When oceans meet: a teleost shows secondary intergradation at an Indian–Pacific interface

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The Indo-West Pacific is characterized by extraordinary marine species diversity. The evolutionary mechanisms responsible for generating this diversity remain puzzling, but are often linked to Pleistocene sea level fluctuations. The impact of these sea level changes on the population genetic architecture of the estuarine fish *Lates calcarifer* are investigated via a natural experiment in a region of the Indo-West Pacific known to have undergone considerable change during the Pleistocene. *L. calcarifer*, a coastline-restricted catadromous teleost, provides an excellent model for studying the effects of sea level change as its habitat requirements potentially make it sensitive to the region's physical history. Evidence was found for a large phylogenetic break (4% mtDNA control region; 0.47% ATPase 6 and 8) either side of the Torres Strait, which separates the Western Pacific and Indian Oceans, although some mixing of the clades was evident. This suggests clinal secondary introgression of the clades via contemporary gene flow. Further, populations on Australia's east coast appear to have passed through a bottleneck. This was linked to the historical drying of the Great Barrier Reef coastal lagoon, which resulted in a significant loss of habitat and forced retreat into isolated refugia. These results suggest that historical eustatic changes have left a significant imprint on the molecular diversity within marine species as well as among them in the Indo-West Pacific.

Keywords: marine biogeography; mtDNA; control region; secondary intergradation; Pleistocene; population genetics

1. INTRODUCTION

Global regions with high biodiversity have historically attracted biologists' inquiries into mechanisms responsible for the origin and distribution of extant species. In the marine realm, the Indo-West Pacific (IWP) may be unrivalled for its species richness (McManus 1985). Accordingly, the level of biogeographic investigation has also been rich (reviewed by Rosen 1988). Despite a long-term focus on the region, few convincing reasons for this diversity have emerged.

Recent evidence from molecular genetics suggests diversification of some IWP marine taxa occurred as late as the Pleistocene (Palumbi 1996). Under the assumption of a molecular clock, the mtDNA lineages of several congeneric species of urchin (Palumbi 1996) and butterflyfishes (McMillan & Palumbi 1995) coalesce in the Pleistocene (Palumbi 1996). These results suggest that conditions favouring speciation were present in the IWP during the Pleistocene. Generally, one of two earth history events has been proposed as providing conditions favourable for diversification: tectonic collisions during the Miocene (Springer 1982) or sea level fluctuations during the Pleistocene (Rosen 1988).

In light of the 'recent' speciation of urchins and butterflyfishes in the IWP, an investigation into the population genetic consequences of those Pleistocene sea level changes for IWP marine taxa is warranted. Small changes in sea level can result in significant alterations in archipelago shape, with some land bridges resulting from falls of less than 10 m from the present mean sea level (McManus 1985). One such region is the confluence of the Indian and Pacific Ocean via the Arafura Sea and Torres Strait, between northern Australia and Papua New Guinea. Sea level changes were sufficient to open and close this passage on several occasions during the Pleistocene (Chappel & Shackleton 1986). Thus, marine populations which presently exchange genes between the Pacific and Indian Oceans via this route exclusively may have been isolated when this passage was closed. This intermittent barrier provides a natural experiment to investigate some of the effects of sea level change on marine taxa.

Species expected to have experienced strongest vicariance are those incapable of gene flow around the north of the island of New Guinea. One such species is *Lates calcarifer*, a coastline-restricted catadromous teleost which inhabits estuaries, rivers, and bays from East Africa through the south-east Asian archipelago to Australia's east coast. *L. calcarifer* does not occur along Papua New Guinea's eastern coastline where the continental slope is steep and a coastal lagoon is absent (Keenan 1994). Genetic analyses of contemporary structure suggest isolation-by-distance along the Australian coastline and that

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Figure 1. Geographical distribution of regions sampled for *Lates calcarifer*.

dispersal is somewhat limited (Chenoweth *et al.* 1998). Such attributes make *L. calcarifer* potentially sensitive to the regions' physical history.

We sampled the spatial distribution of, and estimated the genealogical relationships among, hypervariable mtDNA control region sequences in *L. calcarifer* throughout an oceanic confluence in the IWP. Three biological scenarios and expected patterns of mtDNA haplotype geography and genealogy can be proposed: (1) no evidence for vicariance—the mtDNA phylogeny will not be obviously partitioned and mtDNA lineages will be randomly distributed across the sampled range; (2) evidence for vicariance with no contemporary gene flow—the mtDNA phylogeny will be monophyletic either side of the Torres Strait, no mtDNA lineages will be shared between sides; or (3) vicariance followed by contemporary gene flow—the mtDNA phylogeny will contain two monophyletic clades, but their geographic distribution will overlap and reflect patterns of secondary intergradation.

2. MATERIALS AND METHODS

(a) Sampling design

The spatial distribution of mtDNA lineages was characterized with balanced sampling in each of three regions along the northern Australian coastline, the western Arafura Sea, the Gulf of Carpentaria and the Coral Sea. Within each region, 30 individuals from each of three locales were collected, making a

total sample size of 90 individuals per region (figure 1). A sample size of 90 individuals is sufficient to detect at least one copy of all haplotypes which occur at 1% frequency with 95% confidence (Schwager *et al.* 1990). This sampling power provided a necessary check for the sympatric distribution of independently evolving mtDNA lineages.

(b) mtDNA analysis

We screened a PCR-amplified 270 bp fragment of region 1 of the mitochondrial control region for sequence variation using temperature gradient gel electrophoresis and automated DNA sequencing. Methods for DNA extraction, amplification, screening, and sequencing can be found in Chenoweth & Hughes (1997) and Chenoweth *et al.* (1998).

(c) Statistical analysis

Haplotype diversity was calculated using the package REAP (McElroy *et al.* 1992), nucleotide diversity statistics and their standard errors were calculated using the algorithm of Nei & Jin (1989) with patristic distances generated by the neighbour-joining method of Saitou & Nei (1987). Two classes of statistical test of the neutral evolution hypothesis were performed, Tajima's (1989) test which uses information at the nucleotide sequence level and Chakroborty's (1990) test which is based on the number and relative frequency of haplotypes regardless of nucleotide sequence (ARLEQUIN 1.0, Schneider *et al.* 1997).

Due to the large numbers of haplotypes, a reconstruction of the phylogenetic relationships among them was performed using maximum likelihood by quartet puzzling, which is

computationally expedient compared with traditional likelihood methods (Strimmer & von Haeseler 1996). A Tamura & Nei (1993) model of evolution was assumed with an empirically derived transition to transversion ratio of 13 ± 2.36 . One thousand quartet puzzling steps were performed on a UNIX workstation.

3. RESULTS AND DISCUSSION

(a) *Vicariance and contemporary gene flow*

From the 270 individuals screened, 63 putative haplotypes were resolved. No haplotypes were shared among regions. Maximum likelihood phylogeny reconstruction yielded strong support for two monophyletic clades, A and B, which differed by approximately 4% sequence divergence (figure 2). The geographic distributions of clades A and B are not restricted to particular sides of the Torres Strait; instead, clades occur in near-reciprocal frequencies in each region. Clade A has the highest frequency in the Coral Sea and the lowest frequency in the Arafura Sea, while the frequency of clade B decreases from east to west, having the highest frequency in the western Arafura Sea (figure 3). Randomized χ^2 tests (Roff & Bentzen 1989) of the spatial distribution of individuals bearing clade A and B haplotypes indicate that clades are non-randomly distributed across the sampled range ($p < 0.001$).

The observed pattern of mtDNA genealogy and the spatial distribution of haplotypes best fit our third scenario. A large break in the phylogeny indicates that a vicariant history may partially explain the pattern of mtDNA variation in *L. calcarifer*. The break between clades A and B could also be a consequence of random lineage sorting. Under simulations, random sorting of mtDNA lineages can lead to 'breaks' in intraspecific phylogenies in a continuous population when dispersal is limited (Saunders *et al.* 1986). However, random lineage sorting appears unlikely in this case for the following three reasons.

First, data from independent allozyme loci are consistent with the pattern of mtDNA variation. In a study of *L. calcarifer* allozyme variation along the northern Australian coastline, genetic distances between populations either side of the Torres Strait were greater than comparisons among more distant populations on any given side of the strait (Keenan 1994). Furthermore, rare 'western' and 'eastern' alleles were found to coexist at several sites in the Gulf of Carpentaria (Keenan 1994).

Second, there are obvious causes for a vicariant event. Throughout the Pleistocene the Torres Strait has opened and closed several times due to sea level changes (Chappel & Shakleton 1986). The most recent reopening occurred only 7000 years BP after a closure of *ca.* 108 000 years, while previous closures persisted for over 200 000 years (Galloway & Kemp 1981; Torgersen *et al.* 1985). The fact that *L. calcarifer* does not occur along the eastern coastline of Papua New Guinea (PNG) also lessens the likelihood of gene flow occurring around PNG during times of low sea level.

Third, a vicariance interpretation is strengthened by the existence of Indian-Pacific phylogenetic breaks in other marine species. The coconut crab, *Birgus latro*, exhibits a 2% total mtDNA break between Pacific and Indian ocean populations (Lavery *et al.* 1996).

Furthermore, two species complexes of *Chaetodon* butterflyfishes, the *rhombochaetodon* and *punctatofasciatus* groups exhibit approximately 2% divergence in mtDNA cytochrome *b* sequences between Pacific and Indian Ocean endemics (McMillan & Palumbi 1995). It should be noted that these species have been sampled more sparsely and at wider spatial scales than *L. calcarifer*. Species sampled on spatial scales more closely matched to that of *L. calcarifer* also exhibit significant divergence among mtDNA clades either side of the Torres Strait. The estuarine mud crab, *Scylla serrata*, exhibits 2% divergence in cytochrome oxidase I (COI) sequences (D. Gopurenko and C. Keenan, unpublished data). The marine prawns, *Penaeus esculentus* and *P. merguensis*, are also differentiated by 0.6 and 1.3% COI sequence divergence, respectively, between Arafura and Coral Seas (S. Lavery, unpublished data).

As vicariance appears to be the most likely force responsible for the generation of the two mtDNA clades A and B, we may question the timing of divergence. Due to the occurrence of extreme mutation rate heterogeneity in the control region relative to the remainder of the mitochondrial genome (Wakely 1993), a more reliable estimate of the level of sequence divergence between the two clades may be gained from sequence data comprising the entire ATPase 8 gene and codons 1–203 of the ATPase 6 gene (760 bp) for each of 20 haplotypes representing the two control region clades (S. F. Chenoweth, unpublished data). These results suggest the two clades only diverge by 0.47%, implying a near tenfold evolutionary rate discrepancy between the *L. calcarifer* control region and the ATPase genes.

In fishes, the ATPase genes have been suggested to diverge at a rate of *ca.* 1.4% per million years (at all sites) as calibrated by the rise of the isthmus of Panama (Bermingham *et al.* 1997). This divergence rate suggests that clade A and B individuals shared a common maternal ancestor around 335 000 years BP, which suggests that eustatic changes have led to the split sometime during the Pleistocene.

Gene flow appears to have transported haplotypes from both clades from their likely origins across the sampled range. Direct investigations of *L. calcarifer* movements using mark-recapture techniques suggest a mean single generation dispersal distance of 15.3 km in Coral Sea populations (Russell & Garrett 1988). Although this figure may have a large variance and is not exactly estimating gene flow, it can be used as a crude measure to determine if the secondary intergradation can be explained by the most recent reopening of the Torres Strait. The maximum distance that clades have moved is 4835 km, assuming a generation time of five years (Keenan 1994), 316 generations, or 1580 years are required to transport either clade across the sampled range. As the Torres Strait last reopened 8000 years BP, a more than adequate amount of time has elapsed to account for the observed pattern.

(b) *Low diversity and non-neutrality in the Coral Sea*

An unexpected yet interesting result was the low mtDNA diversity and departure from neutrality in the Coral Sea. There was an overall pattern of decreasing diversity indices from west to east. The Coral Sea

Figure 2. Unrooted consensus tree of 1000 quartet puzzling steps of maximum likelihood generated quartet trees among *L. calcarifer* haplotypes. The nucleotide sequence for the most common haplotype appears in GenBank (accession no. U85194). The bifurcation between clades A and B occurred also in supported trees constructed using the neighbour-joining method with bootstrapping (Saitou & Nei 1987) and its resolution was insensitive to corrections for unequal rates of nucleotide substitutions among sites (Tamura & Nei 1993) using gamma parameters ranging from 0.1 to 1.0.

Figure 3. Geographical distribution of two major *L. calcarifer* mtDNA clades, A and B.

contained the lowest number of haplotypes, significantly lower haplotype diversity than other regions, and significantly lower nucleotide diversity than the Arafura Sea (table 1). The hypothesis of neutral evolution could not be rejected with Tajima's test in any region, but was rejected with the Chakraborty test for the Coral Sea sample (table 1). A significantly greater number of haplotypes than expected under neutrality and a single haplotype at a high frequency of 42% characterizes the pattern of haplotype diversity in the Coral Sea. Three hypotheses can be proposed for the above phenomenon including hidden population subdivision within the sample, a selective sweep, or a bottleneck during recent *L. calcarifer* evolution (Chakraborty 1990).

The first explanation seems unlikely because although population subdivision has been reported within this region (Chenoweth *et al.* 1998), it has also been detected among locales within the other two regions sampled (mean ϕ_{st} values among locales within regions: Coral Sea=0.098, Gulf of Carpentaria=0.087, and Western Arafura Sea=0.075). Thus, it appears that population subdivision alone will not adequately account for the pattern.

The mitochondrial control region is itself non-coding, but is linked to other coding genes in the mitochondrial

genome; therefore, a selective sweep favouring a single haplotype, followed by an expansion of the population in the Coral Sea, is possible. One would have to postulate different selection regimes operating in different oceanic regions under this hypothesis. Although there is no *a priori* reason to suspect this, we cannot rule out this possibility with a single locus such as mtDNA. An investigation of nucleotide sequence variation at independent nuclear loci would permit a robust test of this hypothesis.

Population demography can mimic patterns expected under selection, thus information on past demographic events can be gained from a significant test of neutrality (Rand 1996). In the present study, an interplay between the bathymetry of the Coral Sea and the habitat requirements of *L. calcarifer* lends support for a population bottleneck hypothesis. At times when the sea level is low, due to a shallow gradient, the Great Barrier Reef coastal lagoon dries and fully oceanic conditions are created along the east coast of Australia. This is an unsuitable habitat for *L. calcarifer*, as is evident by its absence from such areas along the eastern coastline of PNG. It is likely that during times of low sea level a single large estuary acted as a refuge for *L. calcarifer* in the Coral Sea (Keenan 1994). In such a scenario, genetic drift would have been able to overcome the effect of homogenizing gene flow and sort many haplotypes to extinction, thus facilitating the increase in frequency of a single common haplotype. As sea levels began to rise, large amounts of shallow coastal habitat would have been created due to the shallow gradient of the Great Barrier Reef coastal lagoon (Galloway & Loffler 1974), a requisite habitat for *L. calcarifer* juvenile survival (Russell & Garrett 1985). A concomitant increase in *L. calcarifer* effective population size would allow novel haplotypes to persist at low frequencies because the effect of random lineage extinction is less severe in an expanding population than a stable one (Avice *et al.* 1984).

4. CONCLUSION

The extent to which historical environmental changes have influenced *L. calcarifer* mtDNA genealogy and geography appears to be significant. Vicariance facilitated by low sea levels during the Pleistocene resulted in reciprocal monophyly of mtDNA lineages in populations on either side of the Torres Strait. Following inundation of the barrier, gene flow transported haplotypes from each clade in both directions through the strait, producing the

Table 1. Indices of intraregional mtDNA diversity with their standard errors and tests of neutrality for *Lates calcarifer*

(k_{obs} refers to the number of observed haplotypes, while k_{exp} is the number of haplotypes expected under the neutral model.)

region	diversity indices		neutrality tests		
			Tajima	Chakraborty	
	haplotype diversity	nucleotide diversity	D	k_{obs}	k_{exp}
Coral Sea	0.763±0.044	0.024±0.005	−0.4594 n.s.	17 ^a	9.5
Gulf of Carpentaria	0.914±0.013	0.032±0.005	0.1206 n.s.	22	22.5
Western Arafura Sea	0.933±0.011	0.050±0.008	1.7566 n.s.	24	26.7

^a $p < 0.001$.

pattern of secondary intergradation. Coral Sea populations apparently experienced a loss of mtDNA diversity either due to a bottleneck in response to lowered sea levels or a selective sweep on the mitochondria.

Accumulation of intraspecific molecular data from IWP marine taxa remains in its genesis but the comparisons drawn in the present study provide compelling evidence that strong evolutionary forces, most likely aided by sea level changes, were present during the Pleistocene. Additional comparative data sets will hopefully clarify these patterns.

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REFERENCES

- Avise, J. C., Neigel, J. E. & Arnold, J. 1984 Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *J. Molec. Evol.* **20**, 99–105.
- Bermingham, E., McCafferty, S. S. & Martin, A. P. 1997 Fish biogeography and molecular clocks: perspectives from the Panamanian isthmus. In *Molecular systematics of fishes* (eds T. D. Kocher & C. A. Stepien), pp. 113–128. San Diego: Academic Press.
- Chakraborty, R. 1990 Mitochondrial DNA polymorphism reveals hidden heterogeneity within some Asian populations. *Am. J. Hum. Genet.* **47**, 87–94.
- Chappell, J. & Shackleton, N. J. 1986 Oxygen isotopes and sea level. *Nature* **324**, 137–140.
- Chenoweth, S. F. & Hughes, J. M. 1997 Genetic population structure of the catadromous Perciform, *Macquaria novemaculeata*. *J. Fish. Biol.* **50**, 721–733.
- Chenoweth, S. F., Hughes, J. M., Keenan, C. P. & Lavery, S. 1998 Concordance between mitochondrial gene flow and dispersal: isolation by distance in the tropical teleost, *Lates calcarifer*. *Heredity*. (In the press.)
- Galloway, R. W. & Löffler, E. 1974 Aspects of geomorphology and soils in the Torres Strait Region. In *Bridge and barrier: the natural and cultural history of Torres Strait* (ed. D. Walker), pp. 11–28. Canberra: Australian National University.
- Galloway, R. W. & Kemp, E. M. 1981 Late Cainozoic environments in Australia. In *Ecological biogeography of Australia* (ed. A. Keast), pp. 51–80. Junk: The Hague.
- Keenan, C. P. 1994 Recent evolution of population structure in Australian barramundi, *Lates calcarifer* (Bloch): an example of isolation by distance in one dimension. *Aust. J. Mar. Freshwater Res.* **45**, 1123–1148.
- Lavery, S., Moritz, C. & Fielder, D. R. 1996 Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. *Molec. Ecol.* **5**, 557–570.
- McElroy, D., Moran, P., Bermingham, E. & Kornfield, I. 1992 REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. *J. Hered.* **83**, 157–158.
- McManus, J. W. 1985 Marine speciation, tectonics and sea-level changes in south east Asia. *Proc. 5th. Int. Coral Reef Con.* **4**, 133–138.
- McMillan, W. O. & Palumbi, S. R. 1995 Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. *Proc. R. Soc. Lond. B* **260**, 229–236.
- Nei, M. & Jin, L. 1989 Variances of the average numbers of nucleotide substitutions within and between populations. *Molec. Biol. Evol.* **6**, 290–300.
- Palumbi, S. R. 1996 What can molecular genetics contribute to marine biogeography? An urchin's tale. *J. Exp. Mar. Biol. Ecol.* **203**, 75–92.
- Rand, D. M. 1996 Neutrality tests of molecular markers and the connection between DNA polymorphism, demography and conservation biology. *Conserv. Biol.* **10**, 665–671.
- Roff, D. A. & Bentzen, P. 1989 The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small sample size. *Molec. Biol. Evol.* **6**, 539–545.
- Rosen, B. R. 1988 Progress, problems and patterns in the biogeography of reef corals and other tropical marine organisms. *Helgolander Meeresunters.* **42**, 269–301.
- Russell, D. J. & Garrett, R. N. 1985 Early life history of barramundi, *Lates calcarifer* (Bloch). *Aust. J. Mar. Freshwater Res.* **36**, 203–218.
- Russell, D. J. & Garrett, R. N. 1988 Movements of juvenile barramundi, *Lates calcarifer* (Bloch), in North-Eastern Queensland. *Aust. J. Mar. Freshwater Res.* **39**, 117–123.
- Saitou, N. & Nei, M. 1987 The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* **4**, 406–425.
- Saunders, N. C., Kessler, L. G. & Avise, J. C. 1986 Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus*. *Genetics* **112**, 613–627.
- Schneider, S., Kueffer, J., Roessli, D. & Excoffier, L. 1997 *ARLEQUIN: exploratory molecular population genetics software, v. 1*. University of Geneva.
- Schwager, S. J., Mutchler, M. A., Federer, W. T. & Scully, B. T. 1990 The effect of linkage on sample size determination for multiple trait selection. Technical Report No. BU-1031-MA., Ithaca, New York: Biometrics Unit, Cornell University.
- Springer, V. G. 1982 Pacific plate biogeography, with special reference to shorefishes. *Smithsonian contributions to zoology*, no. 367. Washington: Smithsonian Institution Press.
- Strimmer, K. & von Haeseler, A. 1996 Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. *Molec. Biol. Evol.* **13**, 964–969.
- Tajima, F. 1989 Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–595.
- Tamura, K. & Nei, M. 1993 Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molec. Biol. Evol.* **10**, 512–526.
- Torgersen, T., Jones, M. R., Stephens, A. W., Searle, D. E. & Ullman, W. J. 1985 Late Quaternary hydrological changes in the Gulf of Carpentaria. *Nature* **313**, 785–787.
- Wakeley, J. 1993 Substitution rate variation among sites in hypervariable region 1 of human mitochondrial DNA. *J. Molec. Evol.* **37**, 613–623.