

# Connectivity of populations within and between major biogeographic regions of the tropical Pacific in *Conus ebraeus*, a widespread marine gastropod

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**Abstract** Information on genetic connectivity and structure of populations in the tropical Pacific is critical for making inferences about the origins and maintenance of diversity in this region. Sequences of the mitochondrial COI gene from 92 individuals of the trans-Pacific gastropod *Conus ebraeus* from eight localities spanning the tropical Pacific were analyzed to determine whether populations in the western, central, and eastern Pacific exhibit genetic structure, to examine the demographic histories of populations, and to infer patterns of gene flow. A total of 43 unique haplotypes were recovered, including a common haplotype that occurred in six of the eight populations examined. AMOVA and pairwise *F*-statistics showed that populations in the western and central Pacific were significantly differentiated from populations in the eastern Pacific, but no other evidence of structure. Bayesian isolation–migration (IM) analysis suggested that populations in the western and central Pacific separated from those in eastern Pacific during the Pleistocene. Examination of mismatch distributions and results from IM revealed that populations in the western and central Pacific expanded during the Pleistocene. Gene flow across the East Pacific Barrier appears to occur predominantly westward.

**Keywords** Phylogeography · East Pacific Barrier · *Conus* · Gene flow · Population structure

## Introduction

Distributions of tropical shallow water marine organisms are generally restricted to four well-defined biogeographic regions: the Indo-West Pacific, the eastern Pacific, and the (western and eastern) Atlantic regions (Briggs 1974). These distributions were presumably strongly influenced by past vicariant events due to various barriers to dispersal that separated formerly widespread species. Since the Mesozoic, numerous geophysical events have shaped marine biotas. The closure of the Tethys Seaway, the establishment of the Benguela upwelling, and emergence of the Isthmus of Panama isolated the Atlantic from the Indo-West Pacific (defined as the entire Indo-Pacific area, except the eastern Pacific). The mid-Atlantic and east Pacific barriers (wide stretches of water, difficult to cross by planktonic larvae) hindered dispersal of species across the entire width of each of the two oceans. Phylogenetic relationships among species of tropical marine taxa that occur in multiple biogeographic regions illuminate the importance of these phenomena in determining broad-scale historic patterns of divergence (Lessios et al. 1999, 2001, 2003; Colborn et al. 2001; Muss et al. 2001; Meyer 2003; Williams and Reid 2004; Duda and Kohn 2005; Meyer et al. 2005).

The East Pacific Barrier (EPB), a 4,000–7,000 km stretch of deep water between the easternmost islands of the central Pacific and the offshore islands of the Americas, is perhaps the most formidable oceanic barrier to dispersal for tropical marine organisms. It has presumably been in place throughout the Cenozoic (Grigg and Hey 1992). Indo-West Pacific and eastern Pacific shallow-water benthic biotas are

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nearly completely distinct, and few benthic and demersal tropical marine species have disjunct, trans-Pacific distributions (Briggs 1974). These distributions are often assumed to reflect relatively recent jump dispersals across this barrier that originated in the Indo-West Pacific (e.g., Briggs 1961, 1967; Emerson 1991, but see Robertson et al. 2004), but the direction of the initial dispersal event and of subsequent gene flow has only been extensively examined in fishes (Lessios and Robertson 2006).

Among prosobranch gastropods, 33 species are known to occur in both the Indo-West Pacific and eastern Pacific (Emerson 1991). All 33 trans-Pacific species possess planktotrophic larvae, and their populations in the eastern Pacific are assumed to represent ephemeral sink populations, which depend for their long-term persistence on recruitment from Indo-West Pacific populations (Emerson 1978, 1991; Emerson and Chaney 1995). Because most transpacific species [including gastropods (Emerson 1991)] have much wider distributions west of the EPB, the eastern Pacific populations are thought to have resulted from range expansion initiated in the Indo-West Pacific (Briggs 1974). However, reconstruction of phylogenetic relationships of members of the gastropod genus *Conus* revealed several historic breaches of the EPB that may have occurred in either direction during at least the past 10 million years (my) (Duda and Kohn 2005). Moreover, there is little reason for assuming that the initial introduction occurred in the same direction as subsequent gene flow. Phylogeographic analyses of 20 trans-Pacific fish species showed that directionality of gene flow across this barrier is not as universal as had been assumed; while some fishes exhibit bidirectional gene flow, others show predominant patterns of migration in either westward or eastward directions (Lessios and Robertson 2006). These results challenge the long assumed view (Ekman 1953; Briggs 1974; Dana 1975; Vermeij 1978; Glynn et al. 1983; Rosenblatt and Waples 1986; Vermeij 1991) that initial colonization and subsequent gene flow across the EPB occur exclusively in an eastward direction and call for further investigations of patterns of genetic exchange of populations separated by this barrier.

Understanding patterns of divergence of tropical marine organisms *within* biogeographic regions is also critical for making inferences about the origins of biodiversity. These patterns are best illuminated through comparison of the distributions of genotypes of populations of widespread species. For example, even though a number of unrelated taxa show concordant genetic breaks between Indian and Pacific populations, presumably caused by historical isolation of the two basins during times of low sea level stands (Benzie and Stoddart 1992; Mcmillan and Palumbi 1995; Lavery et al. 1996a; Miya and Nishida 1997; Williams and Benzie 1997, 1998; Duke et al. 1998; Benzie 1999; Duda

and Palumbi 1999b; Lessios et al. 1999, 2001; Williams et al. 2002; Bay et al. 2004; Teske et al. 2005; Reid et al. 2006; Crandall et al. 2008), there is no equivalent concordance in breaks between the western and central Pacific areas of the Indo-West Pacific, or between localities within either of these areas. Vicariant events that could have influenced the genetic divergence between populations in the western and the central Pacific are not apparent in geologic history. Some species, including clams (Benzie and Williams 1997), sea urchins (Palumbi et al. 1997; Lessios et al. 1999, 2001), lancelets (Kon et al. 2006), and fishes (Winans 1980; Bernardi et al. 2001; Planes and Fauvelot 2002; Bay et al. 2004; Ravago-Gotanco and Junio-Meñez 2004), exhibit patterns of genetic differentiation between these two regions that suggest isolation by distance. However, there is no obvious consistency among species in patterns of gene flow among populations. Other species, including gastropods (Crandall et al. 2008), crustaceans (Williams et al. 2002), and echinoderms (Lessios et al. 2001), show evidence of limited structure between these areas. The lack of concordance in the distributions of genotypes and patterns of gene flow of species in the western and central Pacific implies that differences in species traits, possibly related to life history, dispersal ability, or other aspects of their biology, or unique demographic histories have affected their current genetic population structures.

Three *Conus* species currently exhibit trans-Pacific distributions: *C. chaldaeus*, *C. ebraeus*, and *C. tessulatus* (Emerson 1991). In the Indo-West Pacific these species range from the eastern shores of Africa to the easternmost islands of the central Pacific, although *C. chaldaeus* and *C. tessulatus* have not been reported from Easter Island, and *C. tessulatus* does not occur in the Hawaiian Archipelago (Röckel et al. 1995). These three species also occur at offshore islands in the eastern Pacific, including Clipperton Atoll (all three species), Isla del Coco (all three species), the Galapagos Islands (*C. chaldaeus* and *C. ebraeus*), the Revillagigedo Islands (*C. tessulatus* only), as well as at several nearshore islands off of the coast of Central America (Emerson 1991). The phylogeography of one of these three trans-Pacific *Conus* species, *C. ebraeus*, was examined to interpret levels of differentiation and patterns of gene flow of this species in the tropical Pacific. The range of *C. ebraeus* is summarized in Fig. 1 based on data presented in Röckel et al. (Röckel et al. 1995). Planktotrophic larvae of *C. ebraeus* have a minimum pelagic period of 25–27 days (Kohn and Perron 1994) and thus have the potential to disperse relatively large distances. The following questions were addressed: Do populations of *C. ebraeus* show evidence of genetic subdivision within the western and central Pacific? Are populations of *C. ebraeus* in the eastern Pacific and the Indo-West Pacific genetically differentiated from

each other? Is gene flow between eastern Pacific and Indo-West Pacific populations occurring and, if so, is it directionally biased?

## Methods

Sequences of a fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene were obtained from 92 individuals of *C. ebraeus* from locations spread through the tropical Pacific (Fig. 1). Although *C. ebraeus* occurs in the eastern Pacific, it is not particularly abundant in some locations, so only two specimens were obtained from islands in the Gulf of Chiriquí in western Panama and 10 specimens from Clipperton Island. Tissues were preserved in 75–95% ethanol. All specimens that were specifically collected for this work were deposited in the collections of the Mollusk Division of the University of Michigan Museum of Zoology (UMMZ). Tissues from specimens from Enawetak and Clipperton Island were acquired from collections of the UMMZ and the Santa Barbara Museum of Natural History, respectively. While most specimens were from recent collections and were preserved and stored in 75–95% ethanol, specimens from Enawetak were collected in 1960 and initially preserved in propylene phenoxetol. Previously unpublished sequence data were deposited in GenBank under accession numbers EF547559–EF547649.

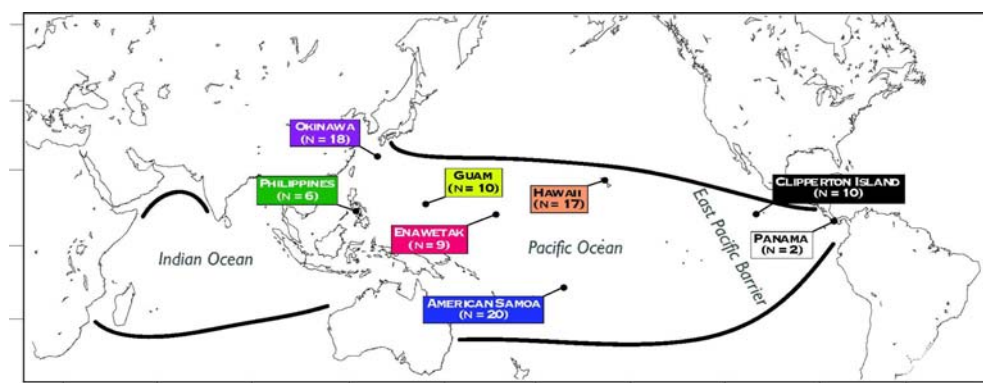
DNA was extracted from approximately 25 mg of foot tissue using methods described in Duda and Palumbi (1999a), or with the E.Z.N.A.<sup>TM</sup> Mollusc DNA Kit (Omega Bio-Tek, Doraville, Georgia, USA). Tissues from specimens from Enawetak that were originally preserved in propylene phenoxetol had a gelatin-like consistency; extractions of these tissues were also performed with the Omega Bio-Tek kit; amplifications from the resultant DNAs from these samples were by and large successful. Amplification of a fragment of the mitochondrial COI gene was accomplished using universal COI primers LCO1490 and HCO2198 (Folmer et al. 1994). Unincorporated dNTPs and primers were removed from amplified sequences with

the Qiaquick PCR Purification Kit (Qiagen, Valencia, California, USA). Approximately 611 bases of the amplified fragments were sequenced in both directions. Chromatograms were analyzed, and sequences were aligned to a sequence previously determined from an individual of *C. ebraeus* from American Samoa (GenBank accession number AY588175, Duda and Rolán 2005) with Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, Michigan, USA).

The most appropriate model for nucleotide substitution was determined with Modeltest 3.7 (Posada and Crandall 1998). Sequences were inspected to identify haplotypes that are shared among individuals with MacClade 4.0 (Maddison and Maddison 2000). To examine the distribution of haplotypes among populations, a haplotype network was constructed with TCS version 1.21 (Clement et al. 2000). Arlequin version 2.0 was used (Schneider et al. 2000) to calculate haplotype frequencies, haplotype diversity statistics, Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992), and pairwise  $\Phi_{ST}$  statistics using Tamura and Nei (1993) distances. Although hierarchical likelihood ratio tests in Modeltest selected the HKY model (Hasegawa et al. 1985) as the most appropriate model for nucleotide substitution, this model is not available in Arlequin and so Tamura-Nei distances were used; HKY and Tamura-Nei distances differ at most at the fifth decimal place. About 10,010 permutations were used to assess the degree to which obtained estimates were different than those obtained from a random assignment of haplotypes to populations. Arlequin was also used to examine mismatch distributions (Rogers and Harpending 1992; Rogers 1995) to investigate the demographic history of populations.

To distinguish whether genetic similarity between populations from the eastern Pacific and from the rest of the Pacific is due to recent separation or to recurrent gene flow after initial separation, and to determine the direction of gene flow, a Bayesian procedure, developed by Nielsen and Wakeley (2001); Hey and Nielsen (2004) was employed. This isolation–migration (IM) method uses coalescence to estimate effective population size of ancestral and daughter

**Fig. 1** Map of the Indian and Pacific Oceans with the range of *Conus ebraeus* (delimited by thick lines), collection locations and sample sizes (*n*)



populations, the time since their initial separation (i.e., the time since vicariance or the last massive invasion), and the migration rate in each direction. Specifically, the algorithm estimates the time of separation  $t$  (number of generations, scaled by mutation rate,  $\mu$ ) between populations,  $\theta = N_e\mu$  (where  $N_e$  is the effective population size of the ancestral and the two daughter populations, each estimated separately), and the scaled migration rate  $m = m/\mu$  in each direction. Analyses were implemented by pooling sequences from the eastern Pacific in one sample, those from the rest of the Pacific in another, using the HKY (Hasegawa et al. 1985) model of nucleotide substitutions, and assuming constant population size. From the results of IM, twice the number of females moving through the barrier per generation ( $M$ ) was calculated as  $M = 2N_e m_f = \theta m/2$  (where  $m_f$  is the female migration rate).

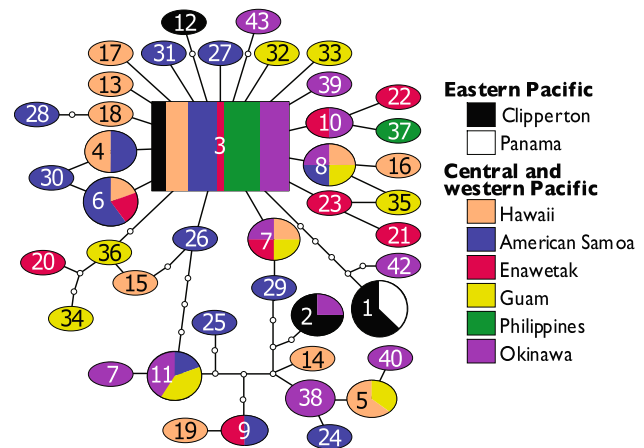
To perform the IM analysis, wide limits for prior values for each parameter were selected, then multiple runs were carried out, starting from simple unheated runs with burn-in intervals of  $10^5$  steps, and continuing by increasing the number of steps, the burn-in time, and the complexity of the heating scheme until complete (or nearly complete) posterior likelihood curves were obtained for each parameter. Runs were initiated with different random seeds. The final run, from which estimates are shown here, involved geometric heating of 20 Markov chains, a burn-in period of  $5 \times 10^6$  steps, continuous calculations of  $2.85 \times 10^8$  steps beyond the burn-in, and a minimum effective sample size (ESS) of 907.

## Results

### Sequences and haplotypes

Among the 92 individuals sequenced, 43 unique COI haplotypes were detected. These sequences differed by a maximum of 12 out of a total of 61 variable sites. Of these, 31 haplotypes were unique to single individuals, another occurred in more than one individual but was unique to a single location (haplotype 38, Fig. 2) and the remaining 11 occurred in more than one individual from more than one location (haplotypes 1–11). Haplotype diversity in western and central Pacific sites with  $N > 6$  ranged from 0.93 to 1. Clipperton in the eastern Pacific and combined eastern Pacific sites had lower haplotype diversity (Table 1).

The haplotype network constructed from the COI sequences (Fig. 2) reveals that the most common, presumably ancestral, haplotype (no. 3) occurred at all locations except for Guam and Panama. The distribution of this haplotype thus extends from the Philippines and Okinawa, all the way to the Clipperton Atoll, a distance of approximately 13,500 km. Many other haplotypes in the western



**Fig. 2** Statistical parsimony (Templeton et al. 1992) network of COI haplotypes joining 92 individuals of *Conus ebraeus* from the tropical Pacific at the 95% confidence level. The ancestral haplotype according to “outgroup weight” (Castelloe and Templeton 1994) is depicted as a rectangle. Numbers inside the shapes are designating individual haplotypes. The area of each shape is proportional to the frequency of each haplotype (haplotype 1:  $n = 6$ , 2:4, 3:19, 4:4, 5:3, 6:5, 7:4, 8:4, 9:2, 10:2, 11:5 and 38:3). Hypothetical haplotypes are indicated with empty small circles. Localities are shown in the legend

Pacific differ by one or two mutations from this common haplotype. Several other haplotypes differ by more than four sites from the ancestral haplotype.

Analysis of molecular variation within and between regions

AMOVA comparison of genetic variation within and between the eastern Pacific samples versus the western and central Pacific ones revealed that nearly 74% of the variance in COI was within localities, and only 1% between localities within regions (Table 2). Approximately one-fourth (25.2%) of the variance was between regions.

Pairwise  $F$ -statistics

As is to be expected given the AMOVA results, pairwise  $\Phi_{ST}$  values among localities show evidence of significant genetic separation between eastern Pacific and western + central Pacific samples (Table 3). Within the western and central Pacific, samples from each site show no evidence of structure, and pairwise  $\Phi_{ST}$  values are considerably smaller than those observed between western + central Pacific site and the Clipperton Island samples; none of the comparisons within the western + central Pacific are significantly different from zero (Table 3).

Historical demography of populations

The mismatch distribution of haplotypes from the entire western and central Pacific was not significantly different

**Table 1** Sample sizes and haplotype diversity of *Conus ebraeus* from several sites in the Pacific

	Number of individuals	Number of haplotypes	Haplotype diversity (SE)
<i>Eastern Pacific</i>			
Clipperton	10	4	0.778 (0.091)
Panama	2	1	0.000 (0.000)
Combined	12	4	0.712 (0.105)
<i>Central and western Pacific</i>			
Hawaii	17	13	0.963 (0.033)
American Samoa	20	14	0.947 (0.034)
Enawetak	9	9	1.000 (0.052)
Guam	10	9	0.978 (0.054)
Philippines	6	2	0.333 (0.215)
Okinawa	18	12	0.935 (0.041)
Combined	80	41	0.942 (0.018)

Standard errors are indicated in parentheses

**Table 2** Analysis of molecular variance (AMOVA) and  $\Phi$ -statistics of populations of *Conus ebraeus* from the eastern Pacific and western + central Pacific

Category	Observed partition				
	Variance	% explained	$\Phi$ -statistics	<i>P</i>	df
Among regions	0.631	25.2	$\Phi_{CT} = 0.252$	<0.00001	1
Among populations within regions	0.026	1.0	$\Phi_{SC} = 0.014$	0.244	6
Within populations	1.845	73.7	$\Phi_{ST} = 0.263$	<0.00001	84

**Table 3** Pairwise  $\Phi_{ST}$  values for all populations of *Conus ebraeus* in the tropical Pacific with *n* > 5

	Eastern Pacific	Central and western Pacific					
	Clipperton	Hawaii	American Samoa	Enawetak	Guam	Philippines	Okinawa
<i>Eastern Pacific</i>							
Clipperton	–						
<i>Central and western Pacific</i>							
Hawaii	0.189***	–					
American Samoa	0.210****	–0.025	–				
Enawetak	0.194**	–0.021	–0.009	–			
Guam	0.157**	–0.019	0.006	–0.015	–		
Philippines	0.248*	–0.011	–0.010	–0.047	0.039	–	
Okinawa	0.112*	0.017	0.034	0.039	–0.021	0.085	–

Probabilities that observed  $\Phi_{ST}$  values deviate from a null hypothesis of no difference between populations were determined from the proportion of 10,100 permutations of haplotypes between populations that gave  $\Phi_{ST}$  values greater than or equal to the observed  $\Phi_{ST}$  (\* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.005, \*\*\*\* *P* < 0.001)

from the unimodal distribution expected from recent expansion (*P* = 0.249) (Fig. 3). Similar results were also obtained when samples from sites in the western and central Pacific were examined individually by location. Estimates of initial and current  $\theta$  following population expansion ( $\theta_0$  and  $\theta_1$ , respectively, where  $\theta = 2N_e\mu$ ,  $N_e$  is the effective population size and  $\mu$  is the mutation rate) of the combined western and central Pacific sites are  $\theta_0 = 2.752$  (95% confidence interval = 0.000–8.400) and  $\theta_1 = 1062.5$  (95% confidence interval = 20.994–7586.3).

The estimated value of  $\tau$  (the number of generations scaled by mutation rate) for the combined data from samples in the western and central Pacific is 1.399 (95% confidence interval: 0.352–5.500). On the other hand, the mismatch distribution for data from the two sites in the eastern Pacific is bimodal, and the least squares fit is significantly different than one determined for a simulated recently expanded population (*P* = 0.005). Nonetheless, because the sample size from this region is small this result is not robust.

Despite IM run durations longer than 1 year, it proved impossible to obtain a distinct inflection point in the posterior probability density curve of  $\theta$  for the populations from the western and central Pacific, indicating that the data from this single locus did not contain the information necessary for the estimation of this parameter. The curve of posterior probability density for this parameter rose from a low value to a plateau, such that all estimates of  $\theta$  greater than 2,000 had the same approximate probability, and so it is reasonable to consider 2,000 as a minimum estimate of  $\theta$  (see Hey 2005). Convergence was reached for all other parameters. Based on these results,  $\theta$  for combined data from the western and central Pacific ( $\theta_{W+CP} > 2000$ ) was considerably larger than estimates of  $\theta$  for the eastern Pacific [ $\theta_{EP} = 0.045$  (highest posterior probability interval HPPI): 0.045–1.04] and of the ancestral population ( $\theta_A = 11.11$ , HPPI: 0.027–69.09). These results were consistent with those from analyses of mismatch distributions in which the population from the western and central Pacific appears to have undergone recent population

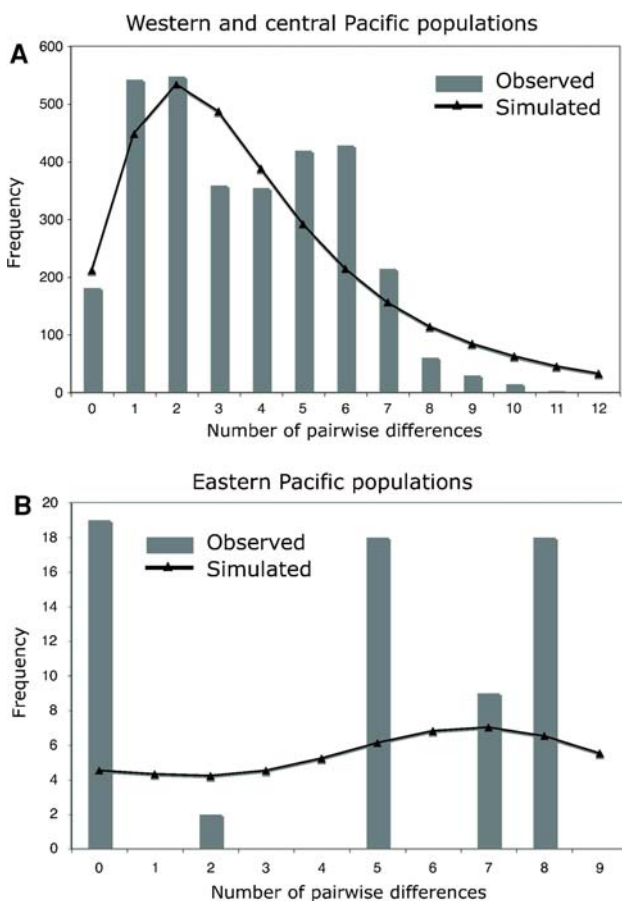
expansion while the population from the eastern Pacific has not. While the scaled probability of migration into the eastern Pacific was higher ( $m_{EP} = 40.2$ , HPPI: 0.25–394.25) than that into the western and central Pacific ( $m_{W+CP} = 0.05$ , HPPI: 0.05–8.68), the number of female propagules ( $M = 2N_e m = \theta m/2$ ) that the western and central Pacific has received ( $M_{W+CP} > 50.0$ ) was much higher than the number that the eastern Pacific has received ( $M_{EP} = 0.92$ ) if the estimation of  $\theta_{W+CP}$  is reasonable. Also, the proportion of time during the IM run that  $M_{W+CP}$  was greater than  $M_{EP}$  was 0.99. The estimated scaled time of divergence of populations ( $t = g/u$ ,  $g$  is number of generations) was 0.315 (HPPI: 0.075–3.375).

## Discussion

### Connectivity among western and central Pacific populations

Even though all samples of *C. ebraeus* in the western and central Pacific included haplotypes unique to particular sites, the majority of these haplotypes were only a few mutational steps removed from the most common haplotype. Many other haplotypes were shared among populations (Fig. 2). Thus, COI in *C. ebraeus* indicates high levels of on-going gene flow among populations west of the EPB, including those from Hawaii, or else that *C. ebraeus* recently expanded in these regions. Pairwise estimates of  $\Phi_{ST}$  between populations in this region also reveal an overall lack of structure among these populations (Table 3). No  $\Phi_{ST}$  value between any pair of populations from the western and central Pacific was significant. Analysis of molecular variance further demonstrates that the majority of the genetic variation occurs within populations and only approximately 1% of the variation is partitioned among populations within regions when considering populations from the western + central and eastern Pacific (Table 2).

The genetic structure of *C. ebraeus* in the western and central Pacific perhaps best resembles that of several other Indo-West Pacific species: the nerite gastropods *Nerita albicilla* and *N. plicata* (Crandall et al. 2008) and the sea urchins *Tripneustes gratilla* (Lessios et al. 2003) and *Diadema savignyi* (Lessios et al. 2001). As in *C. ebraeus*, several mitochondrial haplotypes are shared among populations throughout the range of each species. Pairwise estimates of  $\Phi_{ST}$  are generally low, though in *Tripneustes* some are significant. Larvae of *Diadema* and *Tripneustes* have planktonic phases that last from one to three months, and so, similar to *C. ebraeus*, they may achieve high rates of gene flow due to great dispersal abilities of their planktonic larval stage.



**Fig. 3** Mismatch distributions of COI haplotypes of populations of *Conus ebraeus* from **a** the western and central Pacific and **b** the eastern Pacific. Curves depicting frequency distributions of pairwise sequence differences simulated under a model of population expansion are illustrated for each set of haplotypes

Results from analysis of  $F$ -statistics are incapable of distinguishing between low levels of gene flow among recently derived populations and higher levels of gene flow among long separated populations. The mismatch distribution of combined haplotype sequences of western and central Pacific populations of *C. ebraeus* (Fig. 3) is consistent with a model of recent expansion within this region. COI sequences of a pair of transisthmian *Conus*, *C. gladiator* and *C. mus*, given by Duda and Rolán (2005) and an estimated time of divergence of these species at 6.9 my (Duda and Kohn 2005) provides a mutation rate of 3.7 substitutions per million year for the amplified fragment of COI. Based on this rate and the estimated value of  $\tau$ , expansion of western and central Pacific populations presumably took place during the last 190 thousand years; the 95% confidence interval for the timing of the expansion is 50–740 thousand years. Accordingly, fossils of *C. ebraeus* are known from Pleistocene deposits in the Seychelles (Braithwaite et al. 1973), Australia (Kohn 1997), and Hawaii (Kosuge 1969) but have not been reported from earlier deposits. The coconut crab, *Birgus latro* (Lavery et al. 1996b), and the sea urchin *T. gratilla* (Lessios et al. 2003) also show similar mismatch distributions as those of *C. ebraeus* and apparently also recently expanded in the western Pacific during the Pleistocene. Based on a variety of Bayesian analyses, the nerites *N. albicilla* and *N. plicata* also appear to have recently expanded in the Pacific during the Pleistocene (Crandall et al. 2008). Few other phylogeographic studies have specifically examined the demographic histories of species in the western and central Pacific, but gene trees of several, including some echinoderms, such as *D. savignyi*, *D. setosum* and *D. paucispinum* (Lessios et al. 2001) and a fish (*Chlorurus sordidus*, Bay et al. 2004), exhibit a star-like pattern that is suggestive of recent population expansion. Thus, the limited degree of genetic differentiation of populations of these species may simply reflect recent expansion, rather than current levels of gene flow and this pattern appears to be shared by many other western and central Pacific marine taxa.

#### History and origins of populations in the eastern Pacific

Trans-Pacific distributions of tropical prosobranch gastropod species that are widely distributed and abundant in the Indo-West Pacific are often assumed to indicate relatively recent range expansions from these areas into the eastern Pacific (e.g., Emerson 1991). However, if speciation is often peripatric (Mayr 1970), there is also the possibility that a new species would arise in the margins of the ancestral distribution, then spread into the center. In *C. ebraeus* the ancestral haplotype is found in the western, central and eastern Pacific, so it provides no information regarding the direction of invasion across the East Pacific

Barrier. In 13 out of 20 trans-Pacific fishes examined by Lessios and Robertson (2006) source populations were not obvious for the same reason.

#### Patterns of gene flow between western + central Pacific and eastern Pacific populations

The results of the IM analysis are tentative, not only because it proved impossible to obtain a good estimate of effective population size for the western + central Pacific, but also because a single locus does not necessarily provide good estimates of population history. Nevertheless, the estimate that more larvae cross the EPB in a westerly direction and contribute genes to populations in the western and central Pacific is similar to results recently obtained for a variety of fishes (Lessios and Robertson 2006). Out of 15 comparisons of trans-Pacific populations of fishes across the EPB in which directionality of migration could be determined, Lessios and Robertson (2006) found that 11 showed a higher rate of gene flow westward. They attributed this trend to the higher probability that migrants across this large oceanic stretch would successfully mate and leave their genes behind when they arrived at the denser and more widespread populations in the west. The time of separation between eastern and western + central Pacific populations of *C. ebraeus* estimated by IM, when calibrated with the same substitution rate of *Conus* as the estimates obtained from the mismatch analysis, is approximately 84 thousand years. If this estimate is correct, there was a large pulse of migration, obliterating any genetic differences that might have existed between populations within the last millennium. Given that the sample from the eastern Pacific is composed almost entirely of *C. ebraeus* collected at the Clipperton Atoll, the westernmost island in the eastern Pacific, the fauna of which is a mixture of eastern and central Pacific species (Emerson 1994; Robertson and Allen 1996), this scenario is not entirely implausible.

#### Sample sizes, geographic coverage and single locus analysis

Because of the difficulty of obtaining *C. ebraeus* from many places, sample sizes from some localities are small, and geographic coverage, albeit widespread within the Pacific, is not dense. Nonetheless, the inclusion of COI data from additional individuals or localities would not be likely to produce results that contradict the main interpretations concerning the lack of structure within the western + central Pacific and distinctiveness of the eastern Pacific population even if inferences of demographic histories are more tentative. For example, despite low sample sizes from some sites, pairwise  $\Phi_{ST}$  values estimated between sites in the western and central Pacific are consistently not

significant, whereas those estimated between sites in the western + central Pacific and eastern Pacific are consistently significant. These interpretations are also based solely on examination of a single mitochondrial gene. Presumably variation in the marker is effectively neutral, but if this were not the case (e.g., if a recent selective sweep of mitochondrial DNA had occurred), then the inferences on demographic histories would be in error. Examination of additional loci would be useful in evaluating these interpretations.

The phylogeography of *C. ebraeus* contrasts greatly with that of another gastropod, *Astrarium rhodostomum* (Meyer et al. 2005). Like *C. ebraeus*, *A. rhodostomum* has a wide distribution (from Thailand to Polynesia) and possesses a planktonic larva. But unlike the planktonic larvae of *Conus*, those of *Astrarium* are unable to feed, and thus spend less time in the plankton. Possibly due to this difference, *A. rhodostomum* displays a high degree of fine-scale endemism, with 30 separate clades, many of them geographically isolated from each other. Meyer et al. suggest that such “species” that are morphologically uniform but genetically differentiated between archipelagos are common among gastropods. *C. ebraeus* definitely does not fit this pattern, and its phylogeography is more similar to that of other marine taxa than it is to *Astrarium*. Thus, each marine species, independently of its phylogenetic affinities, has its own unique history and its own biological characteristics that can affect its phylogeographic history. Many more studies of widespread species will be needed before the factors that contribute to modern-day patterns can be elucidated.

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