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# Reconciling geography and genealogy: phylogeography of giant freshwater prawns from the Lake Carpentaria region

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**Abstract**

**There is convincing geological evidence for the historical existence of an ancient lake on the Australian–New Guinea continental shelf during the late Pleistocene. Lake Carpentaria was a vast fresh- to brackishwater lake that would presumably have provided habitat for, and facilitated gene flow among, aquatic taxa that tolerate low to moderate salinities in this region. Moreover, it has been argued that the outflow of Papua New Guinea's Fly River was diverted westward into Lake Carpentaria during this period, although this hypothesis is controversial. We predicted that these events, if a true history, would have promoted gene flow and population growth via range-expansion events in the giant freshwater prawn (*Macrobrachium rosenbergii*) and restricted gene flow subsequently by way of a vicariant event as sea levels rose during the late Pleistocene, and a marine environment replaced Lake Carpentaria. We tested these hypotheses using phylogeographical and phylogenetic analyses of mitochondrial DNA variation in *M. rosenbergii* populations sampled from the Lake Carpentaria region. Our results support the hypothesis that Lake Carpentaria facilitated gene flow among populations of *M. rosenbergii* that are today isolated, but contest claims of a westward diversion of the Fly River. We inferred the timing of initial expansion in the 'Lake Carpentaria lineage' and found the timing of this event to be broadly concordant with geological dating of the formation of Lake Carpentaria. Reconciling geological and molecular data, as presented here, provides a powerful framework for investigating the influence of historical earth history events on the distribution of biological (i.e. molecular) diversity.**

*Keywords*: biogeography, Fly River, Lake Carpentaria, nested clade analysis, phylogeography, range expansion

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## Introduction

The recent development of statistical phylogeographical methodologies (e.g. Templeton *et al*. 1995; Knowles & Maddison 2002) has enabled researchers to distinguish between competing biological (e.g. dispersal) or earth history (e.g. vicariance) events that may have influenced patterns of genetic variation. Intraspecific phylogeographical studies have specifically tested biogeographical hypotheses and the role of earth history events on the distribution of taxa and genetic variation (e.g. Bermingham & Martin 1998; Avise 2000; Waters *et al*. 2001; Sponer & Roy 2002; Waters & Roy 2003). The intraspecific approach has been implemented to great effect along the northern Australian coastline in diverse marine taxa (Benzie *et al*. 1992; Keenan 1994; Norman *et al*. 1994; Elliott 1996; Fitzsimmons *et al*. 1997; Begg *et al*. 1998; Chenoweth *et al*. 1998; Gopurenko & Hughes 2002), and has highlighted the important role that vicariance has played in structuring populations that were effectively isolated by the closure of the Torres Strait, that separates Papua New Guinea from Australia, during Pleistocene low sea-level stands. Several of these studies (Benzie *et al*. 1992; Keenan 1994; Chenoweth *et al*. 1998) also demonstrated that subsequent dispersal events lead to admixture between divergent lineages, and influenced intraspecific genetic diversity in the region after inundation of the Torres Strait land bridge by rising sea levels.

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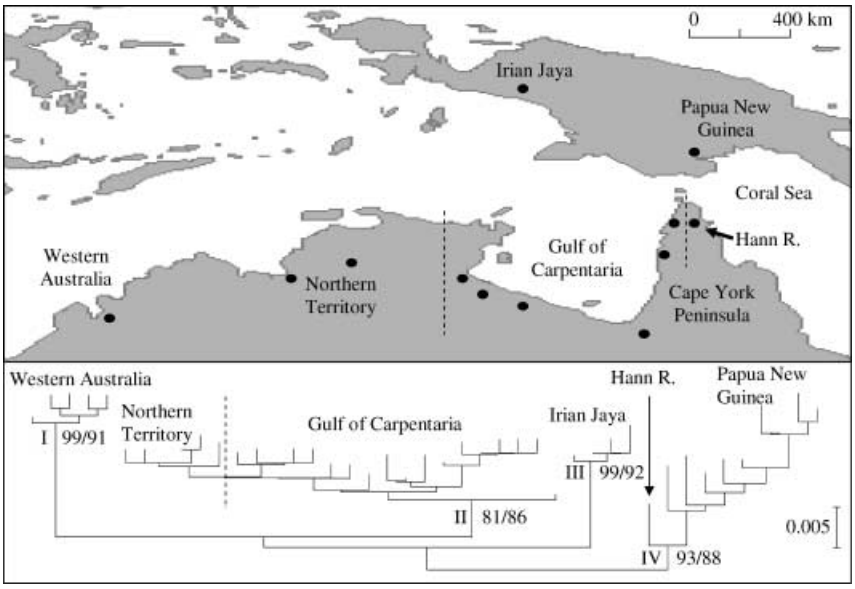


**Fig. 1** Study region and phylogenetic relationships (neighbour joining tree) among *M. rosenbergii* COI mtDNA lineages. Sampling sites indicated by black dots as per Fig. 2. Phylogenetic relationships among haplotypes indicated by location as per map. Major lineages indicated by Roman numerals to the left of nodes (see text for details), while bootstrap support indicated by numbers to the right of nodes (neighbour-joining analysis first, maximum-likelihood analysis second). GenBank Accession nos for haplotypes: AY614545–AY614587.

In contrast, few molecular studies have addressed the effects of eustasy on freshwater aquatic taxa in this region (Macaranas *et al*. 1995; McGuigan *et al*. 2000). While gene flow was effectively disrupted for marine taxa by the closure of the Torres Strait, the same event may actually have had the opposite effect and facilitated gene flow among fresh- and brackishwater tolerant taxa, due to the formation of a substantial lake on the Australia–New Guinea continental shelf during this period (Smart 1977; Torgersen *et al*. 1983; Torgersen *et al*. 1985; Jones & Torgersen 1988). Lake Carpentaria was a vast (approximate maximum size = 600 × 300 km; Chivas *et al*. 2001) intermittently fresh- to brackishwater lake hypothesized to have existed from approximately 80 000–8500 years before present (BP), before absolute marine conditions were once again restored by rising sea-levels cresting the Arafura Sill (Torgersen *et al*. 1983; Torgersen *et al*. 1985; Jones & Torgersen 1988; Chivas *et al*. 2001). Lake Carpentaria would presumably have provided habitat for (Torgersen *et al*. 1983; McGuigan *et al*. 2000), and facilitated connectivity among many Australian and New Guinean fresh- and brackishwater tolerant aquatic taxa that are today separated by a marine barrier (Figs 1 and 2). A second significant factor that may have influenced gene flow among populations of these taxa in the region was the historical pattern and direction of flow of New Guinea's Fly River, which has been hypothesized (Blake & Ollier 1969; Torgersen *et al*. 1983; Torgersen *et al*. 1988) to have drained into Lake Carpentaria until it diverted to its present-day easterly course into the Coral Sea (Fig. 2) some 40–35 000 years BP (Blake & Ollier 1969; Torgersen *et al*. 1988). This hypothesis is controversial, however, and has been disputed by Harris *et al*. (1996; see Voris 2000) who found no evidence for a past westward diversion of the Fly River, but argued that the outflow of the river in 'recent' geological time has always remained on an easterly course into the Coral Sea (Fig. 2).

To determine the roles that Lake Carpentaria and the Fly River have played in the evolutionary history of freshwater organisms in this region, we examined mitochondrial DNA variation in the giant freshwater prawn, *Macrobrachium rosenbergii* (eastern form; *sensu* de Bruyn *et al*. 2004). We collected samples from rivers that are believed to have drained into Lake Carpentaria (Voris 2000), as well as from rivers within the region that apparently remained isolated during this period, for comparative analyses (Fig. 2). *M. rosenbergii* is a commercially important (FAO 2000) freshwater crustacean that migrates to estuaries to spawn, as juveniles require brackishwater for survival and development. Laboratory experiments indicate that adults and juvenile *M. rosenbergii* can survive in brackishwater for extended periods of time, but do not tolerate full marine conditions for more than a week as adults and approximately 3 weeks as postlarvae (Sandifer *et al*. 1975). *M. rosenbergii* would therefore appear to be an ideal model organism for investigating the influence of an historical fresh- to brackishwater lake on the biogeographical history of the region. Taxonomists (Short 2000; D. Wowor, pers. comm.) recognize two distinct 'races' of Australian *M. rosenbergii*; a northwestern Australian race distributed from the Fitzroy (northern Western Australia) to the Keep Rivers, and a northeastern Australian race distributed from the Roper to the Normanby (NE Cape York Peninsula) Rivers, with an intermediate form found between these two regions (Figs 1 and 2). Thus, a further aim of the present study was to determine whether

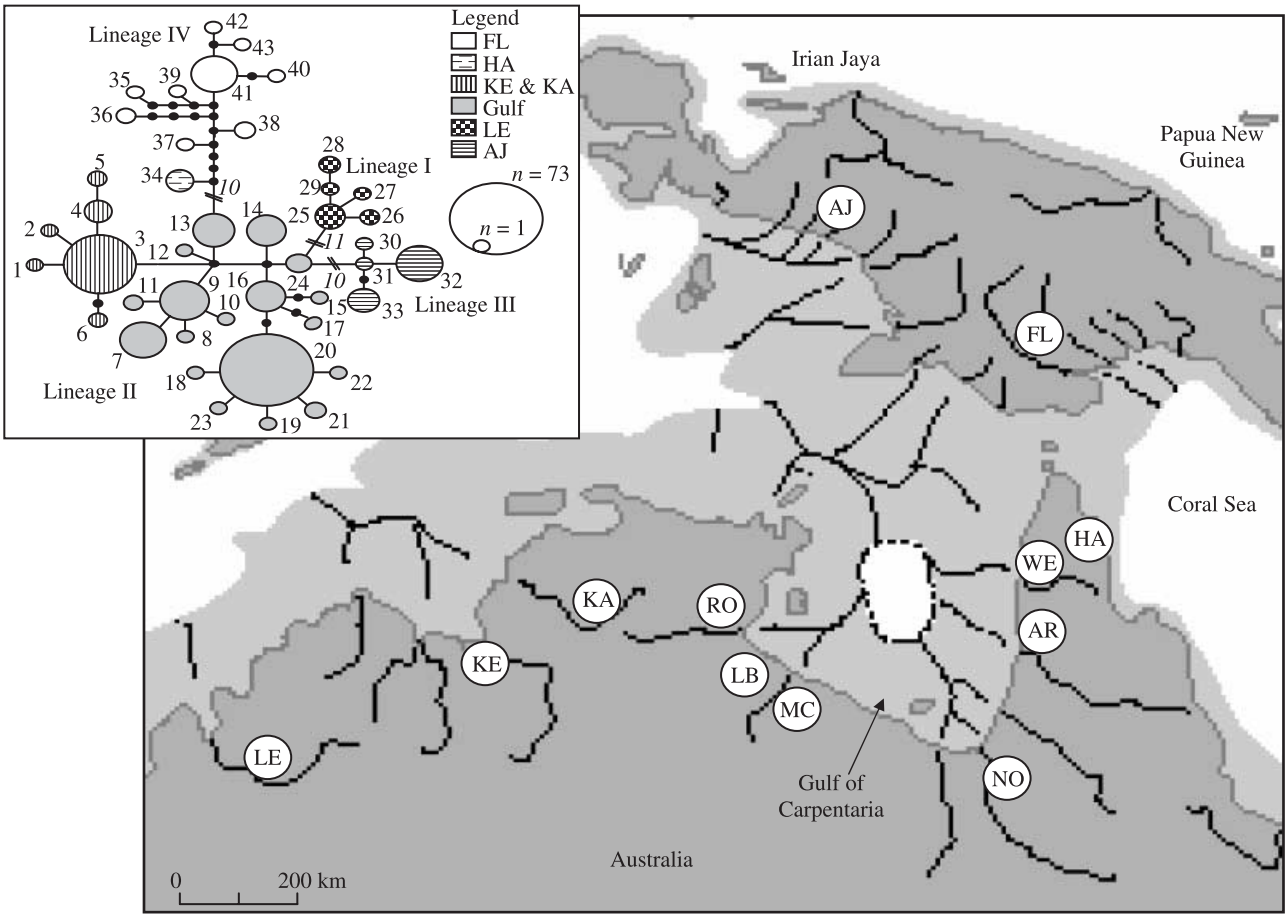


**Fig. 2** Minimum-spanning network and study region indicating Lake Carpentaria and sea levels for much of the Pleistocene. Circle size for each haplotype (1–43) indicates overall frequency. Small black circles indicate inferred missing haplotypes not observed in the data set. Site location abbreviations as per Table 1. Light grey shading on map indicates –75 m sea-level contour. Pleistocene drainage basins indicated on map (map adapted with kind permission Harold K. Voris and the Field Museum of Natural History, Chicago, USA; Voris 2000).

relationships based on molecular data presented here were consistent with previous studies based on morphological characters.

If Lake Carpentaria facilitated gene flow in *M. rosenbergii* in the 'recent' past, populations sampled from rivers that formerly drained into Lake Carpentaria (Fig. 2) should display the molecular signatures of 'recent' genetic interchange, a 'recent' range expansion, and a corresponding population expansion that followed the formation of the Lake. Additional evidence may be expected for subsequent vicariance when a marine environment replaced the Lake, presumably restricting gene flow as sea levels crested the Arafura Sill. Similarly, a signature of 'recent' genetic interchange and subsequent vicariance between Fly River and Gulf of Carpentaria populations might be expected if the Fly River did indeed flow into Lake Carpentaria in 'recent' times.

We therefore documented the phylogeography of the giant freshwater prawn *M. rosenbergii* from the Lake Carpentaria region to determine:

1 If molecular evidence indicates that Lake Carpentaria acted as a conduit for gene flow, and provided habitat for *M. rosenbergii* during the low sea level stands of the late Pleistocene (the outcome of which might be cautiously generalized to other fresh- and brackishwater tolerant taxa in the region).
2 If gene flow was subsequently restricted by rising sea levels that inundated Lake Carpentaria.
3 If molecular evidence supports the westward diversion of the Fly River into Lake Carpentaria during the late Pleistocene.
4 If relationships based on molecular data concord with those based on morphological variation.

**Table 1** Sampling sites and their abbreviations, distribution of mtDNA COI haplotypes, and sample sizes used in this study

| | Location and abbreviation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplotype | Katherine KA | Keep KE | Roper RO | McArthur MC | Wenlock WE | Norman NO | Archer AR | Limmen Bight LB | Lennard LE | Ajkwa AJ | Hann HA | Fly FL |
| 1 | 4 | | | | | | | | | | | |
| 2 | | 1 | | | | | | | | | | |
| 3 | 28 | 21 | | | | | | | | | | |
| 4 | | 7 | | | | | | | | | | |
| 5 | | 2 | | | | | | | | | | |
| 6 | 1 | | | | | | | | | | | |
| 7 | | | 22 | 7 | | | | | | | | |
| 8 | | | | | 1 | | | | | | | |
| 9 | | | | | | 31 | | | | | | |
| 10 | | | | 1 | | | | | | | | |
| 11 | | | | | | 2 | | | | | | |
| 12 | | | | | | 1 | | | | | | |
| 13 | | | | | 21 | | | | | | | |
| 14 | | | | | | | 17 | | | | | |
| 15 | | | | | | 1 | | | | | | |
| 16 | | | | | 14 | 3 | | | | | | |
| 17 | | | | 1 | | | | | | | | |
| 18 | | | | | | | | 1 | | | | |
| 19 | | | | | | | | 1 | | | | |
| 20 | | | | 40 | | 6 | 3 | 24 | | | | |
| 21 | | | | | 3 | | | | | | | |
| 22 | | | | | 1 | | | | | | | |
| 23 | | | | | | | | 1 | | | | |
| 24 | | | | | | | 5 | | | | | |
| 25 | | | | | | | | | 10 | | | |
| 26 | | | | | | | | | 2 | | | |
| 27 | | | | | | | | | 1 | | | |
| 28 | | | | | | | | | 4 | | | |
| 29 | | | | | | | | | 1 | | | |
| 30 | | | | | | | | | | 1 | | |
| 31 | | | | | | | | | | 1 | | |
| 32 | | | | | | | | | | 29 | | |
| 33 | | | | | | | | | | 11 | | |
| 34 | | | | | | | | | | | 8 | |
| 35 | | | | | | | | | | | | 1 |
| 36 | | | | | | | | | | | | 2 |
| 37 | | | | | | | | | | | | 1 |
| 38 | | | | | | | | | | | | 3 |
| 39 | | | | | | | | | | | | 1 |
| 40 | | | | | | | | | | | | 1 |
| 41 | | | | | | | | | | | | 28 |
| 42 | | | | | | | | | | | | 1 |
| 43 | | | | | | | | | | | | 1 |
| *n* | 33 | 31 | 22 | 49 | 40 | 44 | 25 | 27 | 18 | 42 | 8 | 39 |

## Materials and methods

### *Sample collection and molecular analyses*

A total of 378 individuals collected from 12 sites in Australia, Papua New Guinea and Irian Jaya were included in the analyses (Table 1; Figs 1 and 2). Tissue samples (muscle or pleopod) were stored in 70% ethanol until required for molecular analyses. For DNA extraction, a small piece of tissue was first rehydrated for 30 min in 1 mL GTE buffer (100 mM glycine, 10 mM Tris, 1 mM EDTA). Tissue samples were then incubated overnight at 55 °C in 500 μL extraction buffer [100 mM NaCL, 50 mM Tris, 10 mM EDTA, 0.5% sodium dodecyl sulphate (SDS)]

containing 20 μL of 10 μg/μL proteinase K (Sigma Co.). Total genomic DNA was extracted using standard phenol:chloroform extraction methods, and collected by ethanol precipitation. Amplification of a fragment of the mtDNA cytochrome *c* oxidase subunit I (COI) gene was carried out using primers LCO1490 and HCO2198 (Folmer *et al*. 1994). Each 50 μL amplification reaction consisted of 400 ng of template DNA, 5 μL of 10× buffer containing $MgCl_2$ (Roche), an additional 2 μL of 25 mM $MgCl_2$ (Roche), 0.5 units of *Taq* polymerase (Roche), 0.8 μL of each primer (10 μM final conc.), 0.2 mM of each dNTP and 38.95 μL autoclaved $ddH_2O$. Samples that proved difficult to use polymerase chain reaction (PCR) were amplified using Ready-to-Go® beads (Pharmacia Biotech). Thermal cycling was performed on a PTC-100 thermocycler (MJ Research, Inc.) under the following conditions: 3 min denaturation at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C, 30 s at 72 °C, and a final 10 min extension at 72 °C, before cooling to 4 °C for 10 min. Negative controls were included in all PCR runs, and sterile procedures were adhered to throughout. PCR amplifications were confirmed with agarose gel electrophoresis on a 1% gel. Screening for intrapopulation variation was carried out using temperature gradient gel electrophoresis (TGGE) combined with outgroup heteroduplex analysis (OHA) (Campbell *et al*. 1995). This method proved to be sensitive enough to distinguish consistently among haplotypes that varied by a single base pair (bp). Multiple examples (~2–3) of PCR products from haplotypes identified as unique using TGGE/OHA were purified using a Qiagen QIAquick PCR purification kit and sequenced. DNA sequencing of 602 bp of the COI gene was conducted on an ABI 3730 automated sequencer at the Australian Genome Research Facility at the University of Queensland, Brisbane, Australia. Both strands of the PCR product were completely sequenced.

### *Data analyses*

Sequences were aligned in CLUSTALX (Thompson *et al*. 1997). Initial data exploration and standard diversity indices were calculated in ARLEQUIN (version 2.0; Schneider *et al*. 2000). To determine whether the mitochondrial region employed in the present study was evolving according to neutral expectations, we employed neutrality tests (Tajima 1989; Fu & Li 1993) in DNASP version 4.00 (Rozas *et al*. 2003). We investigated whether sequences had reached substitution saturation by plotting separately the number of transitions or transversions between pairs of haplotypes vs. the Kimura 2-parameter genetic distances that corrects for multiple hits. Population structure was investigated in ARLEQUIN using $\Phi_{ST}$ statistics, analysis of molecular variance (AMOVA) with statistical significance determined by permutation analyses (Excoffier *et al*. 1992) and the construction of a minimum-spanning network (MSN; Excoffier & Smouse 1994). We determined that the TrN model of substitution (Tamura & Nei 1993) plus invariable sites (*I*) and a gamma distribution (Γ) of rate heterogeneity across variable sites provided the best fit to our data set with the program MODELTEST 3.06 (Posada & Crandall 1998). The estimated parameters under this model were $\Gamma = 0.5930$, $I = 0.6928$ and $Ti/Tv = 4.69$. We used the neighbour-joining method of tree construction with bootstrap analysis (1000 replicates; Felsenstein 1985) to evaluate support for relationships, implemented in PAUP* 4.0b10 (Swofford 2002). As a comparison with the neighbour-joining method, we also constructed trees using maximum likelihood methods. We adopted these methods in an attempt to minimize the potential for error that may arise from the assumptions inherent in phylogeny reconstruction methods. A single *M. rosenbergii* individual from Bali (western form; de Bruyn *et al*. 2004) was used as an outgroup. We calculated maximum-likelihood distances among haplotypes in PAUP*. To test for adherence to a clock-like evolution of the mtDNA sequences, a log-likelihood ratio test was carried out in PAUP* that compared trees generated under the assumption of a molecular clock, to trees unconstrained by any such assumption (Felsenstein 1988). The timing of cladogenesis identified in the phylogeny was then inferred by way of molecular clock approximation. Although the accuracy of dates of divergence based on a molecular clock are debatable (Marko 2002), they do none the less provide a relative time frame for investigating phylogeographical relationships. To determine relationships among haplotypes, and factors that may have influenced these relationships, we employed nested clade analysis (NCA; Templeton *et al*. 1995). A 95% probability haplotype cladogram was constructed according to Templeton & Sing (1993), Crandall (1996) and Templeton (1998) in TCS version 1.13 (Clement *et al*. 2000). This network was then converted into a nested design and analysed in GEODIS version 2.0 (Posada *et al*. 2000), with the null hypothesis of no geographical association among haplotypes. Templeton's (2004) latest inference key was used to infer processes involved in any statistically significant association observed. To address the question of changes in historical population (lineage) size, we employed mismatch distribution analyses (Rogers & Harpending 1992; Rogers 1995) in DNASP and ARLEQUIN. If the distribution identified is unimodal and fits the sudden expansion model, it is possible to estimate the time to the onset of population expansion.

## Results

### *Genetic diversity*

A total of 602 bp of the COI mitochondrial gene were amplified successfully for all samples analysed for a total of 378 *M. rosenbergii* individuals, resulting in 43 unique

**Table 2** Pairwise $\Phi_{ST}$ values and exact test of population differentiation among sites. $\Phi_{ST}$ above diagonal based on nucleotide content and haplotype frequencies (all values significant; $P < 0.05$). Exact test probabilities of nondifferentiation below the diagonal based on 10 000 Markov permutational steps, significance values indicated by $*P < 0.05$ and $***P < 0.0005$ (i.e. all pairwise comparisons significant). See Table 1 for sampling site codes

| | KA | KE | RO | MC | WE | NO | AR | LB | LE | AJ | FL | HA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KA | — | 0.16 | 0.94 | 0.76 | 0.55 | 0.56 | 0.76 | 0.95 | 0.94 | 0.94 | 0.93 | 0.97 |
| KE | * | — | 0.90 | 0.74 | 0.54 | 0.57 | 0.72 | 0.92 | 0.93 | 0.93 | 0.92 | 0.96 |
| RO | *** | *** | — | 0.77 | 0.65 | 0.49 | 0.82 | 0.98 | 0.95 | 0.95 | 0.93 | 1.00 |
| MC | *** | *** | *** | — | 0.45 | 0.53 | 0.54 | 0.10 | 0.89 | 0.89 | 0.89 | 0.89 |
| WE | *** | *** | *** | *** | — | 0.30 | 0.37 | 0.63 | 0.86 | 0.88 | 0.87 | 0.85 |
| NO | *** | *** | *** | *** | *** | — | 0.49 | 0.72 | 0.88 | 0.89 | 0.89 | 0.88 |
| AR | *** | *** | *** | *** | *** | *** | — | 0.77 | 0.89 | 0.90 | 0.90 | 0.91 |
| LB | *** | *** | *** | * | *** | *** | *** | — | 0.95 | 0.94 | 0.93 | 0.99 |
| LE | *** | *** | *** | *** | *** | *** | *** | *** | — | 0.92 | 0.91 | 0.93 |
| AJ | *** | *** | *** | *** | *** | *** | *** | *** | *** | — | 0.91 | 0.92 |
| FL | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | — | 0.80 |
| HA | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | — |

haplotypes defined by 59 variable sites. All unique sequences have been deposited with GenBank (Accession nos: AY614545–AY614587). The sequences were aligned unambiguously with no insertions or deletions observed in the data set. No significant deviations from those expected under neutrality were identified when all haplotypes were analysed together (Fu & Li 1993; $D = -1.12$, $P > 0.10$; $F = -1.08$, $P > 0.10$; Tajima 1989; $D = -0.53$, $P > 0.10$), or when phylogenetic lineages (Fig. 1) or individual populations were analysed separately (statistics not presented here). Haplotype diversity ranged from 0.00 to 0.66 (mean 0.376). Maximum-likelihood distances ranged from 0.002 to 0.05. No evidence was found for saturation in transitions or transversions in our data set (graphs not shown). Conversion of the nucleotide sequences into amino acid sequence indicated that nearly all polymorphisms were silent substitutions. Only three amino acid changes were inferred, two from Irian Jayan sequences and one from a Western Australian sequence. Moreover, no stop codons were identified and these data supported our view that pseudogenes were absent from the data set.

### *Phylogeny reconstruction*

The neighbour-joining tree (Fig. 1) constructed using the complete data set of 43 haplotypes resulted in a well-resolved phylogeny, defined by 37 phylogenetically informative sites. Four major clades were identified, referred to hereafter as lineages I–IV, supported by high bootstrap values (Fig. 1). These relationships were also supported strongly by the maximum-likelihood analysis (Fig. 1), although within lineage relationships varied depending on the method of tree construction. The geographical distributions of all identified lineages were discrete. Lineage II had the broadest geographical distribution, and was represented by specimens that were collected from Australian rivers that discharge into the Gulf of Carpentaria (Figs 1 and 2) and from two more westerly Australian sites (Keep and Katherine Rivers, Northern Territory). The Northern Territory populations formed a subgroup nested within this clade, although bootstrap support for this relationship was low (neighbour-joining bootstrap value = 44). Lineage I was restricted to the Western Australian population, the Irian Jayan population comprised lineage III, while the Papua New Guinean and the Hann River (Australia) populations together comprised lineage IV (Fig. 1). Bootstrap support was low for within lineage variation so we examined these relationships further as described below.

### *Genetic structuring among and within lineages*

AMOVA identified 75% of the variation to be present among phylogenetic lineages, 16.5% of the variation to be among populations within lineages and only 8.5% of the variation to be within populations. This evidence for restricted gene flow among populations representing discrete phylogenetic lineages was supported by pairwise $\Phi_{ST}$ and exact test values (Table 2). These data suggest that gene flow among populations representing discrete lineages has not taken place for a significant period of evolutionary time. Even within lineages, little or no ongoing gene flow among populations was suggested by pairwise $\Phi_{ST}$ and exact test values, as all populations were significantly differentiated from each other (Table 2). The Katherine and Keep River populations (lineage II) were genetically most similar ($\Phi_{ST} = 0.161$), while the Roper and Hann River populations (lineages II and IV, respectively) were most dissimilar ($\Phi_{ST} = 1.000$). Interestingly, these four populations were all collected from Australian sites (Figs 1 and 2).

*Network estimation and nested clade analysis*

Relationships among haplotypes in the MSN (Fig. 2) supported the presence of the four discrete lineages identified in the phylogenetic reconstruction (Fig. 1). Lineage II (Australian Gulf of Carpentaria and Northern Territory populations) formed a central clade dominated by four haplotypes (haplotypes 3, 9, 16 and 20) occurring at high frequencies at the centre of a star-like radiation, separated from one another by 1–5 bp differences (Fig. 2). Of these four haplotypes, three were found in more than one geographical location (haplotype 3 = Keep and Katherine Rivers; haplotype 16 = Wenlock and Norman Rivers; haplotype 20 = Norman, McArthur, Archer and Limmen Bight Rivers), while the fourth was restricted to the Norman River (haplotype 9; Table 1). Only one other haplotype from the total data set (haplotype 7) was identified at multiple geographical locations, namely in the Roper and McArthur River populations. There were few intermediate missing haplotypes, and lineage II was separated by 11 bp from lineage I and 10 bp from both lineages III and IV, respectively. Lineages I and III formed two well-resolved clades with only a single missing haplotype evident in lineage III. Relationships within lineage IV were more complex, with a number of missing intermediate haplotypes. The Fly River (Papua New Guinea) population had the greatest number of haplotypes (nine) compared with all other populations (Table 1). The Hann River population from the eastern Cape York Peninsula (Australia) was only 5 bp divergent from the Fly River population, but a minimum of 11 bp divergent from any other Australian haplotype (Fig. 2). We adopted a conservative approach, and analysed lineage II only by approximation of a 95% parsimony network, followed by nested clade analysis (Templeton *et al*. 1995). This decision was made based on the extensive intermediate (unsampled) geographical areas between populations from each respective lineage, which may result in an ambiguous outcome in NCA (Templeton 2004), and the large number of inferred missing haplotypes among lineages identified in the MSN (Fig. 2). In contrast, lineage II's range was well sampled and there were few inferred missing haplotypes (Fig. 3). NCA enabled us to reject the null hypothesis of no association between the distribution of haplotypes and geography for a number of clades, and we therefore inferred probable causes for the observed patterns using Templeton's (2004) latest inference key (see Table 3 for nested clade distances and inferred processes). For clades 1-5, 2-1 and 2-3 the inference key did not allow us to distinguish between contiguous range expansion, long-distance colonization or past fragmentation. The inference key suggested contiguous range expansion for both clades 1-10 and 2-2, while at the entire cladogram level either isolation by distance or long distance dispersal was suggested.

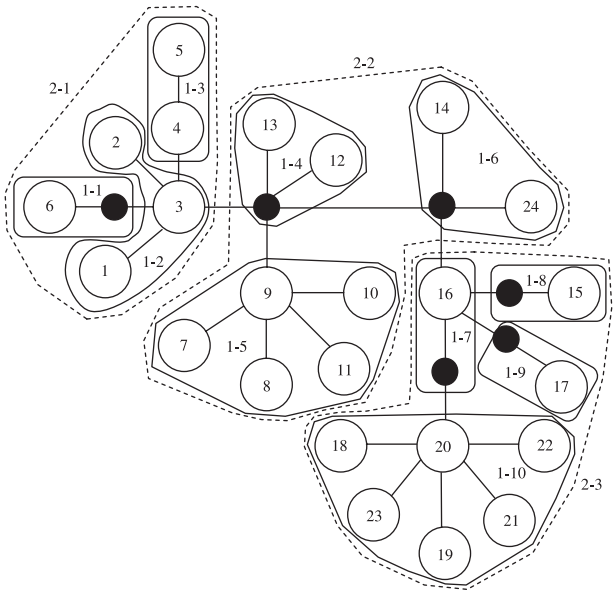


**Fig. 3** Statistical parsimony cladogram (95%) for lineage II estimated from the *M. rosenbergii* COI data. Small black circles indicate inferred missing haplotypes not observed in the data set. See Fig. 2 and Table 1 for haplotype frequencies.

*Timing of cladogenesis and lineage expansions*

A log-likelihood ratio test could not reject the hypothesis that lineages were evolving according to a clock-like model of evolution ($-\ln L = 1576.76$ with molecular clock enforced vs. $-\ln L = 1553.55$ without molecular clock enforced, $\chi^2 = 46.42$, d.f. = 41, $P > 0.10$). Knowlton & Weigt (1998) calibrated a caridean shrimp (caridea is the infraorder of *M. rosenbergii*) COI molecular clock at $1.4 \times 10^{-8}$ based on the rise of the Isthmus of Panama. Assuming a molecular clock and applying this divergence rate to: first, the uncorrected genetic distances and second, the corrected maximum-likelihood distances (as per Knowlton & Weigt 1998) between lineages, coalescence between the central lineage II and lineages I, III and IV, respectively, dates back to the early Pleistocene (1–1.4 million years ago (Mya) uncorrected; 1.2–1.7 Mya corrected). To determine whether lineages fitted the predicted distribution under a sudden expansion model, we employed mismatch distribution analyses. The validity of the model was tested using the parametric bootstrap approach in ARLEQUIN, where $P$ = (number of SSDsim = SSDobs)/B (Schneider & Excoffier 1999). Lineages II and IV fitted well the predicted distribution under a sudden expansion model, but the fit for lineages I and III were rejected. Mismatch distributions (Slatkin & Hudson 1991; Rogers & Harpending 1992) and *P*-values are presented in Fig. 4. To determine the approximate timing of the expansion of lineages II and IV the equation for tau ($\tau = 2ut$) was rearranged to solve for $t$ (generations since expansion). The product of μ and 602

**Table 3** Results of nested clade analysis showing clade (*Dc*), nested (*Dn*) and interior to tip clade (I–T) distances. Only clades with significant permutational $\chi^2$ probabilities for geographical structure have been included

| Nesting level | Haplotype/ clade no. | Location | *Dc* | *Dn* | $\chi^2 - P$ | Inference key conclusion |
|---|---|---|---|---|---|---|
| 1-2 | 1 | Tip | 0 | 154.89 | 0.0730* | RG-IBD |
| | 2 | Tip | 0 | 211.11 | | |
| | 3 | Interior | 180.73$^{L*}$ | 179.85$^{L*}$ | | |
| | | I–T | 180.73$^{L*}$ | 13.71 | | |
| 1-5 | 9 | Interior | 0$^{S}$ | 453.44$^{L}$ | 0.0000 | CRE/LDC/PF? |
| | 7 | Tip | 55.09$^{S}$ | 306.57$^{S}$ | | |
| | 8 | Tip | 0 | 658.26$^{L}$ | | |
| | 11 | Tip | 0 | 453.44 | | |
| | 10 | Tip | 0 | 107.05 | | |
| | | I–T | –48.41$^{S}$ | 133.35$^{L}$ | | |
| 1-10 | 20 | Interior | 165.02$^{S}$ | 182.07$^{S}$ | 0.0000 | CRE |
| | 18 | Tip | 0 | 155.39 | | |
| | 23 | Tip | 0 | 155.39 | | |
| | 19 | Tip | 0 | 155.39 | | |
| | 21 | Tip | 0 | 677.99$^{L}$ | | |
| | 22 | Tip | 0 | 677.99 | | |
| | | I–T | 165.02 | –271.95$^{S}$ | | |
| 2-1 | 1-1 | Tip | 0 | 183.23 | 0.0020 | CRE/LDC/PF? |
| | 1-2 | Interior | 178.65$^{S}$ | 183.04$^{L}$ | | |
| | 1-3 | Tip | 0$^{S}$ | 182.77$^{S}$ | | |
| | | I–T | 178.65$^{L}$ | 0.22$^{L}$ | | |
| 2-2 | 1-4 | Interior | 48.30$^{S}$ | 385.25$^{S}$ | 0.0000 | CRE |
| | 1-5 | Tip | 365.84$^{S}$ | 463.70$^{L}$ | | |
| | 1-6 | Interior | 0$^{S}$ | 393.00$^{S}$ | | |
| | | I–T | –341.69$^{S}$ | –74.58$^{S}$ | | |
| 2-3 | 1-7 | Interior | 0$^{S}$ | 587.24$^{L}$ | 0.0000 | CRE/LDC/PF? |
| | 1-8 | Tip | 0 | 477.40 | | |
| | 1-9 | Tip | 0 | 167.61 | | |
| | 1-10 | Tip | 201.29$^{S}$ | 261.67$^{S}$ | | |
| | | I–T | –196.44$^{S}$ | 324.11$^{L}$ | | |
| 3-1 | 2-1 | Tip | 183.00$^{S}$ | 653.09$^{L}$ | 0.0000 | IBD/LDD? |
| | 2-2 | Interior | 426.99 | 426.40$^{S}$ | | |
| | 2-3 | Tip | 399.36$^{S}$ | 391.67$^{S}$ | | |
| | | I–T | 102.49$^{L}$ | –55.71 | | |

Significantly small or large values for *Dc*, *D*n and I–T (*Dc* and *Dn*) are indicated by superscript 'S' and 'L', respectively, and * indicates $P < 0.08$. Inference key conclusions using Templeton's updated key for NCA (Templeton 2004). Conclusions as follows: RG–IBD, restricted gene flow with isolation by distance; CRE, contiguous range expansion; LDC, long-distance colonization; PF, past fragmentation; IBD, isolation by distance; LDD, long distance dispersal. '?' indicates inconclusive outcome discriminating between the listed conclusions.

nucleotide sites used in this study replaced the parameter $u$ in the equation. Substituting these values into the equation and assuming a generation time of 6 months–1 year, it is suggested that the initial timing of these expansion events took place approximately 154 000–77 000 years BP for lineage II, and 370 000–185 000 years BP for lineage IV.

## Discussion

Four discrete genealogical lineages of giant freshwater prawns were identified in this study (Fig. 1): a Western Australian lineage (lineage I); a Gulf of Carpentaria/ Northern Territory lineage (II); an Irian Jayan lineage (III); and a Papua New Guinean/NE Cape York lineage (IV). This relationship coincides with the phylogeographical structuring of a number of marine organisms sampled from Australian waters that have displayed genetic breaks between (i) Western Australia, (ii) NE Australia and/or (iii) Northern Territory/Gulf of Carpentaria (Mulley & Latter 1981; Benzie *et al*. 1992; Johnson & Joll 1993; Keenan 1994; Norman *et al*. 1994; Elliott 1996; Fitzsimmons *et al*. 1997; Begg *et al*. 1998; Chenoweth *et al*. 1998; Brooker

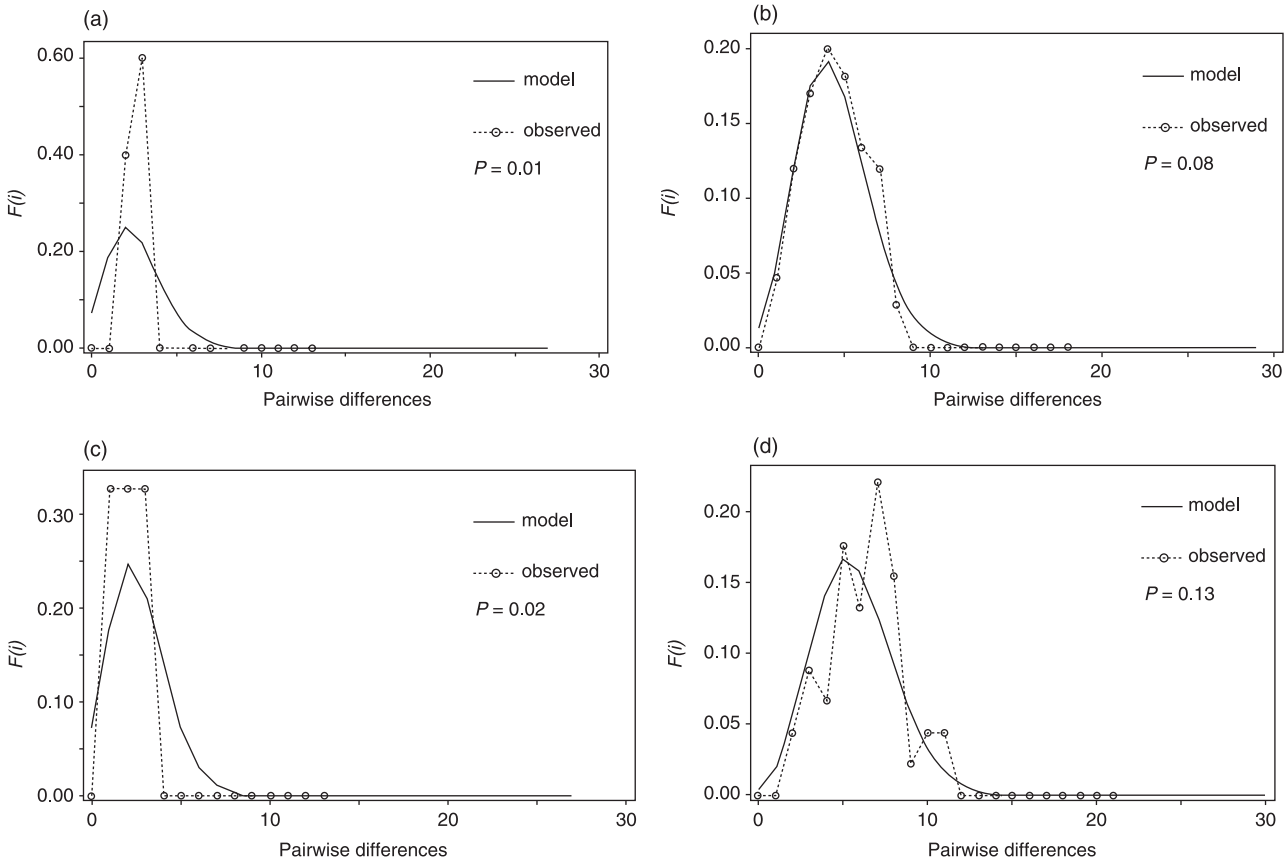


**Fig. 4** Distribution of pairwise differences among mtDNA COI haplotypes for lineages I–IV. *F*(*i*): relative frequency of haplotypes with *i* differences. (a) Lineage I; (b) lineage II; (c) lineage III; (d) lineage IV.

*et al*. 2000; Benzie *et al*. 2002; Gopurenko & Hughes 2002; Ovenden *et al*. 2002). It also coincides with results from the only two molecular studies undertaken on freshwater taxa in this region to date. The first identified low levels of divergence between populations of redclaw crayfish from the Gulf of Carpentaria and the Northern Territory (Macaranas *et al*. 1995), while the second found a close phylogenetic relationship between rainbowfish from southern Papua New Guinea and northern Australia indicative of recent speciation and range expansion events (McGuigan *et al*. 2000). Morphological variation among Australian *M. rosenbergii* populations also appears broadly concordant with genetic relationships presented here. While the reciprocal monophyly of the northwestern and northeastern Australian races (lineages I and II, respectively) are supported in the phylogenetic and phylogeographical reconstructions (Figs 1 and 2), the intermediate form, while comprising a distinct subgrouping (Northern Territory haplotypes), remains nested within the northeastern Australian lineage (lineage II; Fig. 1). Based on molecular data, the Keep River specimens collected for the present study do not represent the northwestern race, as suggested by the morphological data (Short 2000), but rather the intermediate form. Alternatively, specimen collections for these two studies may have been from two geographically isolated locations from the Keep River drainage, thus the Keep River region may be a zone of contact between the northwestern race and the intermediate form. This hypothesis warrants further investigation, and we propose to investigate this further using microsatellite markers in the near future. In direct contrast to the molecular data, no morphological divergence was reported between NW and NE Cape York populations (Short 2000).

Analysis of molecular variance indicated that the regional structuring of the four lineages explained much of the total genetic variance presented here. Complete lineage sorting and the high $\Phi_{ST}$ values (Table 2) among populations from discrete lineages suggest that these lineages are on independent evolutionary pathways, and have been so for a considerable time frame. Indeed, a molecular clock estimate of the time to coalescence between these groups indicates that events during the early Pleistocene, some 1–1.7 Mya, initiated the regional structuring of haplotypes. Any inferences regarding the timing of colonization

based on a partial phylogeny such as that presented here, however, may not reveal a true history. Hence, we did not attempt to elucidate the chronology of ancestry among these lineages, as our sampling was limited to a fairly small part of *M. rosenbergii*'s (eastern form) known distribution (de Bruyn *et al*. 2004). We propose to investigate ancestral relations among lineages in more detail in the future.

Within-lineage variation was also highly structured, with all populations differentiated significantly from each other in the two lineages that were represented by more than a single population (lineages II and IV; Table 2). In population genetic terms, these data suggest that gene flow among populations within regions is not sufficient to counter the effects of random genetic drift. Of a total of 43 unique haplotypes, only four were distributed in multiple populations, all of which represented lineage II. Sharing of haplotypes among the Keep and Katherine Rivers, the Wenlock and Norman Rivers, the Norman, McArthur, Archer and Limmen Bight Rivers and the Roper and McArthur Rivers, respectively, could indicate either the retention of ancestral polymorphisms or alternatively that low levels of gene flow occur(ed) among these populations. That three of these four haplotypes were interior (ancestral) haplotypes (Fig. 3) would suggest that the retention of ancestral polymorphisms is a more probable scenario.

Within lineage IV, the close phylogenetic relationship between the Hann River population (NE coast of Cape York Peninsula, Australia) and the Fly River population (Papua New Guinea) was surprising, given the much greater geographical distance between the Hann and Fly Rivers compared with that between the Hann River and western Cape York sites (e.g. Wenlock and Archer Rivers, see Fig. 2). None the less, the Hann and Fly River populations do not share haplotypes, and it would appear (Figs 1 and 2; Table 3) that the relationship between them is historical, and not a consequence of contemporary processes. The lack of evidence for 'recent' genetic interchange between the Fly River and the Gulf of Carpentaria populations challenges the hypothesis (Blake & Ollier 1969; Torgersen *et al*. 1983; Torgersen *et al*. 1988) that the Fly River drained into Lake Carpentaria for an extensive period of time during the late Pleistocene. Rather, the data support the alternative view (Harris *et al*. 1996), that the Fly River remained on an easterly course draining into the Coral Sea during this time. Indeed, an estimate of the time to coalescence between populations from the Fly River and the Gulf of Carpentaria dates back some 1.5 Mya. Additional molecular studies of freshwater taxa sampled from southern New Guinea rivers (that are believed to have drained into Lake Carpentaria; see Fig. 2) are warranted to further elucidate historical connectivity between Australia and New Guinea. Interestingly, the time frame estimated here for coalescence between lineages restricted to western and eastern flowing rivers on the Cape York Peninsula (lineages II and IV, respectively) concords with that identified for divergence between populations of the estuarine mud crab, *Scylla serrata*, sampled from either side of the Cape York Peninsula (~1 Mya; Gopurenko *et al*. 1999).

The hypothesis that Lake Carpentaria provided habitat for, and facilitated gene flow among, giant freshwater prawn populations during the late Pleistocene is supported by our analyses. NCA of lineage II strongly indicated a range expansion event at both one-step and two-step clade levels for present-day Gulf of Carpentaria populations (Table 3). This expansion is also evident in the star-like structuring of lineage II haplotypes (Fig. 3; Slatkin & Hudson 1991). Inferring the timing of this lineage expansion (154 000–77 000 years BP) indicated that the formation of Lake Carpentaria some 80 000 years BP (Jones & Torgersen 1988) may have initiated this event. Subsequently, Lake Carpentaria was replaced by a marine environment some 8500 years BP as sea-levels rose (Torgersen *et al*. 1983; Torgersen *et al*. 1985; Jones & Torgersen 1988; Chivas *et al*. 2001), which evidently restricted gene flow among populations formerly connected by the Lake. This subsequent restriction of gene flow is suggested by significant values for all pairwise tests of nondifferentiation among populations from the Gulf region (Table 2), while the low number of nucleotide differences and the lack of geographical structuring among these haplotypes indicates that divergence was 'recent'. This fragmentation event highlights one recognized limitation of NCA, i.e. the fragmentation event was too recent to detect using NCA (see Masta *et al*. 2003 and Templeton 2004 for discussion). This problem can be circumvented, however, by incorporating frequency-based analyses (e.g. $F_{ST}$s; A. R. Templeton, pers. comm.) as illustrated here. It would appear that Northern Territory sites included in this study were either colonized shortly after the time of Lake Carpentaria's formation, or *in situ* haplotypes were replaced by 'Lake Carpentaria type' haplotypes that have diverged subsequently in apparent isolation (Figs 1 and 2).

In conclusion, Lake Carpentaria appears to have played an important role in the evolutionary history of aquatic taxa during the late Pleistocene (this study; Macaranas *et al*. 1995; McGuigan *et al*. 2000), and may also prove to have been a significant influence on prehistoric human migrations in the region. Moreover, our results do not support the hypothesis (Blake & Ollier 1969; Torgersen *et al*. 1983; Torgersen *et al*. 1988) of a westward diversion of the Fly River during this time. The combination of geological and molecular data presented here provides a powerful framework for investigating the influence of historical earth history events on the distribution of biological (i.e. molecular) diversity.

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This research forms part of Mark de Bruyn's PhD thesis on the evolutionary history of giant freshwater prawns, and the application thereof in addressing questions related to earth history events. John Wilson (Mark's co-supervisor) and members of his group focus mainly on the ecology and population dynamics of small mammal populations, particularly in northern Queensland. Peter Mather leads research in the QUT Ecological Genetics laboratory, which often focuses on the management implications of molecular variation in fishes and crustaceans, although studies on other animals, and occasionally plants, are also tolerated.

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