorphosis if necessary (as was suggested for habitat specialists in a recent survey of the literature, Bishop *et al*., 2006).

Although we cannot make any inferences regarding the degree of larval retention with the current data set, our results show that at least some larvae from both amphidromous gastropod species are able to delay larval metamorphosis for longer than many marine species (>50 days in *N. canalis* and >115 days in *N. dilatatus*). Other amphidromous species found on oceanic islands have a similarly lengthy PLD (Radtke *et al*., 2001; Kano, 2006; Hoareau *et al*., 2007b), an absence of genetic structure across long pelagic distances (Myers *et al*., 2000; Waters *et al*., 2000; Hoareau *et al*., 2007a) and the

molecular signature of recent colonization events (Myers *et al*., 2000; Kano and Kase, 2004; Cook *et al*., 2008). The pelagic larva has only been lost from the life history a few times among the extant freshwater Neritids, and only once in an island species (Holthuis, 1995), even though pelagic larvae have been lost multiple times in other families of marine invertebrates (Duda and Palumbi, 1999; Hart, 2000). Given their unstable habitat, this makes sense, as the evolution of adaptations for larval retention in a freshwater species would lead to its restriction to individual islands and a heightened risk of species extinction (Hansen, 1978; Jablonski and Lutz, 1983).

If instability of lotic habitats on oceanic high islands promotes long-distance larval dispersal, then we should see the opposite pattern in amphidromous species living in continental watersheds. In these geologically older and more stable lotic environments, selection against dispersal from the natal habitat should be unopposed. Consistent with this prediction, phylogeographic studies of amphidromous Atyid shrimp (Page *et al*., 2005, 2007; Cook *et al*., 2006) and Galaxiid fishes (Waters and Wallis, 2001) from Australia and New Zealand show evidence for multiple losses of amphidromy, with widespread basal amphidromous lineages giving rise to multiple freshwater lineages that are restricted to watersheds. Within the Neritidae, the only genus with benthic development in fresh water (*Theodoxus*) occurs in Eurasia (Bunje and Lindberg, 2007), further supporting the prediction that the relative stability of continental riverine habitats supports the loss of planktonic larvae.

Conclusions

An absence of genetic structure across three archipelagoes in the Western Pacific shows that both *N. canalis* and *N. dilatatus* have a capacity for long-distance larval dispersal that is as good or better than many marine species. Furthermore, these species have colonized Central Pacific archipelagos that lie over 2000 km away from the nearest freshwater habitat. Coalescent analysis suggests that these colonization events occurred independently, and relatively recently in comparison with the age of the archipelagos. Predominantly westward gene flow in *N. dilatatus* seems to have continued after colonization, whereas it has more or less ceased in *N. canalis*. Long-lived larvae, and low levels of genetic structure among oceanic island populations of many amphidromous species, combined with the frequent loss of amphidromy in continental watersheds, support theoretical predictions (Johnson and Gaines, 1990; Holt and Mcpeek, 1996; Elkin and Marshall, 2007) that temporal instability of habitats plays a major role in promoting the evolution of dispersal ability.

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Conflict of interest

The authors declare no conflict of interest.

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