Population structure of *Macrobrachium australiense* (Decapoda: Palaemonidae) in Western Queensland, Australia: the role of contemporary and historical processes

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Rivers of Western Queensland, Australia, represent a discontinuous and variable aquatic habitat for the freshwater fauna of the region. Rivers periodically fluctuate between being highly fragmented, with numerous disconnected waterholes and ephemeral channels, and being highly connected by a dominant system of anastomosing channels. We used mitochondrial sequences to investigate the genetic structure and inferred patterns of dispersal associated with this flow regime for the freshwater prawn, *Macrobrachium australiense* (Decapoda: Palaemonidae), sampling 28 localities throughout eight catchments. Based on a 505 base pair fragment of mitochondrial cytochrome *c* oxidase subunit I, we identified 98 haplotypes in a sample of 402 individuals. The haplotypes clustered into two main clades correspond-

ing geographically to the major drainages: the Lake Eyre and Murray–Darling basins. Populations of *M. australiense* inhabiting the two basins appear to have diverged around 800 000 years ago (estimated sequence divergence of 1.6%). Analysis of population differentiation indicated contemporary high levels of genetic subdivision and restricted gene flow among populations within and among catchments. Phylogenetic analysis detected a series of historical range expansions in the region and we suggest that climate fluctuations during the Pleistocene have resulted in extensive floods that have promoted historical movements of aquatic organisms across catchment boundaries.

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Introduction

Dispersal is the outward spreading of organisms from their point of origin and it can affect the evolution and population genetics of a species, only if successful colonisation is followed by breeding of subsequent generations. Dispersal is essential for the maintenance of gene flow and genetic diversity, and also for colonisation and re-colonisation of habitats. Generally, species with low dispersal capabilities exhibit a tendency towards genetic fragmentation and species with efficient mechanisms of dispersal have limited genetic structuring of their subpopulations (Slatkin, 1987).

Freshwater invertebrates occur in habitats that represent discrete sites surrounded by inhospitable terrestrial landscapes. Some aquatic insects possess a flying stage in their life cycle, which allows them to disperse across drainage boundaries (Hughes *et al.*, 1998; Miller *et al.*, 2002). However, movements of continuously aquatic freshwater species are very much limited by the physical nature and arrangement of the riverine system (Hurwood and Hughes, 2001; McGlashan and Hughes, 2001) and

often species with apparently good dispersal abilities present surprisingly high levels of population subdivision (Hughes *et al.*, in review; Hughes and Hillyer, 2003).

Rivers in arid and semi-arid areas, such as Western Queensland, have a quasi-periodic flow regime with episodic extensive floods and no-flow periods. As a result, such dryland rivers fluctuate between being highly fragmented, with numerous disconnected waterholes where continuously aquatic freshwater species are isolated, and being highly connected by a dominant system of ephemeral channels and floodplains (Knighton and Nanson, 1997). In such discontinuous riverine habitat, passive transport by water currents and active swimming of freshwater invertebrates may be achieved only during wet seasons when water is flowing in rivers and the nearby floodplains. Until recently, the geographical extent of colonisation events of aquatic fauna associated with the quasi-periodic flow regime in Western Queensland was thought to be extensive due to the intense floods and the low topographic relief of the region (Gibling *et al.*, 1998). However, recent studies using molecular markers have provided a novel insight into patterns of dispersal and connectivity of freshwater invertebrates within this region. It was suggested that contemporary gene flow across drainage and catchment boundaries was absent in two species: the freshwater crayfish, *Cherax destructor* (Decapoda: Parastacidae) (Hughes and Hillyer, 2003) and the freshwater snail,

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Notopala sublineata (Gastropoda: Viviparidae) (Carini and Hughes, in review). In addition, Hughes and Hillyer (2003) indicated high levels of dispersal and gene flow within catchments for the freshwater crayfish, but Carini and Hughes (in review) detected very low levels of gene flow and dispersal between populations both within and among catchments for the snails.

The freshwater prawn, *Macrobrachium australiense* (Decapoda: Palaemonidae), appears to have good dispersal capabilities as described by Williams (1980) and suggested by Cook *et al* (2002) in a recent study in Western Queensland. They used mostly allozyme analysis, although a fragment of the cytochrome oxidase I gene was sequenced for a small numbers of individuals. A major division between Lake Eyre and Murray–Darling basin was detected, but low levels of genetic differentiation between populations from the same catchment. Nevertheless, Hughes *et al* (in review) demonstrated that, using mtDNA markers, species with apparently efficient mechanisms of dispersal could display low levels of gene flow between populations within catchments in the same geographic region. Thus, our aim was to use mtDNA to investigate the genetic variation among populations of *M. australiense* and to infer contemporary levels of dispersal and gene flow in Western Queensland. Due to the haploid nature and the maternal mode of inheritance, mtDNA has a four-fold smaller effective population size than nuclear markers, increasing its sensitivity to any limited gene flow between populations (Birky *et al*, 1989). Therefore, it would be more likely to detect genetic structure of the species using mtDNA than allozymes.

In addition, by assessing information from rapidly evolving molecular markers, we intended to determine the extent to which the population structure of *M. australiense* in Western Queensland results from recurrent processes, such as ongoing gene flow, *versus* historical processes, such as fragmentation or range expansion (Templeton *et al*, 1995). Recent studies within the region (Hughes and Hillyer, 2003; Hughes *et al*, in review; Carini and Hughes, in review) have suggested that climate oscillations of the Pleistocene may be directly correlated with historical high levels of connectivity and therefore dispersal across catchment boundaries. We hypothesised that levels of connectivity and dispersal between populations of *M. australiense* might have been higher in ancient times.

Materials and methods

Study area and sampling design

Sampling sites are localised within the Lake Eyre basin and the northern regions of the Murray–Darling basin (Figure 1). The Lake Eyre basin is an endoreic system and it is characterised by temporary streams and rivers (Knighton and Nanson, 1994). All the catchments terminate at Lake Eyre except for the Bulloo, which terminates in a series of wetlands in northern New South Wales. The Murray–Darling basin is exoreic, drawing water from two major systems: the Murray and the Darling rivers, ultimately draining to the sea through river systems along the South Australian coast. Western Queensland rivers are not conventional systems due to their highly variable hydrology. During floods, dispersal

presumably occurs not only ‘upstream–downstream’ but also laterally via the network of ephemeral channels because of the low topographic relief of the region and the intense water flow. We sampled *M. australiense* from waterholes in the four major catchments (Bulloo, Cooper, Diamantina and Georgina) and tributaries within the Lake Eyre basin as well as in four catchments (Balonne–Condamine, Border rivers, Warrego and Paroo) and tributaries within the Murray–Darling basin. The freshwater prawns were caught using a hand-hauled seine net, placed in a liquid nitrogen container and then transferred into a -80°C freezer, until required for further analysis.

Study species: *M. australiense*

The freshwater prawn family Palaemonidae is found throughout the world in a variety of habitats (Lee and Fielder, 1983). In particular, the genus *Macrobrachium* is widely distributed in Australia, but apparently only one species (*M. australiense*) occurs in the arid and semiarid streams of Western Queensland (Williams, 1980). *M. australiense* is the most common and abundant macro-invertebrate species in these water systems (Lee and Fielder, 1983; Sheldon and Walker, 1998). It completes all the life stages in water, including a planktonic larval phase (Williams, 1980), but it does not possess any strategies, such as resistant eggs or ability to construct burrows, to overcome desiccation (Williams, 1980). *M. australiense* is thought to be able to disperse across terrestrial environments (Lee and Fielder, 1984). However, such terrestrial dispersal across watersheds seems to be unlikely in Western Queensland due to the extreme aridity of the region.

Mitochondrial DNA extraction, PCR and sequencing

Mitochondrial DNA was extracted from the muscle tissue of *M. australiense* using the alkaline lysis protocol outlined in Tamura and Aotsuka (1988). A 710 base pair fragment of mitochondrial DNA cytochrome *c* oxidase subunit I (COI) was amplified using polymerase chain reaction (PCR) with primers LCO1490 and HCO2198 (Folmer *et al*, 1994). Reactions contained 0.5 μl of 10 mM dNTPs, 1.25 μl of 50 mM MgCl_2 , 2.5 of $10 \times$ polymerase reaction buffer, 0.1 μl of *Taq* polymerase (Biotech International Limited), 1 μl of each primer (10 μM), 4 μl of mtDNA template and adjusted to a final volume of 25 μl with ddH₂O. PCR was performed on the Geneamp PCR System 9700 (PE Applied Biosystem); DNA was initially denatured at 94°C for 5 min, followed by 35 cycles of: denaturation at 94°C for 30 s; annealing temperature at 55°C for 30 s and extension at 72°C for 45 s. The final extension at 72°C for 7 min terminated each reaction. A negative control was always included to eliminate the possibility of contamination. PCR product was purified using a Quiaquick agarose gel purification kit (Qiagen Pty Ltd, Victoria, Australia) and directly sequenced on an automated 377 Applied Biosystems sequencer. Each individual was sequenced using LCOI 1490; a few samples per waterhole were sequenced in both directions to check sequence accuracy. Sequences were aligned using the SEQUENCHER™ package (1995, Gene Codes Corporation, Inc.).

Figure 1 Study area and sampling sites for *M. australiense*. Site names and catchment groupings are: Georgina catchment: Hamilton river at Bulla Bulla waterhole (22), Georgina river at Boulia (23), Burke river at Boomerang waterhole (24), Eyre creek at Cuttaburra crossing (25); Diamantina catchment: Wockingham creek at Winton (17), Diamantina river at Combo waterhole (18), Diamantina river at Verdun Valley (19), Diamantina river at Diamantina Lakes (20), Diamantina river at Monkira (21); Cooper catchment: Thompson river at Noondah (11), Barcoo river at Retreat (12), Kyabra creek at Springfield (13), Cooper creek at Windorah (14), Cooper creek at Tanbar (15), Wilson river at Noccundra (16); Bulloo catchment: Bulloo river at Thargomindah (10); Paroo catchment: Paroo river at Cheepie (8), Paroo river at Eulo (9); Warrego catchment: Ward river at Charleville (3), Warrego river at Quilberry (4), Warrego river at Glencoe (5), Warrego river at Thurulgoona (6), Cuttaburra creek at Tinnenburra (7); Balonne-Condamine catchment: Condamine river at Brigalow (1), Balonne river at St George (2); Border Rivers catchment: Whalan creek at Salt Bush (26), Weir river at Kanowna (29), Gnungarah creek at Gnungarah (28) and Commoroon creek at Iron Bark (28).

Genetic variability

Cytochrome *c* oxidase subunit I (COI) is a mitochondrial coding gene, so we tested for its neutrality prior to genetic analysis. We performed Tajima's *D* test for selective neutrality (1989) in the program Arlequin version 2.0 (Schneider *et al*, 2000). Molecular diversity estimates were calculated using the package Arlequin version 2.0 (Schneider *et al*, 2000).

Population structure

The spatial distribution of genetic variation was examined using an analysis of molecular variance (AMOVA; Excoffier *et al*, 1992) and nested clade analysis (NCA; Templeton *et al*, 1995). AMOVA was performed to test the null hypothesis that genetic variation was not associated with drainage structure, using Arlequin version 2.0 software (Schneider *et al*, 2000). Φ -Statistics (based on haplotype frequencies and molecular divergence) and *F*-statistics (haplotype frequencies only) were used to

assess population subdivision. Hierarchical partitioning was calculated between and within the two drainages: Lake Eyre basin and Murray–Darling basin. In addition, separate hierarchical AMOVAs were calculated within and among catchments for both the Lake Eyre and Murray–Darling basins. Significance was tested using a nonparametric permutation procedure (Excoffier *et al*, 1992), incorporating 10 000 permutations.

Phylogeographic analysis

Nested clade analysis was used to test the null hypothesis of no association between clades and geographic locations. We constructed a haplotype network displaying evolutionary relationships among sampled haplotypes using the program TCS 1.13 (Clement *et al*, 2000), which incorporates the cladogram algorithm described by Templeton *et al* (1992). The nesting information, sample size for each haplotype and geographical location of each clade were then entered into the software

package GeoDis 2.0 (Posada *et al*, 2000). Where significance was detected, we used the updated version (2001) of the Inference Key by Templeton (1998) to determine the likely cause of the association (downloaded from the Crandall home page: http://inbio.byu.edu/faculty/kac/crandall_lab/Computer.html)

We analysed pairwise migration rates and divergence times between populations with a coalescent-based approach using the program MDIV (Nielsen, 2002). MDIV is a program that simultaneously estimates divergence times and migration rates between two populations. We chose to run the program under the 'finite model', which does not assume that only one mutation occurs at each site but takes into account the possibility of multiple hits, differences in the nucleotide frequencies and the presence of transition/transversion bias (Nielsen and Wakeley, 2001). MDIV estimated values for theta ($\theta = 2N_e\mu$), migration rate ($M = 2N_e m$), time of population divergence ($T = t/N_e$) and expected time to the most common ancestor (TMRCA = tu) (Nielsen and Wakeley, 2001). We then ran each simulation 5×10^6 times with a 10% burn-in period as suggested by Nielsen (2002). Likelihood values for θ , M and T were calculated and the value with the highest posterior probability accepted as the best estimate. Values for T and TMRCA were calculated using a generation time of 1 year (Williams, 1980) and a mutation rate estimated at 6×10^{-6} substitutions/locus/year (Shank *et al*, 1999).

Results

Our mitochondrial data did not deviate significantly from predictions of neutrality (Tajima's D : -1.02079 ; $P = 0.14$; Tajima, 1989). We acquired sequence data for 402 specimens of *M. australiense* collected from eight catchments, using direct sequencing. Based on a 505 base pair fragment of COI of unambiguous sequence, we identified 98 unique haplotypes (Appendices 1–3), of which 63% represented single individuals. Of the 505 base pairs sequenced, 76 (15%) sites were polymorphic. The transition (Ts) to transversion (Tv) substitution ratio was 5.3:1. Haplotype gene diversity values (mean: 0.923 ± 0.0075) were high and variable; reflecting the large number of haplotypes detected at most sites.

Population structure

Analysis of molecular variance of a total of 402 *M. australiense* samples detected significant levels of genetic variation at all the spatial scales examined and no haplotypes were shared between the two basins. There were highly significant levels of genetic differentiation of populations between and within the major drainages and among all catchments (Table 1). Within the Lake Eyre basin, we detected significant levels of genetic differentiation of populations between the two groups formed by adjacent catchments: Cooper–Bulloo and Diamantina–Georgina. Also, we observed significant values among and within the four catchments (Table 2). Within the Murray–Darling basin, estimates of the genetic variation were significant among populations within catchments, but not significant among the four catchments (Φ_{CT} : 0.04805; $P = 0.16 \pm 0.0043$) (Table 3). Moreover, within the Murray–Darling basin, the estimate of Φ_{SC} was higher than Φ_{CT} , suggesting that genetic variation is higher between populations within catch-

Table 1 Results of the analysis of molecular variance for populations of *Macrobrachium australiense*, displaying F-statistics and Φ -statistics

Level of partitioning	F-statistics	Φ -statistics
Among drainages (Lake Eyre Basin) (Murray–Darling Basin)	F_{CT} : 0.139*	Φ_{CT} : 0.84254*
Among Catchments (Cooper) (Diamantina) (Georgina) (Bulloo) (Paroo) (Balonne–Condomine) (Warrego) (Border Rivers)	F_{CT} : 0.24*	Φ_{CT} : 0.80559*

The levels of partitioning investigated included: between the two drainages, the Lake Eyre Basin and the Murray–Darling, and also, among all the eight catchments, * $P < 0.001$.

Table 2 Results of the analysis of molecular variance for populations of *Macrobrachium australiense*, displaying the F-statistics and Φ -statistics

Level of partitioning	F-statistics	Φ -statistics
Among groups within Lake Eyre Basin (Cooper–Bulloo) (Diamantina–Georgina)	F_{CT} : 0.288*	Φ_{CT} : 0.387*
Among Catchments within Lake Eyre Basin (Cooper) (Diamantina) (Georgina) (Bulloo)	F_{CT} : 0.295*	Φ_{CT} : 0.41128*
Among populations within catchments	F_{SC} : 0.032*	Φ_{SC} : 0.06393*

The levels of partitioning investigated included: between the groups correspondent to adjacent catchments (Bulloo–Cooper and Diamantina–Georgina) and among and within the four catchments of the Lake Eyre Basin, * $P < 0.001$.

Table 3 Results of the analysis of molecular variance for populations of *Macrobrachium australiense*, displaying the percentage variation and associated Φ -statistics and the F-statistics

Level of partitioning	F-statistics	Φ -statistics
Among catchments within Murray–Darling Basin (Paroo) (Balonne–Condomine) (Warrego) (Border Rivers)	F_{CT} : 0.042	Φ_{CT} : 0.04805
Among populations within catchments	F_{SC} : 0.155*	Φ_{SC} : 0.12058*

The levels of partitioning investigated included: among and within the four catchments of the Murray–Darling Basin. * $P < 0.001$.

ments than between catchments overall (Table 3). This result seems to be largely due to the fact that the population from the Glencoe site in the Warrego (number 5 in Figure 1) did not share any haplotypes with other populations from the same catchment (Appendix 3). Indeed, when the Glencoe site was removed from the analyses, both Φ_{SC} and F_{SC} were not significant and lower than Φ_{CT} and F_{CT} , respectively (Φ_{CT} : 0.0985* and

F_{CT} : 0.1267*, $P < 0.001$; Φ_{SC} : 0 and F_{SC} : 0.02583, not significant).

Phylogeographic structure and NCA

It was evident that the haplotype network was divided into two distinct groups corresponding to the Lake Eyre and Murray–Darling basins. The Murray–Darling clade was more closely related to the Diamantina–Georgina clade than to the Cooper clade (Figure 2, Appendix 2 and 3).

Within the Lake Eyre basin, two further groups could be distinguished. The first group contained mostly samples collected in the Diamantina, the Georgina and the Bulloo catchments (Figure 1), but a single haplotype (H-2) was also found in the Cooper catchment. The network indicated that haplotype 2 was at the centre of this group (Figure 2 and Appendix 2). The second group included haplotypes from throughout the Cooper catchment. Haplotype 37 was the centre of this group and was

detected in all sites with the exception of the Wilson river at Noccundra (Figure 1 and Appendix 2). The Wilson river population (site number 16 in Figure 1) represented a unique subclade.

The group represented by the Murray–Darling haplotypes was complex and there was no clear grouping of samples according to catchments. In fact, there was extensive haplotype sharing between waterholes among adjacent and nonadjacent catchments. Haplotypes 90 and 76 were internal to the network and widespread across the Murray–Darling basin. Haplotype 76 occurred in the Warrego, Border rivers and Balonne–Condamine catchments, while haplotype 90 was widespread across all the catchments within the Murray–Darling basin (Figure 2 and Appendix 3), except for the population of Glencoe in the Warrego (site number 5 in Figure 1). In fact, samples from this population contained a unique subclade (Appendix 3).

Figure 2 Haplotype and genealogy network, indicating the catchment distribution of each haplotype (1–98). Circle size for each haplotype is proportional to the overall frequency in our sample. Note that proportions of colours corresponding to catchment, within each pie chart, are representative of the relative number of that haplotype from each catchment. Haplotypes not detected in the sample are indicated by a small solid black circle.

The 98 haplotypes were partitioned into 42 one-step clades, 11 two-step clades, four three-step clades and two four-step clades (Figures 2 and 3). The haplotype cladogram revealed two divergent clades: 4-1 and 4-2 (Figure 2). Haplotypes within clade 4-1 (3-2 and 3-1) belonged to the Lake Eyre basin, whereas haplotypes within clade 4-2 (3-3 and 3-4) belonged to the Murray-Darling basin (Figure 2, and Appendices 2 and 3). The level of genetic divergence between these clades was approximately 1.6% (uncorrected *P*-distance).

Contingency analyses indicated no significant associations between nested clades and geography ($P > 0.05$) for any one-step clades, except 1-36 (Table 4). The Inference Key (Templeton, 1998) indicated a pattern of geographic variation in clade 2-11 (from the Murray-Darling basin) consistent with restricted gene flow with isolation by distance. For clades 2-1 and 2-3 (from the Diamantina/Georgina and Cooper, respectively), range expansion with long distance colonisation was suggested. Continuous range expansion was indicated for clade 3-3 (from the Murray-Darling). Range expansion with long-distance colonisation was suggested for clade 4-1 (from the Lake Eyre basin) and restricted gene flow with isolation by distance was indicated for clade 4-2 (from the Murray-Darling). The outcome for the total cladogram was inconclusive (Figure 3 and Table 4).

One population of approximately 10–15 sequences was randomly chosen from each catchment and we estimated values for θ , M , T , and *TM*RCa for 28 sets of pairwise

comparisons. Estimates based on time since the populations have diverged (*T*) ranged between roughly 30 000 years ago for populations from different catchments of the Murray-Darling basin to around 800 000 years ago for populations from the two major basins.

It was estimated that, within the Lake Eyre basin, populations in different catchments became separated between 260 000 and 50 000 years ago. Populations from catchments within the Murray-Darling appeared to have been separated more recently, between 80 000 and 13 000 years ago (Table 5).

Estimates of gene flow based on migration rate (*M*) between populations from the two major basins and between those within the Lake Eyre basin were very low. Estimates of *M* between populations from the Murray-Darling were low to moderate, ranging from a value of 1.24 (between the Border Rivers and the Paroo catchments) to 23.25 (between the Warrego and the Balonne-Condamine catchments) (Table 5).

Discussion

Isolation and subsequent genetic divergence can occur in populations of continuously aquatic freshwater species inhabiting separate drainages, because the terrestrial habitat can represent an insurmountable barrier, precluding dispersal and connectivity among drainages. Populations of *M. australiense* clustered into two main clades, corresponding geographically to the two major

Figure 3 Two-, three- and four-step clade nesting for the hypothesised network with 95% plausible set of haplotype connections. Clade step delineation is shown next to each nesting box. A solid branch between haplotype 1-98 is representative of a single base pair change in the mitochondrial sequence.

Table 4 Nested clade analysis showing clade (D_C), nested (D_N) and interior-tip clade ($I-T$) distances

Nesting	Location	No.	D_C	D_N	χ^2 -P	Inference key $\Rightarrow$ Conclusion
1–36	One-step	Clades				
	Tip	84	0^s	74.13^s	0.026	1 $\rightarrow$ 2 $\rightarrow$ 3 $\rightarrow$ 5 $\rightarrow$ 6 $\Rightarrow$ Inconclusive
	Tip	85	53.21	275.21^L		
	Tip	86	0	152.31		
	Interior	87	105.02	124.44		
	$I-T$		89.81	–18.30		
2–1	Two-step	Clades			0	1 $\rightarrow$ 2 $\rightarrow$ 11 $\rightarrow$ 12 $\rightarrow$ 13 $\Rightarrow$ RE LDC (Diamantina/Georgina)
	Interior	1–1	149.80^s	166.69^s		
	Tip	1–4	0	154.90		
	Tip	1–5	0^s	209.88		
	Tip	1–6	296.84^L	294.58^L		
	Tip	1–7	143.81	166.68		
	Tip	1–8	170.35	172.20		
	Tip	1–9	0^s	0^L		
	$I-T$		13.11	–64.86 ^s		
2–3	Interior	1–10	76.33^s	81.06^s	0	1 $\rightarrow$ 2 $\rightarrow$ 11 $\rightarrow$ 12 $\rightarrow$ 13 $\Rightarrow$ RE LDC (Cooper)
	Tip	1–11	0	71.76		
	Tip	1–12	21.67^s	30.32^s		
	Tip	1–16	44.60	63.77		
	Tip	1–17	0^s	237.86^L		
	$I-T$		62.80	–53.38 ^s		
2–6	Interior	1–21	148.59	152.54	0.02	1 $\rightarrow$ 11 $\rightarrow$ 17 $\Rightarrow$ Inconclusive
	Tip	1–25	0	23.55		
	Tip	1–37	78.54	191.52		
	Tip	1–31	114.05	164.93		
	Tip	1–26	146.67	186.52		
	Tip	1–36	138.94	239.64^L		
	$I-T$		24.30	–51.52 ^s		
2–11	Interior	1–39	201.30^L	196.32	0.001	1 $\rightarrow$ 2 $\rightarrow$ 3 $\rightarrow$ 4 $\Rightarrow$ RGF IBD (Murray–Darling)
	Tip	1–40	0^s	104.44^s		
	Tip	1–41	57.24	216.20		
	Tip	1–42	0	239.45		
	Tip	1–38	0	251.72		
	$I-T$		192.71^L	61.00^L		
3–3	Three-step	Clades			0.001	1 $\rightarrow$ 2 $\rightarrow$ 11 $\rightarrow$ 12 $\Rightarrow$ C RE (Murray–Darling)
	Interior	2–5	40.20^s	239.25		
	Interior	2–6	172.69^s	210.63		
	Tip	2–7	99.19^s	239.07		
	Tip	2–8	182.73	206.74		
	Tip	2–11	189.54^s	208.56		
4–1	Four-step	Clades			0	1 $\rightarrow$ 2 $\rightarrow$ 11 $\rightarrow$ 12 $\rightarrow$ 13 $\Rightarrow$ RE LDC (Lake Eyre)
	Tip	3–2	100.15^s	186.37^s		
	Interior	3–1	194.29^s	223.92^L		
	$I-T$		94.14^L	37.55^L		
4–2	Interior	3–3	212.95	212.73	0.003	1 $\rightarrow$ 2 $\rightarrow$ 3 $\rightarrow$ 4 $\Rightarrow$ RGF IBD (Murray–Darling)
	Tip	3–4	0^s	157.95^s		
	$I-T$		212.95^L	54.78^L		
Total	Total	Cladogram			0	1 $\rightarrow$ Inconclusive
	Tip	4–1	211.09^s	338.42^s		
	Tip	4–2	211.62^s	396.16^L		

Only clades with significant permutational χ^2 probabilities for geographic structure have been included in the table. Significantly small or large values for ' D_C ', ' D_N ' and ' $I-T$ ' are in bold and indicated by '^s' and '^L', respectively. 'Inference key $\Rightarrow$ Conclusion' refers to the update version of Inference Key (Templeton, 1998), whereby numbers indicate the steps and conclusions are abbreviated as follows: 'RE LDC', range expansion long-distance colonisation; 'RGF IBD' restricted gene flow with isolation by distance; 'C RE' contiguous range expansion.

drainage basins: the Lake Eyre and the Murray–Darling, which appeared to have diverged 800 000 years ago. This major division between these drainages was also detected by Cook *et al* (2002) based on allozyme data. We suggest that gene flow has not occurred recently between the two basins, resulting in the high genetic divergence between the corresponding populations.

Furthermore, habitat fragmentation may reduce exchanges of individuals between adjacent populations,

leading to an increase of differentiation through random drift. The Western Queensland region is a fragmented habitat for aquatic species. During dry periods, water becomes restricted to waterholes and rivers turn into dryland, resulting in a formidable system of barriers for freshwater fauna. It is only during occasional large floods that water fills up the channel system and provides the opportunity for aquatic dispersal to neighbouring waterholes (Knighton and Nanson, 1997). Our genetic data

Table 5 Pairwise of female effective population sizes ($\theta = 2N_{\text{ef}} u$), migration rates ($M = N_{\text{ef}} m$), time since divergence (T) and time to the most common ancestor (TMRCA) based on analysis of mtDNA COI using the MDIV program

<i>Balonne–Condamine</i>	<i>Border Rivers</i>	<i>Warrego</i>	<i>Paroo</i>	<i>Bulloo</i>	<i>Cooper</i>	<i>Diamantina</i>
<i>Border rivers</i>						
$\theta = 3.2$						
$M = 12.55$						
$T = 13\,000$	—					
TMRCA = 270 000 _{YBP}						
<i>Warrego</i>						
$\theta = 2.3$	$\theta = 3.8$					
$M = 23.25$	$M = 19.4$					
$T = 38\,000$	$T = 31\,600$	—				
TMRCA = 788 000 _{YBP}	TMRCA = 52 000 _{YBP}					
<i>Paroo</i>						
$\theta = 2.22$	$\theta = 5.03$	$\theta = 3.57$				
$M = 2.02$	$M = 1.24$	$M = 2.9$				
$T = 14\,800$	$T = 83\,800$	$T = 44\,600$	—			
TMRCA = 765 000 _{YBP}	TMRCA = 436 600 _{YBP}	TMRCA = 588 000 _{YBP}				
<i>Bulloo</i>						
$\theta = 1.83$	$\theta = 4.18$	$\theta = 2.2$	$\theta = 3.54$			
$M = 0.01$	$M = 0.01$	$M = 0.1$	$M = 0.1$	—		
$T = 744\,000$	$T = 724\,500$	$T = 762\,600$	$T = 50\,1500$			
TMRCA = 997 000 _{YBP}	TMRCA = 901 600 _{YBP}	TMRCA = 828 300 _{YBP}	TMRCA = 670 000 _{YBP}			
<i>Cooper</i>						
$\theta = 2.77$	$\theta = 4.73$	$\theta = 2.69$	$\theta = 3.85$	$\theta = 2.59$		
$M = 0.01$	$M = 0.02$	$M = 0.12$	$M = 0.1$	$M = 0.2$	—	
$T = 812\,000$	$T = 780\,000$	$T = 739\,000$	$T = 436\,000$	$T = 203\,000$		
TMRCA = 867 000 _{YBP}	TMRCA = 721 000 _{YBP}	TMRCA = 753 000 _{YBP}	TMRCA = 633 000 _{YBP}	TMRCA = 293 000 _{YBP}		
<i>Diamantina</i>						
$\theta = 2.34$	$\theta = 4.61$	$\theta = 2.62$	$\theta = 3.46$	$\theta = 2.28$	$\theta = 3$	
$M = 0.01$	$M = 0.03$	$M = 0.1$	$M = 0.1$	$M = 0.3$	$M = 0.2$	—
$T = 722\,000$	$T = 814\,400$	$T = 786\,000$	$T = 473\,000$	$T = 157\,700$	$T = 260\,000$	
TMRCA = 903 000 _{YBP}	TMRCA = 863 000 _{YBP}	TMRCA = 833 000 _{YBP}	TMRCA = 633 000 _{YBP}	TMRCA = 273 300 _{YBP}	TMRCA = 270 000 _{YBP}	
<i>Georgina</i>						
$\theta = 1.64$	$\theta = 3.69$	$\theta = 2.1$	$\theta = 3.13$	$\theta = 1.62$	$\theta = 2.33$	$\theta = 2.42$
$M = 0.09$	$M = 0.01$	$M = 0.09$	$M = 0.1$	$M = 0.3$	$M = 0.2$	$M = 0.4$
$T = 1\,060\,000$	$T = 849\,000$	$T = 1\,000\,000$	$T = 750\,000$	$T = 89\,100$	$T = 221\,000$	$T = 50\,400$
TMRCA = 1110 000 _{YBP}	TMRCA = 943 000 _{YBP}	TMRCA = 1 090 000 _{YBP}	TMRCA = 778 000 _{YBP}	TMRCA = 330 000 _{YBP}	TMRCA = 303 000 _{YBP}	TMRCA = 270 000 _{YBP}

Values with the highest likelihood scores for θ and M . Estimates for T and TMRCA are calculated in years before present (YBP).

detected significant levels of genetic variation within and among catchments as well as a high number of haplotypes and unique clades at some sites (Wilson, Lake Eyre basin and Glencoe, Murray–Darling basin; Figure 1), indicating a general pattern of restricted gene flow.

We suggest that the main consequence of this mosaic of aquatic landscape in Western Queensland is the subdivision of *M. australiense* into many arrays of localised populations and that, despite the potential for movement of individuals across catchment boundaries at flood times, almost none occurs. Our interpretation of this genetic subdivision implies that: (a) despite the potential for long-distance dispersal of some aquatic species, the episodic floods do not ensure a good network of connectivity among waterholes and/or (b) during floods the freshwater prawns do not maximise their potential for dispersal and tend to remain in the waterhole of origin. Even if in a completely different type of stream environment, there is some evidence that, during floods, freshwater fauna accumulate into refugial areas of low hydraulic disturbance, thus avoiding dispersal (Winterbottom *et al.* 1997).

During the Pleistocene, periods of high increased rainfall alternated with periods of very dry weather, associated with interglacial and glacial phases, respectively (Kershaw and Nanson, 1993; Alley, 1998). These climate oscillations deeply changed the hydrological flood regimes in Western Queensland, modifying levels of connectivity between populations of aquatic species. We propose that extensive floods and temporary internal lakes resulted in high levels of connectivity between waterholes during interglacial phases, promoting movement of freshwater prawns within but not between the major drainage basins, resulting in a series of population range expansions.

The late Pleistocene signalled the gradual stabilisation of the climate towards the contemporary quasi-periodic arid hydrological regime of Western Queensland (Alley, 1998). It is likely that the reduction in intensity and frequency of the floods as well as ongoing aridity progressively isolated populations, first from different catchments and then even within catchments.

It was estimated that populations of *M. australiense* in different catchments of the Lake Eyre basin became separated much earlier (260 000–50 000 years ago) than populations of the Murray–Darling basin (80 000–13 000 years ago). We suggest that, despite the progressive reduction of rainfall, floods were still able to connect catchments within the Murray–Darling basin until the end of the Pleistocene, due to the combined effect of geographic distance and hydrology patterns. The channels of the Murray–Darling are organised in a dendritic structure, whereby smaller tributary systems feed into increasingly fewer but longer channels (Williams, 1980; Young, 1999). These channels are less ephemeral than those in the Lake Eyre basin, therefore increasing the potential for connectivity between them. Our data also suggest that *M. australiense* colonised the Bulloo and the Cooper from the Georgina/Diamantina, possibly during the Pleistocene. The structure of the haplotype network indicated that the Cooper clade was derived from the Georgina/Diamantina clade, which is internal within the network and connected to the Murray–Darling group. Similarly, all the

haplotypes of the Bulloo cluster with the Georgina/Diamantina clade.

In both historical biogeography and comparative phylogeography (Avice, 2000), the most parsimonious explanation for multiple taxonomic groups to exhibit common spatial patterns of evolutionary subdivision is that they have been subjected to the same environmental history. For example, a general pattern of restricted gene flow was detected in a number of species in Western Queensland (Hughes *et al.*, in review; Hughes and Hillyer, 2003; Carini and Hughes, in review), suggesting that levels of movements of individuals were limited among populations despite potential mechanisms of dispersal of the species. The Wilson site (Cooper catchment, Lake Eyre basin) contained a unique clade for *M. australiense*. Similarly, this pattern was observed for a mussel species (Hughes *et al.*, in review), indicating that a geographic barrier has isolated this site for long enough to allow the local populations of prawns and mussels to evolve a unique set of haplotypes, despite their potential dispersal capabilities.

The phylogenetic break between populations of *M. australiense* of the Lake Eyre and the Murray–Darling basins has also been identified for a mussel species, *Velesunio ambiguus* (Hughes *et al.*, in review), a crayfish, *Cherax destructor* (Hughes and Hillyer, 2003), and a freshwater snail, *Notopala sublineata* (Carini and Hughes, in review). Such congruence between intraspecific phylogeography from different co-distributed species suggests that these communities have undergone substantial periods of independent evolution due to the same vicariant event (Avice, 1992), in this case the historical geographic separation of the two drainages. Also, evidence for some levels of historical gene flow across catchment boundaries has been detected for a number of species (Hughes *et al.*, in review; Hughes and Hillyer, 2003; Carini and Hughes, in review), including *M. australiense*. In all these cases, it has been suggested by the authors that the climate oscillations of the Pleistocene have caused changes in levels of connectivity between species, resulting in population range expansions. Although preliminary, these observations could indicate that aquatic species inhabiting Western Queensland rivers could share a common biogeographic history.

In conclusion, this study indicated that episodic dispersal of *M. australiense* across catchment boundaries was possible during the Pleistocene, but that contemporary movements of individuals are restricted both between and within catchments. Therefore, despite the potential dispersal capabilities of the species, the population of *M. australiense* is fragmented throughout Western Queensland and gene flow is limited among populations.

The outcomes of this study are particularly important when taken in conjunction with the findings from studies of other aquatic species of the region (Hughes and Hillyer, 2003; Carini and Hughes, in review; Hughes *et al.*, in review).

It has been suggested that comparative analysis of phylogeographies for co-distributed species can not only provide insights into the biogeographical history of a region (Avice, 1992; Riddle, 1996), but also extends the use of intraspecific phylogeography to identify evolutionary significant assemblages of species (Avice, 1992; Moritz, 1995). The implications of restricted gene flow

and population fragmentation reported here for *M. australiense* and for other freshwater invertebrates by Hughes and Hillyer (2003), Carini and Hughes (in review) and Hughes *et al* (in review) may affect the long-term survival of these species. We suggest that, if local populations go extinct as a result of modification of their suitable habitat, the chances of recolonisation would be low. The natural habitat for the freshwater fauna of Western Queensland has recently been modified by heavy human impact (eg excessive extraction of water for irrigation, and introduction of alien species such as the carp, *Cyprinus carpio*; Sheldon, personal comment). Some species, such as *N. sublineata*, once widespread, have already apparently disappeared in the Murray–Darling system and are in decline in other areas (Walker, 1996). It has been proposed that comparative analyses of phylogeographies provide a way to investigate geographical patterns of the genetic component of biodiversity as one element of assessing priorities for conservation planning (Avice, 1992; Moritz, 1995). Understanding and combining all the information for freshwater organisms of Western Queensland is essential to develop a suitable management plan for the preservation of such a pristine environment and its endemic fauna.

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Appendix 1

A total of 76 variable sites for 98 haplotypes of COI fragment from 402 *M. australiense* specimens are shown in Table A1.

Table A1

[		111111111	111112222	222222223	333333333	333333344	444444444	444445]
[	1122235566	7111233555	6788913344	6667779900	0112334466	6788999000	0123444456	788990]
[	5814544737	8147928069	5213263736	1470391706	9281692503	6526039235	8763145702	426254]
H-1	ACGTCTCAAT	CTCCCTTATA	CCCAAGTAGT	ATCAGAACCG	CCTAATCCCG	AGCCTAGCCA	ACAAACTTAA	ATGACC
H-3				A			..C.....	
H-4						...T.....	..C.....	
H-5		T.....					..C.....	
H-6		T.T.....					..C.....	
H-7		..T.....					..C.....	
H-8			...G.....				..C.....	
H-2							..C.....	
H-9					..A.....		..C.....	
H-10		C.					..C.....	
H-11						...T.....	..C.....	
H-12							..C...G...	
H-13	...T.....	G					..C...G...	
H-14		G					..C.....	
H-15		..C.....	T.....				..C.....	
H-16			T.....				..C.....	
H-17			T.....				..C.....	
H-18				...A.....			..C.....	
H-19							..C.....	
H-20					T.....		..C.....	
H-21						...C...T...	..C.....	
H-22			...A.....				..C.....	
H-23		...C.....					..C.....	
H-24		..C.....	A.....				..C.....	
H-25			...A.....				..C.....	
H-26					..C.....		..C.....	
H-27	..T.....				..C.....		..C.....G...	
H-28	..T.....				...G.....	...G.....	..C.....G	G.....
H-29	..T.....				...G.....	...G.....	..C.....	
H-30	..T...A...	T.....			...G.....	...G.....	..C.....	
H-31	..T.....				...G.....		..C.....	
H-32	..T.....				...G.....	...A.....	..C.....	
H-33	..T.....					...G.....	..C.....	
H-34						...G.....	..C.....	
H-35	..T.....		...A.....				..C.....	
H-36	..T..T.....						..C.....	

Table A1 Continued

H-37	.T.								C.		
H-38	.T.								C.		G.
H-39	.T.							G	C.		G.
H-40	.T.						C		C.		G.
H-41	.T.							G	C.		
H-42	.T.					G			C.		G.
H-43	.T.					G			C.		
H-44	.TA					G			C.		
H-45	.T.								C.		G.
H-46	.T.		C						C.		
H-47	.T.								C.		T.
H-48	.T.					C			C.		
H-49	.T.			T					C.		
H-50	.T.		T			G			C.		
H-51								A	C.		
H-52							T.A		A	C.	A.
H-53		G		G	CC	GA		C	AT	C	A.
H-54		C	G		G	CC	A	CT	AT	C	A.
H-55		C			G	CC	A	C	AT	C	A.
H-56	G	C			G	CC	A	C	AT	C	A.
H-57		C		G		G	CC	A	C	AT	C
H-58		C	G		G	CC	A	C	AT	CC	A
H-59		C	G		G	CC	A	C	AT	C	A.
H-60		C	G		G	CC	A	TC	AT	C	A.
H-61		C		G		G	CC	A	C	AT	C
H-62		C	G		G	A	CC	G	A	C	A.
H-63	T	C	G		G	CC	G	A	C	AT	C
H-64		C	G		G	A	CC	A	C	T	AT
H-65		C	G		G	CC	A	C	T	AT	CC
H-66		C	G		G	CC	A	C	T	C	AT
H-67		C	G		C		TG	CC	A	C	G
H-68		C	G			G	CC	A	C	A	AT
H-69		C	G			G	CC	A	C	AT	CC
H-70		C	G			G	TC	A	C	G	AT
H-71		CTC	G			G	CC	A	T	C	AT
H-72		CTC	G			G	CC	A	T	C	AT
H-73		CTC	G			G	CC	A	T	C	AT
H-74		C	G		T	G	CC	A	C	AT	C
H-75		C	G			G	CC	A	C	AT	C
H-76		C	G		C		G	CC	A	C	A
H-77		C	G		C		G	CC	A	C	AT
H-78		C	G			TG	CC	A	C	AT	C
H-79		C	G		T		G	CC	A	C	AT
H-80		C	G			G	CC	A	C	G	AT
H-81		C	G			G	CC	A	C	A	AT
H-82		C	G			G	CC	A	C	CT	A
H-83		C	G			G	CC	A	A	C	G
H-84		C	G		C		G	CC	A	C	G
H-85		C	G			G	CC	A	C	G	AT
H-86		C	T	G			G	CC	A	C	T
H-87		C	G			G	CC	A	C	C	T
H-88		C	G			G	CC	A	C	C	T
H-89		C	G			G	CC	A	C	T	AT
H-90		C	G			G	CC	A	T	C	T
H-91		C	G			G	CC	A	C	AT	C
H-92		C	G			G	GCC	A	C	G	AT
H-93		C	G			G	CC	A	C	T	GAT
H-94		C	G		C		G	CC	A	C	T
H-95		C	G			G	CC	T	A	C	T
H-96		C	G			G	CC	A	C	TT	AT
H-97		C	GG			G	CC	A	C	T	AT
H-98		C	G			G	CC	A	C	T	AT

Appendix 2

Distribution of haplotypes detected in 18 waterholes for *M. australiense* within the Lake Eyre basin. For site designation, refer to Figure 1. Catchments: Bulloo (B), Cooper (C), Diamantina (D) and Georgina (G) are shown in Table A2.

Table A2

Catchments sites	B	C	C	C	C	C	C	D	D	D	D	D	D	G	G	G	G
Haplotypes	10	14	13	15	11	12	16	20	30	21	19	18	17	22	25	24	23
H1														1			
H2	2	2						9	2	7	12	5	6	8	7	9	10
H3															1		
H4											1		1				
H5															1	1	
H6											1						
H7													1				
H8										1							
H9														1			
H10															1		
H11								2		1	1	1					
H12								1		1							
H13											1						
H14											1						
H15	1																
H16	4																
H17	1																
H18								1	2	3	3	1	1				
H19									2	2			1				
H20										1							
H21								1									
H22								1		1		1					
H23								2		2							
H24	1																
H25									1			1	1				
H26								2	1	1		1					
H27		2															
H28			1														
H29			1	5													
H30					2												
H31			1														
H32		2		2													
H33				2													
H34				1													
H35						1											
H36		1															
H37		11	13	9	14	5											
H38																	
H39																	
H40																	
H41					1												
H42							1										
H43		2	1														
H44																	
H45					2												
H46					2												
H47			2	1													
H48			2														
H49		1															
H50						1											
H51								1									

Appendix 3

Distribution of haplotypes detected for *M. australiense* within the Murray–Darling basin. For site designation, refer to Figure 1. Catchments: Warrego (W), Paroo (P), Balonne-Condomine (BC) and Border Rivers (BR) are shown in Table A3.

Table A3

Catchments sites	W	W	W	W	W	P	P	BC	BC	BR	BR	BR	BR
Haplotypes	4	6	5	7	3	8	9	1	2	26	29	28	27
H52							1						
H53												1	2
H54								1					
H55											1		1
H56											1		
H57											1		
H58				2									
H59			1										
H60										2	1	1	1
H61				1									
H62	1												
H63										1			
H64									1				
H65			2										
H66			12										
H67			1										
H68			1										
H69			1										
H70			3										
H71			1										
H72			1										
H73					1								
H74		1		2		1	5						
H75										1			
H76	2			1				5	5	3		3	3
H77											1		
H78									1		1	1	
H79												1	
H80								1					
H81				1									
H82	1			2				1					
H83		1											
H84		4											
H85											1	1	
H86	1												
H87	1	1		3	1				1				
H88						1	2						
H89										1			
H90	7	8		8	8		4		1	2	3	3	2
H91							2						
H92												1	
H93													1
H94										1			
H95												1	
H96													1
H97										1	1	1	
H98							1						