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Population genetic studies on the Australian freshwater crayfish, *Cherax destructor* (Crustacea: Parastacidae) using allozyme and RAPD markers

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Abstract – Allozyme and Random Amplified Polymorphic DNA (RAPD) variation was surveyed in the freshwater crayfish *Cherax destructor* Clark, an ecologically and commercially important species that is widespread throughout the freshwater systems of central Australia. At the intra-population level, allozymes revealed a similar level of variation to that found in other freshwater crayfish; RAPDs showed less diversity than allozymes, which was unexpected. At the inter-population level, both techniques revealed significant population structure, both within and between drainages. RAPD results were consistent with phylogeographic patterns previously identified using mtDNA. Although allozyme data showed little geographic pattern in relation to genetic variation based on multidimensional-scaling (MDS) plots on matrices of genetic distance, results of AMOVA and Mantel tests indicated significant population structuring. Each of the mtDNA lineages proposed in a previous study also showed significant genetic structure at similar levels as revealed by RAPDs but different levels by allozymes. These results reject hypotheses previously put forward on genetic homogenisation within the species due to wide-scale translocation. The implications of the findings for conservation and aquaculture of *C. destructor* are also discussed.

Key words: Population genetics / Allozymes / RAPDs / *Cherax destructor*

Résumé – Étude de la diversité génétique des populations de l'écrevisse australienne, *Cherax destructor* (Crustacés : Parastacidés) par les allozymes et les marqueurs RAPD. Le polymorphisme des allozymes et des marqueurs RAPD est étudié chez l'écrevisse *Cherax destructor* Clark, espèce d'importance écologique et commerciale et qui est largement répandue dans tous les bassins hydrographiques de l'Australie centrale. A l'intérieur des populations, les allozymes révèlent un niveau similaire de variation à celui trouvé chez d'autres écrevisses ; les marqueurs RAPD montrent moins de diversité que les allozymes, ce qui est inattendu. Entre les populations, les deux techniques révèlent une structure génétique significative, à la fois au sein d'un même bassin hydrographique et entre bassins hydrographiques. Les résultats obtenus avec les marqueurs RAPD révèlent les mêmes relations phylogéographiques identifiées antérieurement par étude de l'ADNmt. Bien que les données allozymiques révèlent peu de structure géographique en relation avec la variation génétique, basée sur l'analyse en composantes principales et le positionnement multidimensionnel (MDS) des matrices des distances génétiques, les résultats des tests AMOVA et de Mantel indiquent une structure significative des populations. Chacune des lignées d'ADNmt proposées dans une précédente étude montre également une structure génétique significative à des niveaux similaires pour les marqueurs RAPD mais différents pour les allozymes. Ces résultats rejettent les hypothèses posées antérieurement sur l'homogénéisation génétique chez cette espèce suite à des translocations réalisées à grande échelle. Les implications de ces résultats pour la protection et l'aquaculture de *C. destructor* sont aussi discutées.

1 Introduction

Investigations of genetic variation in freshwater crayfish have greatly benefited from rapid developments in molecular techniques. A range of molecular methods has been applied, resolving many questions across a large number of freshwater crayfish taxa (Fetzner and Crandall 2001). The collection of mitochondrial DNA (mtDNA) sequences is the most popular method, and is principally used for addressing systematic questions.

Freshwater crayfish have attracted few population genetic investigations in comparison to molecular systematic studies. Further, the few studies undertaken have investigated population structure of North American (Fetzner and Crandall 1999) and European species (Gouin et al. 2001; Edsman et al. 2002). In Australia, the centre of freshwater crayfish diversity

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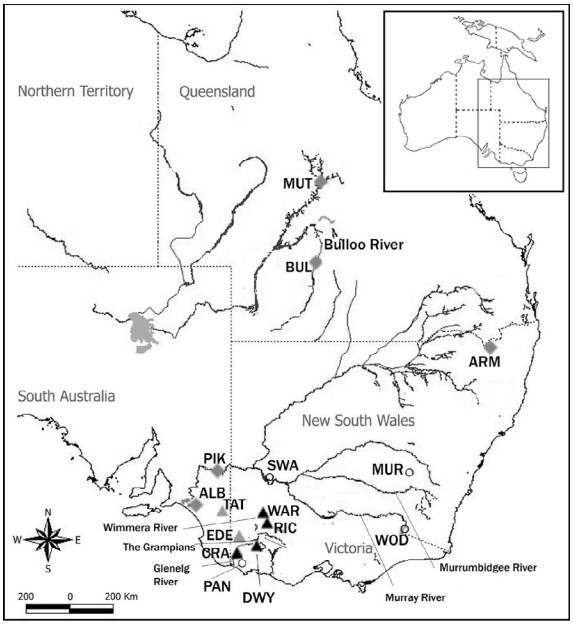

Fig. 1. Sampling localities of crayfish used in the present study. Grey diamond = "northern" *Cherax d. destructor*, open circle = "southern" *C. d. destructor*, black triangle = *C. d. albidus*, grey triangles indicate populations with mixed individuals of *C d. destructor* and *C. d. albidus*.

in the Southern Hemisphere, the only population genetic studies are those by Campbell et al. (1994) and Hughes and Hillyer (2003) on the yabby, *Cherax destructor*, and that of Macaranas et al. (1995) on the redclaw, *Cherax quadricarinatus* (von Martens), the two most important commercial species. Studies by Campbell et al. (1994) and Hughes and Hillyer (2003) were however, limited by sampling mostly within the southern and northern parts of *C. destructor* distribution range, respectively.

Investigation of the population structure of *C. destructor* is of special interest. The species is the most widespread and abundant of all Australian freshwater crayfish, with a natural distribution encompassing freshwater habitats within an area of more than two million square kilometers, from South Australia and the southern Northern Territory in central Australia, to the Great Dividing Range in the east (Fig. 1). *Cherax destructor* is also an ecologically versatile species, inhabiting a wide variety of freshwater systems (e.g., rivers, creeks, lakes, farm dams) and habitats (from still to flowing waters, clear to turbid, clay to rocky substrates). Furthermore, it is also stocked and fished for recreational purposes, used as bait for fishing, and maintained as an aquarium pet, providing further opportunities for anthropogenic dispersal.

Population genetic studies of *C. destructor* are also of particular interest because the species is thought to have been frequently translocated as a food source by Australian aborigines (Horwitz and Knott 1995), and for aquaculture (Austin 1987; Morrissy et al. 1990; Merrick and Lambert 1991). Some authors have suggested that such translocations have contributed to genetic homogenisation of *C. destructor* populations (Campbell et al. 1994; Horwitz and Knott 1995). It is now well documented that translocation events have impacted intraspecific genetic diversity, for example in the European freshwater crayfish (Lörtscher et al. 1997; Grandjean et al. 2001; Gouin et al. 2003), and in such a context there is a need to address these concerns for *C. destructor* also (Nguyen et al. 2004).

Hitherto, implication on population genetic structure of *C. destructor* has been indicated in several studies. Campbell et al. (1994) studied allozyme variation in *C. destructor* and found significant allelic frequency differences between populations. While Campbell et al. (1994) found no clear geographic pattern to this variation, their study was limited to the southern parts of the distribution range. Hughes and Hillyer (2003) examined samples from the northern part of *C. destructor* distribution using mtDNA, and found low level of genetic differences within drainages, but extremely high differentiation between drainages. The results from an analysis of 16S rRNA variation across the entire species range (Austin et al. 2003; Nguyen et al. 2004) revealed a significant phylogeographic pattern, with three geographically non-overlapping clades, namely "northern" *C. d. destructor*, "southern" *C. d. destructor*, and *C. d. albidus*. The "northern" *C. d. destructor* clade has an extensive distribution, encompassing the Murray-Darling, Bulloo and Lake Eyre systems, while the "southern" lineage of *C. d. destructor* is mostly confined to the upper Murray and Murrumbidgee river system *Cherax d. albidus* clade has the most limited distribution of the three clades, restricted to the Wimmera and Glenelg river systems, both of which drain the Grampian Range in the extreme south-east (Fig. 1).

Although the use of mtDNA to study population genetic structure has advantages (i.e., mtDNA is maternally inherited, faster evolving, lacks recombination, and has a smaller effective population size; Avise 1994), data from mtDNA however, suffer from several disadvantages. Firstly, it represents only one locus and inferred gene trees may not show congruence with organismic phylogeny (Avise 1994). Reliance on a single genetic locus greatly diminishes the power to detect significant spatial or temporal structure (Palumbi and Baker 1994). Secondly, mtDNA allows only the reconstruction of maternal lineages (Wilson et al. 1985; Avise et al. 1987). Accordingly, in species that are likely to show gender-biased dispersal, population structure of maternally inherited mtDNA may differ from that of biparentally inherited nuclear DNA (Karl and Avise 1992; Karl et al. 1992).

The present study therefore, was primarily aimed to further evaluate population structure previously revealed by mtDNA data with the presence of three clades (i.e. "northern" *C. d. destructor*, "southern" *C. d. destructor* and *C. d. albidus*) reported by Nguyen et al. (2004) using two nuclear markers, allozyme and RAPD, with samples collected throughout the species distribution range. Population structure among three clades were addressed using analysis of molecular variance (AMOVA) to test for the null hypothesis of no significant population structure among them.

In addition, in the study by Nguyen et al. (2004) using mtDNA data indicated that the three clades within *C. destructor* mentioned above presented true phylogeographic divisions. Also, the issue of genetic differentiation among populations was not addressed due to small sample sizes. As such in the present study, an attempt was made to

Table 1. Sample codes, sample sizes (*n*), localities and coordinates for populations of freshwater crayfish (*Cherax* spp.) used in the present study.

Taxon	Sample code	Locality	*n*	Coordinates
C. d. destructor	BUL	Bulloo River, Queensland	70	144°30'E 25°30'S
	MUT	Muttaburra, Queensland	50	144°42'E 22°40'S
	ARM	Armidale, New South Wales	55	152°02'E 29°10'S
	PIK	Pike Creek, South Australia	35	140°36'E 33°10'S
	URA	Urana, New South Wales	33	146°15'E 35°20'S
	WOD	Wodonga, Victoria	45	149°02'E 35°16'S
	SWA	Swan Hill, Victoria	61	143°42'E 34°55'S
	PAN	Panmure, Victoria	59	142°35'E 38°09'S
	ALB	Lake ALbert, South Australia	50	139°22'E 35°42'S
C. d. albidus	WAR	Warracknabeal, Victoria	50	142°35'E 36°00'S
	RIC	Richardson River, Victoria	40	142°48'E 36°22'S
	DWY	Dwyers Creek, Victoria	50	141°13'E 37°19'S
	CRA	Crawford River, Victoria	50	141°22'E 37°30'S
Mixed populations *	EDE	Edenhope, Victoria	70	141°18'E 57°03'S
	TAT	Tatiara Creek, South Australia	60	140°46'E 36°18'S

* Populations showed mixed mtDNA haplotypes of *C. d. destructor* and *C. d. albidus* according to Nguyen (2004).

Table 2. Enzyme systems studied, Enzyme Commission (E.C.) code number, gel medium (S = starch, C = cellulose acetate) and tissues examined (H = hepatopancreas, M = abdominal muscle). Running conditions for starch and cellulose acetate gels using TG buffer were 200 V for 4 h and 200 V for 20 min, respectively.

Enzyme	Abbreviation	E. C. Number	Gel Medium	Tissue
Aspartate Aminotransferase	AAT	2.6.1.1	C	M
Alcohol Dehydrogenase	ADH	1.1.1.1	S	M
Aldehyde Oxidase	AO	1.2.3.1	C	M
General Proteins	GP	–	S	M
Glucosephosphate Isomerase	GPI	5.3.1.9	S/C	M
Alanine Aminotransferase	GPT	2.6.1.2	C	M
Malate Dehydrogenase	MDH	1.1.1.37	C	M
Peptidase – A*	PEP-A	3.4.11	C	M
Peptidase – D**	PEP-D	3.4.11	C	M
Phosphoglucosemutase	PGM	2.7.5.1	S/C	M
L-Iditol Dehydrogenase	SDH	1.1.1.14	C	H

* Substrate Valine – Leucine (Val-Leu).
** Substrate Phenylalanine – Proline (Phe-Pro).

investigate the finer population structure within each of the clades by testing the null hypothesis of no significant genetic structure among populations within each clade.

2 Materials and methods

2.1 Crayfish samples

A total of 828 individuals representing 15 populations of *C. destructor* were collected during 2000–2002 using drop nets, fish traps or by turning over rocks and the sampling details are given (Table 1, Fig. 1).

2.2 Allozyme electrophoresis

Initially, 4–5 individuals from each population of *C. destructor* were screened for variation using 30 enzyme systems, which included those previously found to be variable (Campbell et al. 1994; Austin 1996). Only variable loci that could be consistently scored were then used to genotype all individuals. Details of loci and running conditions are presented in Table 2. Staining procedures largely followed Richardson et al. (1986) and Hebert and Beaton (1993).

2.3 RAPD analysis

RAPD analyses followed procedures described by Nguyen and Austin (2004). This study also documented Mendelian inheritance of these markers, indicating their suitability for population genetic analysis. Thirty individuals of *C. destructor* from each population were scored for variation, with the exception of Edenhope (70 individuals) and Tatiara (50 individuals), as these populations putatively represent mixtures of individuals from the two subspecies, *C. d. destructor* and *C. d. albidus* (Campbell et al. 1994; Nguyen 2004).

RAPD amplified products were separated in 1.2% agarose gels in 1 × TAE (40 mM Tris base, 20 mM acetic acid, 2 mM

EDTA, pH 8.0), then stained with ethidium bromide and visualized on UV transilluminator. Only bands of high intensity were taken into account as they are the most reproducible (Skroch and Nienhuis 1995). RAPD markers were then scored if they displayed a clear polymorphism (i.e. presence or absence) with no faint amplification. If there were any doubt about the reliability of a band, the reaction was repeated before the final scoring.

2.4 Statistical procedures

2.4.1 Allozyme data

Allozyme variation (allele frequencies, heterozygosity, polymorphism, allelic diversity) was estimated using GenePop version 3.3 (Raymond and Rousset 1995). Fisher's exact tests of deviations from Hardy-Weinberg equilibrium and allele frequency homogeneity among samples employed the same program. For the latter, exact test statistics were combined across loci for each pairwise comparison of populations using Fisher's method, rather than applying Bonferroni correction to individual loci. This "summation" approach has a realised type I error rate that is much closer to the intended α than the "Bonferroni" approach, and hence provides a more powerful test of genetic homogeneity (see Ryman and Jorde 2001). Tests for genotypic disequilibrium among loci were also performed using GenePop. Weir and Cockerham's (1984) θ, an estimator of Wright's (1978) F_{ST}, was calculated for each locus using Fstat version 2.9.3 (Goudet 2001).

2.4.2 RAPD data

For RAPD data, heterozygosity was calculated using PopGene 1.3.1 (Yeh et al. 1999) assuming Hardy-Weinberg equilibrium. Population divergence was assessed by an analysis of molecular variance (AMOVA) for binary data, using the GenAlEx add-in for Microsoft Excel (Peakall and Smouse 2003). In this analysis, RAPD bands were used as a phenotypic marker (i.e., presence or absence) rather than for estimating gene frequency which requires the assumption of Hardy-Weinberg equilibrium for dominant markers (Peakall et al. 1995). To allow comparisons of intra-population diversity between allozyme and RAPD data, Shannon's diversity index was calculated using PopGene 1.3.1. Multidimensional scaling (MDS) was undertaken on matrices of genetic distances (Rogers 1972) for all samples to test for the presence of non-hierachical patterns of relationships among *C. destructor* populations using the SPSS package (Version 10).

2.4.3 AMOVA

In order to examine population genetic structure, estimates of Φ-statistics was conducted via AMOVA using GenAlEx based on three subsets of data that represent three of the mtDNA lineages. The two putative mixed populations (TAT and EDE) were not included in any of these data sets. Details of the three subsets of data are as follows:

- "Northern" *C. d. destructor*: MUT, BUT, ARM, PIK and ALB;
- "Southern" *C. d. destructor*: SWA, MUR, WOD and PAN;
- C. *d. albidus*: WAR, RIC, DWY and CRA.

To facilitate comparisons between the nuclear population genetic structure and phylogenetic relationships inferred from mtDNA sequences, estimates of Φ-statistics were calculated with groups defined on the basis of the three geographically correlated mtDNA clades: "southern" *C. d. destructor*, "northern" *C. d. destructor*, *C. d. albidus* (Nguyen et al. 2004). Multiple tests of the same null hypothesis were subjected to table-wide sequential Bonferroni correction to avoid elevated Type I error rates.

2.4.4 Mantel tests

Mantel tests were used to investigate the concordance of allozyme and RAPD estimates of population divergence with geographic distance using IBD 1.2 (Bohonak 2002), based on $\log_{10}$ geographic distance and Rogers's (1972) genetic distance. Minimum straight-line geographic distance, rather than stream distance, was employed as many of the populations exist in unconnected catchments. The strength of the relationship between geographic and genetic distance was examined via reduced major axis regression using IBD 1.2. This approach is favoured over ordinary least squares regression when there is potentially much larger error associated with the independent variable, and simulations have shown that the ordinary least squares tends to underestimate the true slope of the relationship (Hellberg 1994). Large error in the independent variable could be present given that we are forced to employ straight-line, rather than stream distance.

3 Results

3.1 Allozyme variation

Polymorphism was observed at nine presumptive allozymes, all of which were previously employed by Campbell et al. (1994) and Austin (1996). None of the additional (21) presumptive loci exhibited polymorphism. Polymorphism within *C. destructor* populations varied from no loci (Panmure) to six (Tatiara), and at the 95% criterion ranged 0 to 0.384, and 0 to 0.462 at 99% criterion (Table 3). This pattern was also reflected by average heterozygosity (H_o = 0 to 0.115), Shannon's diversity index (SI = 0 to 0.189), and average number of alleles (A_n = 1.08−1.54) (Tables 3 and 4). Frequencies of heterozygotes were consistent with Hardy-Weinberg expectations ($p > 0.05$). The null hypothesis of genotypic equilibrium was not rejected for any pair of polymorphic loci ($p = 0.097 \pm 0.004$ to 1).

Significant allele frequency heterogeneity was apparent among populations based on pairwise comparisons. A total of 10 pairwise comparisons among populations of "northern" *C. d. destructor*, all of which resulted in highly significant *p*-value. Within "southern" *C. d. destructor* group, only two were non-significant in a total of six pairwise comparisons. For *C. d. albidus*, non-significant heterogeneity was found between WAR, RIC and DWY samples, all of which belong to the Wimmera River system (Table 5).

Table 3. Allele frequencies at polymorphic protein loci in *Cherax destructor*. Numbers in parentheses are sample sizes. Alleles are denoted by relative mobility in relation to the most common allele (designated as 100). A_n: the average number of alleles per locus, $P_{0.99}$ and $P_{0.95}$ are number of polymorphic loci at 99% and 95% levels of significance, respectively. Estimates of allele frequency variance (θ) amongst samples are presented in the last column and their significance of θ values was estimated from 1000 permutations of alleles among samples. All frequency variances were significant ($p < 0.001$).

Locus	Allele	BUL (70)	MUT (50)	ARM (55)	PIK (35)	URA (33)	WOD (45)	SWA (61)	PAN (59)	TAT (60)	ALB (50)	WAR (50)	RIC (40)	DWY (50)	EDE (70)	CRA (50)	θ
AAT	100	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.0	0.88	0.93	1.00	1.00	1.00	1.00	1.00	0.089
	71							0.01			0.07						±0.035
	42							0.02		0.12							
ADH	180						0.04			0.07							
	100	1.00	1.00	1.00	1.00	1.00	0.96	1.00	1.00	0.85	1.00	1.00	1.00	1.00	1.00	1.00	0.122
	20									0.07							±0.051
GP	150																
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.03	0.948
	50															0.97	±0.027
GPI	116		0.01	0.02				0.02								0.02	
	100	1.00	0.99	0.82	0.81	0.98	0.98	0.97	1.00	0.98	0.99	1.00	0.94	1.00	1.00	0.84	0.091
	84			0.16	0.19	0.02	0.02	0.01		0.02	0.01		0.06			0.14	±0.016
GPT	120							0.01									
	100	1.00	1.00	0.99	1.00	1.00	0.98	0.95	1.00	0.95	0.89	1.00	1.00	0.99	0.99	0.99	0.064
	80			0.01			0.02	0.04		0.05	0.11			0.01	0.01	0.01	±0.028
PEP-A	126							0.14									
	113							0.08									0.320
	100	0.98	1.00	0.61	1.00	1.00	0.99	0.78	1.00	1.00	1.00	1.00	0.97	1.00	0.99	1.00	±0.120
	87			0.39									0.03		0.01		
	74	0.02					0.01										
PEP-D	112	1.00	1.00														1.000
	100			1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	±0.000
PGM	113				0.01	0.03						0.04	0.05	0.01	0.03		
	100	1.00	1.00	0.72	0.01	0.97	1.00	1.00	1.00	0.57	0.94	0.96	0.95	0.99	0.94	0.77	0.5234
	87			0.28	0.97					0.43	0.06				0.03	0.01	±0.249
	74															0.22	
SDH	159						0.12										
	100		0.53		0.41	1.00	0.86	1.00	1.00	0.72	1.00	1.00	1.00	1.00	1.00	1.00	0.785
	59	1.00	0.47	1.00	0.59		0.02			0.28							±0.160
	A_n	1.08	1.15	1.38	1.23	1.15	1.46	1.54	1.00	1.31	1.31	1.08	1.23	1.08	1.31	1.46	
	$P_{0.99}$	0.076	0.153	0.384	0.307	0.153	0.462	0.307	0.000	0.462	0.307	0.076	0.231	0.153	0.231	0.231	
	$P_{0.95}$	0.000	0.076	0.230	0.153	0.000	0.076	0.076	0.000	0.384	0.230	0.000	0.153	0.000	0.000	0.153	

Table 4. Within population genetic diversity estimates of *C. destructor* using Shannon Index (SI) and mean heterozygosity (H_e).

Population	Allozyme		RAPD	
	SI	H_e	SI	H_e
BUL	0.008	0.003	0.049	0.034
MUT	0.058	0.040	0.022	0.015
ARM	0.173	0.092	0.051	0.034
PIK	0.130	0.065	0.032	0.021
URA	0.017	0.007	0.025	0.015
WOD	0.093	0.034	0.049	0.032
SWA	0.091	0.044	0.053	0.034
PAN	0.000	0.000	0.000	0.000
TAT	0.189	0.115	0.167	0.111
ALB	0.068	0.035	0.037	0.024
WAR	0.013	0.006	0.023	0.016
RIC	0.042	0.020	0.011	0.007
DWY	0.009	0.003	0.008	0.006
EDE	0.026	0.011	0.025	0.017
CRA	0.094	0.053	0.000	0.000
Mean	0.067	0.035	0.037	0.025

Table 5. Population divergence between samples (Φ_{PT}) based on allozyme (below diagonal) and RAPD (above diagonal) markers for (a) "northern" *Cherax d. destructor*, (b) "southern" *C. d. destructor*, and (c) *C. d. albidus*. Probabilities based on 999 permutations of alleles among samples are all significant after sequential Bonferroni corrections ($p < 0.05$) except for the one in parentheses.

(a)

	BUL	MUT	ARM	PIK	ALB
BUL		0.847	0.852	0.788	0.865
MUT	0.697		0.888	0.893	0.912
ARM	0.815	0.773		0.867	0.849
PIK	0.929	0.854	0.589		0.885
ALB	0.953	0.839	0.743	0.797	

(b)

	URA	WOD	SWA	PAN
URA		0.834	0.794	0.892
WOD	(0.096)*		0.233	0.903
SWA	0.128	0.142		0.780
PAN	0.047*	0.149	0.176	

* Exact test of allele frequency homogeneity not rejected.

(c)

	WAR	RIC	DWY	CRA
WAR		0.847	0.870	0.900
RIC	0.036*		0.921	0.901
DWY	0.015*	0.066*		0.941
CRA	0.821	0.780	0.828	

* Exact test of allele frequency homogeneity not rejected.

Population structure was revealed within each of the mtDNA lineages. Significant values ($p = 0.001$) were observed for AMOVA over all loci ($\Phi_{PT} = 0.823$, 0.146 and 0.779 for "northern" *C. d. destructor*, "southern" *C. d. destructor* and *C. d. albidus*, respectively). Pairwise Φ_{PT} and significant level after Bonferroni correction for allozymes are presented below diagonal in Tables 5a,b and c. Values of θ for each loci indicated that PGM (0.523), SDH (0.785), GP (0.948), PEP-A (0.320) and PEP-D (0.947) exhibited the greatest variation amongst the samples, although θ values for other loci (e.g., GPI, AAT, ADH, GPT) were also significantly greater than zero (Table 3). When analyzing all samples of *C. destructor* with population grouping according to three geographically correlated lineages (i.e., "northern" *C. d. destructor*, "southern" *C. d. destructor* and *C. d. albidus*), AMOVA showed significant allozyme differences among individuals within populations (15%) ($\Phi_{PT} = 0.85$, $p = 0.001$), among populations within groups (44%) ($\Phi_{PR} = 0.74$, $p = 0.001$) and among groups (41%) ($\Phi_{RT} = 0.41$,$p = 0.001$). A significant relationship between Roger's (1972) genetic distance and $\log_{10}$ geographic distances was apparent using the Mantel test ($r = 0.6036$, $p < 0.01$). Reduced major axis regression indicated that 21% of total allozyme variation was explained by geographic distance amongst the samples (Fig. 4). MDS analysis using Rogers's (1972) distance (Fig. 2a) indicates that most of the allozyme variation occurs among the "northern" *C. d. destructor* populations (MUT, BUL, PIK and ARM), with less variation apparent within and between *C. d. albidus* and southern *C. d. destructor*.

3.2 RAPD

A total of 65 RAPD bands could be reliably and consistently scored, based on concordance with Mendelian expectations observed from 11 hatchery reared family lines (Nguyen and Austin 2004). Six bands were monomorphic, giving an overall polymorphism of 0.908 at the 95% criterion. Average heterozygosity and Shannon's diversity for each population are presented (Table 4). AMOVA over all loci showed significant population structure ($p = 0.001$) within each of the mtDNA lineages ($\Phi_{PT} = 0.868$, 0.823 and 0.902 for "northern" *C. d. destructor*, "southern" *C. d. destructor* and *C. d. albidus*, respectively). Pairwise population Φ_{PT} values for RAPDs are presented above diagonal in Tables 5a,b and c. AMOVA with groups defined on the basis of the mitochondrially-defined lineages showed that the majority of the total genetic variance (52%) occurred between the three lineages ($\Phi_{RT} = 0.52$, $p = 0.001$) and among populations within each group (41%) ($\Phi_{PR} = 0.85$, $p = 0.001$), with only 7% ($\Phi_{PT} = 0.93$, $p = 0.001$) representing within population variation. Rogers's (1972) genetic distance between populations based on RAPD markers exhibited a significant relationship with $\log_{10}$ geographic distance ($r = 0.3932$, $p < 0.01$). Reduced major axis regression estimated that 15% of the variation in genetic distance was attributable to geographic distance among populations (Fig. 3).

Relationships among populations are shown in Figure 2b. The first dimension distinguishes "northern" and "southern" *C. d. destructor*. The second dimension differentiates *C. d. albidus* from *C. d. destructor*. In this analysis, sample ALB, along with sample PIK, also from the lower Murray River, fall into an intermediate position between the other "northern" *C. d. destructor* and the "southern" *C. d. destructor* samples. Sample PAN, which shows morphological features consistent with *C. d. albidus* is clearly associated with "southern" *C. d. destructor* samples in both analyses.

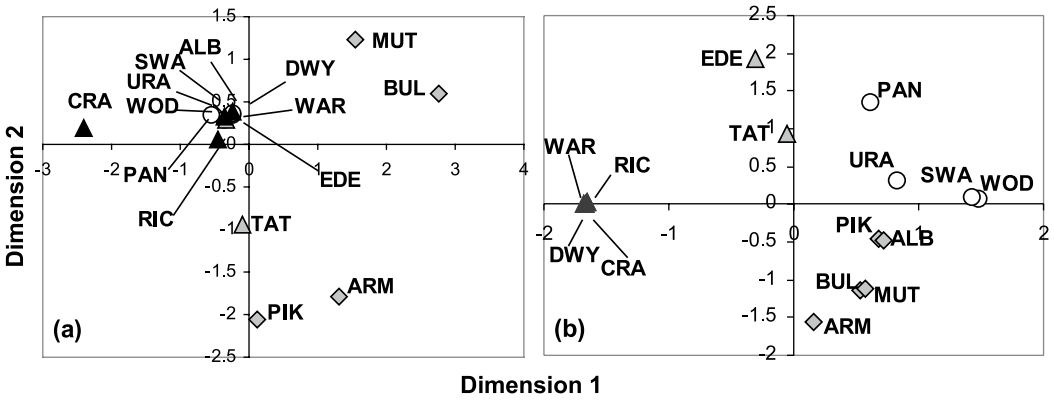

Fig. 2. MDS plot of genetic distance (Rogers 1972) among populations of *Cherax destructor* based on (a) allozyme and (b) RAPD data. Grey diamond = "northern" *C. d. destructor*, open circle = "southern" *C. d. destructor*, black triangle = *C. d. albidus*, grey triangle = mixed populations.

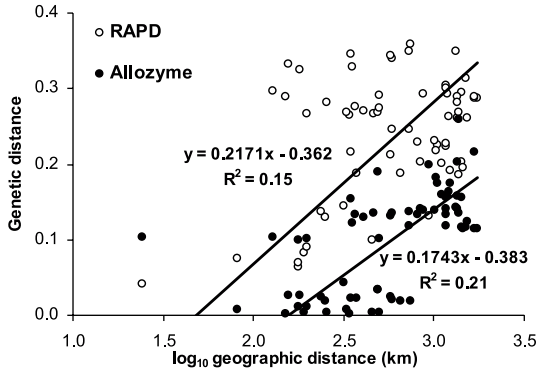

Fig. 3. Reduced major axis regression of genetic distance (Rogers 1972) and geographic distance between samples of *Cherax destructor* for allozyme and RAPD data.

4 Discussion

4.1 Population structure of *Cherax destructor*

4.1.1 Variation within populations

The levels of intra-population allozyme variation in *C. destructor* are low, but consistent with those reported for crustaceans in general (Hedgecock et al. 1979; Nevo et al. 1984). The mean allozyme heterozygosity estimated for *C. destructor* populations, 0.035, is very similar to that reported for other freshwater crayfish (Fetzner and Crandall 2001). There have been few studies of RAPD variation in freshwater decapod crustaceans generally (D'Amato and Corach 1996, 1997), and freshwater crayfish specifically (Macaranas et al. 1995; Souty-Grosset et al. 1999; Schulz 2000; Gouin et al. 2001). These studies report similar levels of diversity to those found in *C. destructor*, except for studies on the European freshwater crayfish *Austropotamobius pallipes* (Souty-Grosset et al. 1999; Gouin et al. 2001), in which much higher levels of intra-population variation were found. Of special relevance to this study are the findings of Macaranas et al. (1995) who examined both allozyme and RAPD variation in *C. quadricarinatus*, which is a widespread species across northern Australia. They found lower heterozygosity in allozymes (0.021) and higher within population variation in RAPDs than those observed in the present study for *C. destructor*. Low levels of heterozygosity in freshwater crayfish are often attributed to low gene flow (Crandall 1997; Fetzner and Crandall 2001) and small effective population size, which erodes allelic diversity via genetic drift (Hedgecock et al. 1979; Avery and Austin 1997).

While intra-population variation was generally low in *C. destructor*, variation in heterozygosity among populations was nevertheless apparent, ranging from 0.000 to 0.115 for allozymes and from 0.000 to 0.111 for RAPDs. The population with the highest diversity for both markers was from Tatiara Creek (TAT), which also has multiple mtDNA haplotypes (Nguyen 2004) and is thought to represent an anthropogenically-induced mixture of *C. d. destructor* and *C. d. albidus* (Campbell et al. 1994).

While both allozyme and RAPD data revealed significant population structure as indicated by results of AMOVA, allozymes showed little geographic pattern based on MDS plots of genetic distances, similar to that reported by Campbell et al. (1994). In contrast, relationships depicted by the RAPD data (Fig. 2b) correspond closely with phylogeographic patterns identified using mtDNA (Nguyen et al. 2004). The RAPD data confirm that *C. destructor* consists of three well-defined geographic groups that represent true divisions within the species. The finding of significant geographic structure to RAPD and mtDNA variation in *C. destructor* indicates that this species has not achieved its distribution in the recent past and that the fragmentation of *C. destructor* into three distinct clades is a Pliocene event (Nguyen et al. 2004).

The contrasting resolving power of the allozyme and RAPD techniques, and the correlation between the RAPD and mtDNA data, is also reflected by AMOVA. The RAPD data indicated that a greater proportion of variation was attributable to variation between mitochondrially-defined groups (52% versus 41%). For species examined using both allozyme and RAPD techniques it has generally been concluded that the latter technique provides greater insight into population structuring (Macaranas et al. 1995; Santucci et al. 1997; Lörtscher et al. 1998; Gouin et al. 2001).

In contrast to the low genetic variation observed within populations of *C. destructor*, substantial inter-population variation was revealed by both allozyme and RAPD data. For allozymes, 85% of the total variation was distributed among

populations and for RAPDs this value was even higher (93%). These results are consistent with general findings that freshwater species show a high degree of genetic structure (Ward et al. 1994; Avise 2000). The few studies on genetic variation in freshwater crayfish report similar estimates of F_{ST} to those herein (Macaranas et al. 1995; Fetzner 1996; Crandall 1997; Gouin et al. 2001), with the exception of an allozyme study on *Astacus astacus* (Fevolden and Hessen 1989), which gave a low estimate of F_{ST}, perhaps reflective of limited geographic sampling.

Significant genetic structure was revealed by allozymes and RAPDs among populations within each of the mtDNA clades. Overall, RAPDs showed high levels of genetic differentiation among populations (pairwise Φ_{PT} = 0.233–0.912) and no differential structuring of the populations was observed between the three clades. It is likely that the population structure of freshwater crayfish will be significantly influenced by the fragmented nature of freshwater environments which limits gene flow and therefore favours inter-population divergence as has been observed in other freshwater crustaceans (Hedgecock et al. 1979; Fuller and Lester 1980; Busack 1988; Fevolden and Hessen 1989). It is also likely that genetic divergence will be further enhanced in freshwater crayfish due to their life history pattern (i.e., direct development without any larval stages) and in Australian species in particular, gene flow may be further restricted by aridity and low river flows typical of their environment.

Allozymes revealed high levels of genetic differentiation among "northern" *C. d. destructor* populations, but limited divergence among populations of "southern" *C. d. destructor* (pairwise Φ_{PT} = 0.096–0.176) and of *C. d. albidus* (pairwise Φ_{PT} = 0.015–0.066, except for the pairwise comparison between CRA to others within this lineage). Low allozyme variation among populations of two latter clades may reflect (a) high level of connectivity within these catchments, as observed by Hughes and Hillyer (2003) for mtDNA variation of *C. destructor* in the northern part of its distribution, or (b) allozymes have been demonstrated to be ineffective in revealing genetic relationship and historical patterns in *C. destructor*, a possibility considered by Horwitz and Knott (1995).

The reason for high levels of genetic structuring of "northern" *C. d. destructor* compared to the other two clades in the southern parts of its distribution revealed by allozymes, can be explained using the hypotheses proposed by Horwitz and Knott (1995) and Sokol (1988). As discussed earlier, it is likely that each of the three main clades reflect the former existence of relict populations that survived in aquatic refugia in three isolated geographic regions during arid periods. Subsequent pluvial periods provided opportunities for significant dispersal both within and between drainages. In the "northern" lineage this involved dispersal on a relatively higher scale compared to other lineages, facilitated by the size and extent of the upper Darling and Lake Eyre drainage basins. Each lineage then had the possibility to apparently undergo significant expansion in the more recent past at different degrees.

It is significant to note that genetic differences were apparent between populations of *C. destructor* within river systems, including the Murray River and the Glenelg River. This indicates that gene flow is constricted in continuous aquatic habitats, once again suggesting that behavioural or life history features play an important role in restricting dispersal and therefore, gene flow in this species. In contrast, Hughes and Hillyer (2003) using mtDNA sequences data from samples collected throughout major river systems the northern part of distribution range of the species, found high levels of connectivity among populations in the same catchments, suggesting recent or contemporary dispersal amongst populations within catchments/river systems.

In general, results of the present study consolidate the findings of previous work using mtDNA markers. Nguyen et al. (2004) found significant phylogeographic structure amongst *C. destructor* populations with three non-overlapping lineages, which is in accordance with the results revealed by RAPD data. In addition, allozyme and RAPD data support the conclusion drawn by Hughes and Hillyer (2003) that *C. destructor* is characterised by a low level of intra-population variation, but high levels of inter-population differentiation. However, Hughes and Hillyer (2003) did not observe population differentiation within catchments, only between them. This could reflect more extensive dispersal within catchments in the northern parts of *C. destructor*'s range than in the southern parts.

4.2 Implications for conservation and aquaculture

The findings of significant genetic structure in *C. destructor* revealed by allozymes, RAPDs and mtDNA (Nguyen et al. 2004) have important implications for the conservation of genetic integrity within *C. destructor* and the utilisation of the species in aquaculture. The three distinct clades of *C. destructor* can be considered as "evolutionary significant units" as they satisfy the criterion of reciprocally monophyletic mtDNA gene trees, and fixed allele frequency differences at nuclear loci (Moritz 1994, 2002; Moritz and Hillis 1996). Transfers of freshwater crayfish from each clade outside of their geographic range should be discouraged as it may threaten the genetic components of species diversity due to interbreeding and competition (Johnson 2000; Austin and Ryan 2002). Impacts of translocation leading to loss of genetic diversity in freshwater crayfish have been recently highlighted in North America (Perry et al. 2001a, 2001b., 2002), Europe (Fratini et al. 2005) and Australia (Austin and Ryan 2002). Within each of the three *C. destructor* groups, distinct "management units" (*sensu* Moritz 1994) should also be recognised, designated by allele frequency differences at nuclear loci.

The findings of the present study also have implications for aquaculture. As there is ongoing interest on selective breeding of *C. destructor* (Lawrence and Morrissy 2000; Jerry et al. 2002), the fixed allelic differences observed among several populations are potentially useful for communal rearing experiments, for monitoring the genetic effects of selection during breeding programs, broodstock management (Carvalho and Pitcher 1995; Cross et al. 2000), and for developing markers for MAS and QTL selection (Liu et al. 1999). The large range of intra-population variation suggests that some populations with high variation (e.g. TAT) may be more effectively used as base-line stocks for selective breeding than others (e.g. PAN). Given the high level of interest in the aquaculture of

C. destructor and the existence of three distinct clades, a prudent strategy for the management of this species would be to restrict translocations for aquaculture to within the geographical boundaries of each of these clades, as has been recommended for other species of freshwater crayfish (Busack 1988; Fetzner et al. 1997).

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