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Genetic diversity of cultured and wild populations of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879) based on microsatellite analysis

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Abstract

Freshwater prawn (*Macrobrachium rosenbergii*) culture in the Western Hemisphere is primarily, if not entirely, derived from 36 individual prawns originally introduced to Hawaii from Malaysia in 1965 and 1966. Little information is available regarding genetic variation within and among cultured prawn stocks worldwide. The goal of the current study was to characterize genetic diversity in various prawn populations with emphasis on those cultured in North America. Five microsatellite loci were screened to estimate genetic diversity in two wild (Myanmar and India-wild) and seven cultured (Hawaii-1, Hawaii-2, India-cultured, Israel, Kentucky, Mississippi and Texas) populations. Average allelic richness ranged from 3.96 (Israel) to 20.45 (Myanmar). Average expected heterozygosity ranged from 0.580 (Israel) to 0.935 (Myanmar). Many of the cultured populations exhibited reduced genetic diversity when compared with the Myanmar and the India-cultured populations. Significant deficiency in heterozygotes was detected in the India-cultured, Mississippi and Kentucky populations (overall F_{is} estimated of 0.053, 0.067 and 0.108 respectively) reflecting moderate levels of inbreeding. Overall estimate of fixation index (F_{st} = 0.1569) revealed moderately high levels of differentiation among the populations. Outcome of this study provide a baseline assessment of genetic diversity in some available strains that will be useful for the development of breeding programmes.

Keywords: freshwater prawn, *Macrobrachium rosenbergii*, genetic diversity, microsatellite

Introduction

Although more than 200 species of prawns belonging to the genus *Macrobrachium* have been described worldwide, only a small number have been evaluated for their potential for commercial production (Holthuis 2000). The Malaysian prawn *Macrobrachium rosenbergii*, also known as the giant freshwater prawn (GFP) or giant river prawn, is the largest *Macrobrachium* species and the most widely cultured prawn species with global production exceeding 200 000 tons in 2006 (FAO (Food & Agriculture Organization of the United Nations) 2009).

Development of modern culture techniques for GFP began in 1959 at the Malaysian Fisheries Research Institute in Penang, Malaysia (Ling 1969). In 1965 and 1966, 36 individuals were imported to Hawaii from Penang, Malaysia in an effort to establish a prawn culture industry (Fujimura

& Okamoto 1972). Shortly after their introduction, researchers at the Anuenue Fisheries Research Center in Honolulu, Hawaii, USA developed mass rearing techniques for commercial scale production of post-larvae (New 2000). This initiated rapid expansion of GFP culture into areas outside the native range of this species. Giant freshwater prawn was introduced to parts of Asia, the Americas, Africa and Europe; however, many of the new populations were derived directly or indirectly from the individuals imported originally to Hawaii in the mid 1960s.

Although the history of GFP cultured stock establishment around the world is relatively well documented, little is known about genetic variation within and among wild and cultured populations. Due to the limited number of individuals used to develop the original Hawaii cultured stock and subsequent derived populations, it is likely that founder effects were compounded, potentially producing sequential population bottlenecks that may have led to loss of genetic diversity. Populations established from only a limited number of individuals also may be exposed to increased inbreeding levels which can negatively affect stock productivity. Inbreeding depression has been discussed widely in relation to GFP culture as it has been suspected to be the cause of declines in production in China (Weimin & Xianping 2002), Taiwan (Mather & de Bruyn 2003), Thailand (Chareontawee, Poompuang, Na-Nakorn & Kamonrat 2007) and India (C. Mohanakumaran Nair, pers. comm.; Kerala Agricultural University, Kerala, India).

Hedgecock, Stelmach, Nelson, Lindenfelser and Malecha (1979) were the first to evaluate genetic diversity in this species based on enzyme polymorphism in wild populations (Sri Lanka, India, Thailand, Java, Sarawak, Philippines, New Guinea and Australia) and the cultured Anuenue strain. The findings of this study suggested that sampled populations exhibited only relatively low levels of enzyme variation. Among population differentiation this was relatively high however; and significant divergence was evident among regional groups of natural populations now referred to as the eastern and western forms of *M. rosenbergii* (Hedgecock *et al.* 1979; de Bruyn, Wilson & Mather 2004). The authors noted that despite the relatively small size of the Anuenue strain founder population this strain did not appear to lack biochemical genetic variation and although diversity levels were slightly lower than those typically found in other decapod species, GFP were still considered to be capable of responding positively to artificial selection (Hedgecock *et al.* 1979).

Since the early 1990s, there have been major advancements in DNA marker technologies that permit genetic diversity to be quantified and characterized routinely and that provide increased sensitivity when estimating levels of genetic variation. Microsatellites or simple sequence repeats is one marker system that recently has become extremely popular for a wide variety of genetic applications in aquaculture (Liu & Cordes 2004). In recent years, a number of GFP specific microsatellite primer sets have been developed from populations in Australia (Chand, de Bruyn & Mather 2005), India (Divu, Khushiramani, Malathi, Karunasagar & Karunasagar 2008; Bhat *et al.* 2008), Malaysia (Bhassu, Hassan, Yusoff, Jamari, Shapor & Guan 2005; Bhassu, See, Hassan, Siraj & Tan 2008) and Thailand (Chareontawee, Poompuang & Na-Nakorn 2006). Although many loci are now available, microsatellite diversity in wild and cultured GFP populations has yet to be widely investigated. Chareontawee *et al.* (2007) evaluated microsatellite diversity in five hatcheries and two wild GFP populations in Thailand. This study revealed no significant differences in the levels of genetic diversity between hatchery and wild populations and suggested that reported declines in cultured GFP productivity in Thailand was unlikely the result of genetic factors (i.e. inbreeding).

Cultured stocks of GFP in the western hemisphere are primarily, if not entirely, derived from the Anuenue strain and the introduction of new wild broodstock to these regions has not been documented. Thus, these stocks have experienced long-term culture without addition of new genetic resources. Genetic diversity is the fundamental resource on which stock improvements rely, therefore, populations can receive priority for selection on the basis of genetic criteria (Petit, El Mousadik & Pons 1998; Vandeputte & Launey 2004). It is important to develop a clear understanding of the levels of diversity in cultured lines and how past management practices have affected diversity before specific stocks are utilized for selective breeding and/or intraspecific hybridization. The goal of the current research was to characterize levels of genetic diversity in GFP cultured lines and wild reference stocks based on microsatellite analysis with an emphasis on North American

cultured lines. These data provide a baseline genetic assessment of some available strains that will be useful in stock improvement programmes.

Materials and methods

Collection and storage of samples

Tissue samples were collected from 45 to 60 individual prawns from each of seven cultured lines and two wild reference populations (Table 1). The cultured lines included Hawaii-1, Hawaii-2, India-cultured, Israel, Kentucky, Mississippi and Texas. The wild populations included India-wild and Myanmar. For the Mississippi and Hawaii-2 samples, whole individuals were shipped frozen to Kentucky State University; upon arrival 2–3 pereiopods (walking legs) were removed from each animal and placed in 95% ethanol. For the Kentucky and Texas samples, pereiopods were removed from live prawns and preserved directly in 95% ethanol. Due to airline restrictions on the shipment of ethanol, a tissue storage buffer consisting of 0.1 M Tris HCl, 0.1 M EDTA Na_2, 0.01 M NaCl and 0.5% with volume SDS detergent with a final pH of 7.5–8.0 (Amato & Lehn 2003) was used to preserve the Hawaii-1, India-wild, India-cultured, Israel and Myanmar samples; whereas 2–3 pereiopods were removed from live prawns and shipped unfrozen to Kentucky State University.

DNA extraction

Genomic DNA was extracted from the pereiopods using the Promega Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) mouse tail extraction protocol. The only deviations from the protocol included incubating the digestion at 65°C instead of 55°C and using >50 mg of tissue to account for the additional weight of the exoskeleton. Pereiopods were cut into small pieces (~3–5 mm in length) to aid digestion. Following extraction, total DNA concentration was determined using a GeneQuant™ *pro* RNA/DNA Calculator Spectrophotometer (GE Healthcare-Life Sciences, Piscataway, NJ, USA). Following quantification, a working solution was prepared by diluting a portion of the total DNA to10 ng μl^{-1} for use as a template source in PCR reactions. Samples preserved in tissue storage buffer were tested for DNA quality using gel electrophoresis, where approximately 1000 ng of genomic DNA was visualized on 2% agarose gels. After electrophoresis, gels were stained with ethidium bromide and visualized under a UV transilluminator. Samples in which genomic DNA was not intact were discarded.

Microsatellite amplification

Primer sets for five dinucleotide microsatellite loci [*Mbr*-1 and *Mbr*-5 (Chareontawee *et al.* 2006); *UVC* 817, *UVC* 819 and *UVC* 823 (P. Mather, pers. comm.; Queensland University of Technology, Brisbane, Queensland, Australia)] were used for amplification of alleles (Table 2). The forward primer sequence was tagged with either a FAM or HEX florophore at the 5′ end. A GTTT sequence was added to the 5′ end of the reverse primer to promote non-template adenylation. Monoplex PCR reactions were performed using a Gene Amp® System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Each reaction contained 40 ng of template DNA, 0.25 pmol of forward and reverse primers (Integrated DNA Technologies, Coralville, IA, USA), 0.25 mM of each dNTP (Promega, Madison, WI, USA), 1.5 mM $MgCl_2$ (Bioline, Taunton, MA, USA), 0.25 U *Taq* DNA polymerase

Table 1 *Macrobrachium rosenbergii* sample origin and source

Population	Origin	Source
Hawaii-1	Cultured	Tamashiro Market, Honolulu, Hawaii, USA; live market supplied by producers in Hawaii
Hawaii-2	Cultured	Commercial Hatchery, Kaneohe, Hawaii, USA
India-cultured	Cultured	Commercial Producer, Andhra Pradesh, India; considered the central region of prawn culture in India
India-wild	Wild	Mahanadi River, Orissa, India
Israel	Cultured	University of Negev, Beer Sheva, Israel
Kentucky	Cultured	Commercial Hatchery, Frankfort, Kentucky, USA
Mississippi	Cultured	Commercial Hatchery, Leland, Mississippi, USA
Myanmar	Wild	Hmaw River, Hlaing River and the Pan Hlaing River; tributaries of the Yangon River, Yangon, Myanmar
Texas	Cultured	Commercial Hatchery, Weatherford, Texas, USA

Table 2 Summary of primer sequence, fluorophore tag, annealing temperature (T_a) and repeat motif for five microsatellite loci

Locus	GenBank Accession no.	Primer Sequence 5′–3′	Fluorophore	T_a (°C)	Repeat motif
Mbr-1*	DQ019863	F: CCCACCATCAATTCTCACTTACC R: TCCTTTTCACATCGTTTCCAGTC	FAM	60	$(GA)_{24}$
Mbr-5*	DQ019867	F: CAAGGCTCGTGTCTCTTGTTTC R: GCTTGTACTTGTTCAGCTTTTGC	HEX	62	$(AG)_{25}$
UVC 817	na	F: ATGGCCAAGATGAAAGATGC R: CTGTCTGTACCGCAGTCGAA	HEX	58	$(CT)_{20}$
UVC 819	na	F: TGATGGGTCGTGTTTTGTGT R: CCCCTCTCGGGAAGAGTAAT	FAM	54	$(CT)_{34}$
UVC 823	na	F: CAAATACGGCATCATACTTTGG R: AAGCAGTGTTTATAAATAACCTTCCA	HEX	52	$(GA)_{37}$

*Primer sequences first reported in Chareontawee *et al.* 2006.

(Bioline, Taunton, MA, USA), 10× PCR reaction buffer (initial concentration) (Bioline, Taunton, MA, USA) and PCR H_20 to a final volume of 10 μL. The PCR profile was initial denaturation at 94°C for 3 min; then 25–35 cycles at 94°C for 30 s, annealing temperature (Table 2) for 45 s, and 72°C for 1 min; followed by a final extension for 7 min at 68°C.

Scoring of amplified products

Amplified products were resolved via capillary electrophoresis using an ABI 3730*xl* DNA analyser (Applied Biosystems). Fragment sizes were determined using GeneMapper® software version 3.5 (Applied Biosystems) by comparison against a GenScan™ 500 ROX™ (Applied Biosystems) internal size standard.

Data integrity

Prior to statistical analysis, genotype data at all five loci were analysed for data integrity using the program MICROCHECKER version 2.2.3 (van Oosterhout, Hutchinson, Wills & Shipley 2004). MICROCHECKER tests for the presence of null alleles, allelic dropout and scoring errors due to stutter.

Data analysis of genetic diversity within populations

Number of alleles per locus (A) and allelic richness (A_r) were computed using Contrib version 1.02 (Petit *et al.* 1998). Allelic richness is a measure of the number of alleles independent of sample size and was computed based on a rarefaction size of $2N$, where $N = 41$, the smallest single locus sample size examined. Observed direct count heterozygosity (H_o) and unbiased expected heterozygosity (H_e) (Nei 1978) were computed using TFPGA version 1.3 (Miller 1997). Differences in values of A_r and H_e between populations were tested for significance ($P \leq 0.05$) using Wilcoxon signed rank test in Statistix version 9 (Statistix Analytical Software, Tallahassee, FL, USA). The number of private alleles (A_p) was determined from allele data computed in TFPGA. Private allelic richness (A_{rp}) was computed using HP-Rare (Kalinowski 2005). Private allelic richness is a measure of the number of private alleles independent of sample size and was computed based on the same rarefaction size described above.

Deviation from Hardy–Weinberg equilibrium (F_{is}) within samples was quantified by Weir and Cockerham's (1984) f using FSTAT version 2.9.3 (Goudet 2002). Significance of f was tested using an exact test in GENEPOP version 4.0 (Rousset 2008). The exact probability (P-value) was estimated using a Markov Chain Randomization method (Guo & Thompson 1992) with parameters set as follows; dememorization = 40 000, batches = 50, and iterations per batch = 40 000. Genotypic linkage disequilibrium was also tested using a Markov Chain Randomization method in GENEPOP employing the same parameters described above. Sequential Bonferroni corrections were applied to adjust significance levels ($P \leq 0.05$) and to account for multiple hypotheses testing (Rice 1989).

Data analysis of genetic diversity among populations

The magnitude of divergence among populations (F_{st}) was quantified using Weir and Cockerham's (1984) θ. Pair-wise θ values were computed for all population pairs using SPAGeDi version 1.2 (Hardy & Vekemans 2002) and significance ($P \leq 0.05$) was estimated using permutation tests (10 000 permutations). A multidimensional scaling (MDS) plot of F_{st} was constructed using SYSTAT® version 13 (Systat Software, Chicago, IL, USA). Homogeneity tests of genotypic differentiation (overall populations and between population pairs) were tested using a Markov Chain Randomization method in GENEPOP employing the same parameters described above. Sequential Bonferroni corrections were applied to adjust significance levels ($P \leq 0.05$) and to account for multiple hypotheses testing. The analogue of Nei's (1978) standard genetic distance (*D*s) $\delta\mu^2$ (Goldstein & Pollock 1997) was calculated between all pairs of populations using SPAGeDi. Contribution to diversity statistics were computed using the program Contrib. The contribution of individual populations to overall genetic diversity was evaluated when diversity is characterized as Nei's (1973) gene diversity (expected heterozygosity) or allelic richness (Petit *et al.* 1998). Estimated contributions to diversity are then divided into two components (Petit *et al.* 1998); one due to the diversity within the population (component of intrinsic diversity) and the other due to population differentiation from the remaining populations (component of uniqueness).

Results

Data integrity

Results of MICROCHECKER analysis revealed no evidence for allelic dropout or scoring error in any population at any loci. Possible presence of null alleles was detected at *Mbr*-1 and *UVC* 819 in the India-cultured population with the number of observed and expected homozygotes being 9 and 4.26, respectively, for *Mbr*-1 and 8 and 3.73, respectively, for *UVC* 819. Since MICROCHECKER did not detect possible presence of null alleles in any other population at these loci and the increase in homozygosity in the India-cultured population could be attributed to cultivation practices, this population was not omitted from additional analyses.

Genetic diversity within populations

All five microsatellite loci screened in the sampled populations were highly polymorphic. A total of 128 unique alleles were amplified across the five sampled loci. Total number of unique alleles per locus was similar at all loci over all populations and ranged from 23 at *UVC* 823 to 28 at *Mbr*-1. Number of alleles per locus, allelic richness and observed and expected heterozygosity varied widely among the sampled populations (Table 3). Average number of alleles per locus ranged from 4.00 ± 0.45 (Israel) to 22.20 ± 1.02 (Myanmar) and average allelic richness ranged from 3.96 ± 0.46 (Israel) to 20.45 ± 0.83 (Myanmar). Average observed heterozygosity ranged from 0.657 ± 0.041 (Kentucky) to 0.919 ± 0.014 (Myanmar) and average expected heterozygosity ranged from 0.580 ± 0.050 (Israel) to 0.935 ± 0.004 (Myanmar). A total of 27 private alleles were detected, 2 private alleles were found in the Israel population, 7 were found in the India-cultured population and 18 were found in the Myanmar population (Table 3). Private allelic richness was estimated in six of the nine populations with the exclusion of the India-wild, Mississippi and Texas populations (Table 3). Microsatellite alleles and their corresponding frequencies in populations are available from the author upon request.

The wild Myanmar and India-cultured populations possessed significantly higher ($P \leq 0.05$) estimates of allelic richness and expected heterozygosity compared with all other populations (Fig. 1). Allelic richness was also significantly higher in the two Hawaii populations compared with the Kentucky, Mississippi, Texas, India-wild and Israel. Allelic richness of the Kentucky, Mississippi, Texas and India-wild populations was not significantly different from each other; however, allelic richness of the Israel population was significantly lower than all other populations with the exception of the Texas population. Expected heterozygosity was not significantly different among the Hawaii-2, Hawaii-1, Mississippi, Kentucky, Texas and India-wild populations; however, expected heterozygosity in the Israel population was significantly lower than for the Hawaii-2, Hawaii-1 and Mississippi populations (Fig. 1).

Significant deviations ($P \leq 0.05$) from Hardy-Weinberg equilibrium were observed in 11 of 45 (5 loci * 9 populations) single locus exact tests before sequential Bonferroni correction. Deviations

Table 3 Sample size (N), number of alleles per locus (A), allelic richness (A_r), number of private alleles (A_p), private allelic richness (A_{rp}), range in base pairs of alleles (R), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficients (F_{is}) and P-values for deviation from Hardy–Weinberg equilibrium (HW) for nine populations of *Macrobrachium rosenbergii* at five microsatellite loci

	Locus					
Population (*N*)	***Mbr*-1**	***Mbr*-5**	***UVC* 817**	***UVC* 819**	***UVC* 823**	**Mean across loci (±SE)**
Hawaii-1 (49)						
A	8	12	13	12	9	10.80 ± 0.97
A_r	7.81	11.76	12.96	11.84	8.81	10.63 ± 0.99
A_p	0	0	0	0	0	–
A_{rp}	0.000	0.025	0.002	0.000	0.297	0.065 ± 0.058
R	279–301	285–329	161–209	180–204	185–229	–
H_o	0.674	0.816	0.891	0.837	0.796	0.803 ± 0.036
H_e	0.755	0.833	0.901	0.895	0.831	0.843 ± 0.027
F_{is}	0.109	0.020	0.011	0.066	0.042	0.048
HW	0.3614	0.1963	0.2028	0.2031	0.5295	0.2269
Hawaii-2 (60)						
A	8	12	15	11	10	11.20 ± 1.16
A_r	7.97	11.54	14.63	10.99	9.38	10.90 ± 1.12
A_p	0	0	0	0	0	–
A_{rp}	0.000	0.010	0.000	0.000	0.018	0.004 ± 0.004
R	279–301	285–329	161–209	180–204	185–229	–
H_o	0.700	0.833	0.898	0.862	0.881	0.835 ± 0.035
H_e	0.738	0.882	0.895	0.896	0.844	0.851 ± 0.030
F_{is}	0.052	0.055	−0.004	0.038	−0.045	0.019
HW	0.0080*	0.3099	0.4704	0.3059	0.8412	0.0932
India-cultured (45)						
A	21	17	20	19	16	18.60 ± 0.93
A_r	20.37	16.64	19.78	19.00	15.90	18.36 ± 0.89
A_p	3	1	2	0	1	1.40 ± 0.51
A_{rp}	3.544	1.222	1.963	1.011	1.404	1.829 ± 0.457
R	253–321	281–335	169–215	152–206	181–231	–
H_o	0.800	0.889	0.932	0.805	0.933	0.872 ± 0.029
H_e	0.916	0.905	0.933	0.920	0.925	0.920 ± 0.005
F_{is}	0.128	0.018	0.001	0.127	−0.009	0.053
HW	0.0020*	0.3333	0.2100	0.0044*	0.0449*	0.0001**
India-wild (50)						
A	7	6	4	4	6	5.40 ± 0.60
A_r	6.65	5.81	4.00	4.00	5.79	5.25 ± 0.53
A_p	0	0	0	0	0	–
A_{rp}	0.000	0.000	0.000	0.000	0.000	–
R	285–303	289–313	195–201	152–190	195–227	–
H_o	0.898	0.776	0.568	0.674	0.620	0.707 ± 0.059
H_e	0.757	0.737	0.634	0.752	0.748	0.725 ± 0.023
F_{is}	−0.189	−0.053	0.104	0.105	0.173	0.025
HW	0.1364	0.8714	0.3674	0.2025	0.1123	0.1810
Israel (50)						
A	3	3	5	5	4	4.00 ± 0.45
A_r	2.84	3.00	4.97	5.00	3.99	3.96 ± 0.46
A_p	1	0	1	0	0	0.40 ± 0.24
A_{rp}	1.000	0.028	1.019	0.000	0.316	0.473 ± 0.226
R	287–307	281–307	167–193	174–192	201–223	–
H_o	0.449	0.837	0.920	0.745	0.592	0.709 ± 0.085
H_e	0.418	0.556	0.722	0.638	0.563	0.580 ± 0.050
F_{is}	−0.075	−0.513	−0.278	−0.169	−0.052	−0.225
HW	0.8057	<0.0001**	<0.0001**	0.0365*	0.1709	<0.0001**

Table 3 (continued)

Population (*N*)	Locus					Mean across loci (±SE)
	Mbr-1	*Mbr*-5	*UVC* 817	*UVC* 819	*UVC* 823	
Kentucky (60)						
A	8	4	7	9	7	7.00 ± 0.84
A_r	7.69	4.00	6.58	8.60	7.00	6.77 ± 0.77
A_p	0	0	0	0	0	–
A_{rp}	0.000	0.000	0.000	0.065	0.000	0.013 ± 0.013
R	253–301	297–317	161–207	160–204	187–231	–
H_o	0.661	0.550	0.583	0.759	0.733	0.657 ± 0.041
H_e	0.827	0.616	0.620	0.800	0.818	0.736 ± 0.049
F_{is}	0.202	0.109	0.059	0.052	0.105	0.108
HW	0.0038*	0.1011	0.0635	0.0789	<0.0001**	<0.0001**
Mississippi (49)						
A	7	5	6	7	8	6.60 ± 0.51
A_r	6.91	4.98	6.00	6.98	7.69	6.51 ± 0.47
A_p	0	0	0	0	0	–
A_{rp}	0.000	0.000	0.000	0.000	0.000	–
R	253–297	297–317	161–207	182–204	187–231	–
H_o	0.733	0.565	0.816	0.714	0.667	0.699 ± 0.041
H_e	0.820	0.587	0.770	0.803	0.767	0.749 ± 0.042
F_{is}	0.107	0.037	−0.061	0.111	0.132	0.067
HW	0.2163	0.9418	0.2519	0.1185	0.0075*	0.0294*
Myanmar (60)						
A	22	24	20	25	20	22.20 ± 1.02
A_r	20.53	22.38	18.75	22.18	18.40	20.45 ± 0.83
A_p	4	5	2	5	2	3.60 ± 0.68
A_{rp}	3.524	4.643	1.699	3.955	1.587	3.082 ± 0.614
R	261–317	283–335	169–211	152–214	187–231	–
H_o	0.900	0.966	0.895	0.932	0.900	0.919 ± 0.014
H_e	0.928	0.949	0.939	0.938	0.924	0.935 ± 0.004
F_{is}	0.030	−0.018	0.047	0.006	0.026	0.018
HW	0.8199	0.5836	0.6866	0.2734	0.3860	0.7513
Texas (50)						
A	7	4	4	8	7	6.00 ± 0.84
A_r	7.00	4.00	3.97	7.99	6.97	5.99 ± 0.84
A_p	0	0	0	0	0	–
A_{rp}	0.000	0.000	0.000	0.000	0.000	–
R	253–297	297–317	169–207	182–204	187–231	–
H_o	0.880	0.480	0.720	0.860	0.740	0.736 ± 0.071
H_e	0.796	0.496	0.597	0.794	0.760	0.689 ± 0.061
F_{is}	−0.107	0.032	−0.209	−0.084	0.027	−0.070
HW	0.0017*	0.2425	0.4049	0.2197	0.0787	0.0045**
All (473)						
A	28	26	25	26	23	25.60 ± 0.81
A_r	18.51	17.81	19.19	15.54	17.08	17.63 ± 0.63
R	253–321	281–335	161–215	152–214	181–231	–
H_o	0.744	0.746	0.803	0.799	0.762	0.771 ± 0.013
H_e	0.773	0.729	0.779	0.826	0.798	0.781 ± 0.016
F_{is}	0.040	−0.019	−0.030	0.032	0.044	0.014

*P-value significant at the $\alpha = 0.05$ level before sequential Bonferroni adjustment.
**P-value significant at the $\alpha = 0.05$ level after sequential Bonferroni adjustment.

were observed in all cultured populations with the exception of the Hawaii-1 population. Only three deviations were significant, however, after sequential Bonferroni correction. Significant deviations were observed in the Israel population at *Mbr*-5 and *UVC* 817 and a single deviation was observed in the Kentucky population at *UVC* 823. Overall locus estimates of F_{is} revealed significant departures

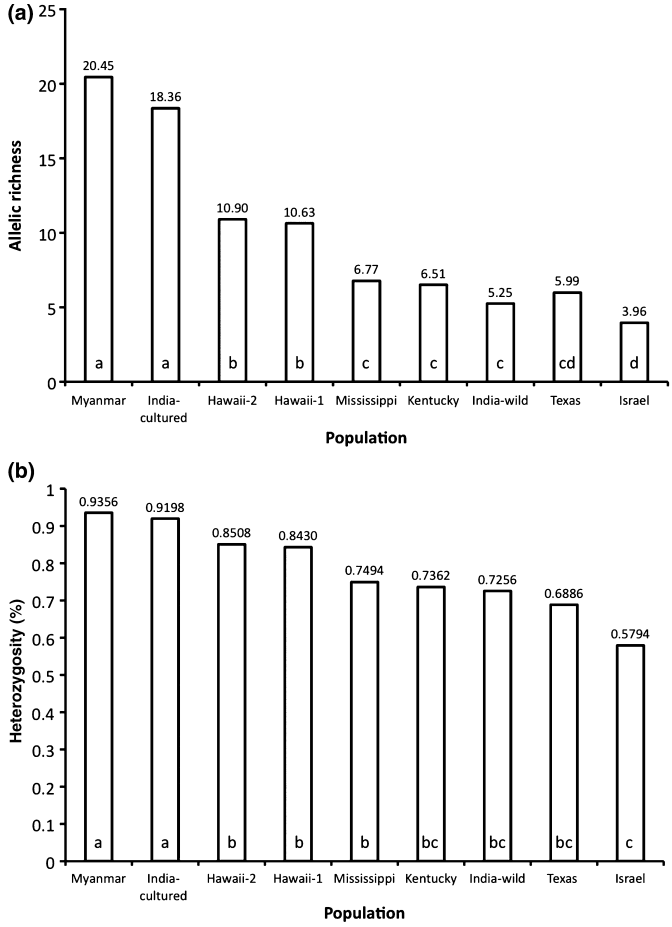

Figure 1 Mean values of allelic richness (±SE) (a) and mean expected heterozygosity (±SE) (b) for nine populations of M*acrobrachium rosenbergii* at five microsatellite loci; lower case letters in bars that differ denote a significant difference ($P \leq 0.05$) between respective populations detected by Wicoxon signed rank test.

from Hardy-Weinberg in five of nine populations before sequential Bonferroni correction and four of nine populations after sequential Bonferroni correction (Table 3). Significant deficiency in heterozygotes was detected in the India-cultured, Mississippi and Kentucky populations (Overall F_{is} estimates of 0.053, 0.067 and 0.108 respectively) and significant excess in heterozygotes was detected in the Texas and Israel populations (Overall F_{is} estimates of −0.070 and −0.225 respectively).

Significant deviations ($P \leq 0.05$) from linkage equilibrium were observed in 32 of 90 (10 locus combinations * 9 populations) locus pair exact tests before sequential Bonferroni correction. All significant deviations were observed in cultured populations in at least one locus pair, whereas wild populations showed no evidence for linkage disequilibrium. Only the Hawaii-2, India-cultured, Israel, Kentucky and Texas populations showed evidence for linkage disequilibrium in at least one locus pair after sequential Bonferroni correction.

Genetic diversity among populations

Exact homogeneity tests among populations showed significant genotypic differentiation across all five loci ($P < 0.0001$). Homogeneity tests between population pairs indicated significant genotypic differentiation in 172 of 180 (36 population combinations * 5 loci) population pair exact tests before sequential Bonferroni correction and 168 population pair exact tests after sequential Bonferroni correction. Of the twelve population pairs where homogeneity was detected after Bonferroni correction, five observations were between the Hawaii populations at all loci, four were between the Texas and Kentucky populations at *Mbr*-1, *Mbr*-5, *UVC* 819 and *UVC* 823, one was between the Kentucky and Mississippi populations at *UVC* 819, one was between the Mississippi and Texas populations at *UVC* 819 and one was between the India culture and Myanmar populations at *UVC* 819.

The magnitude of genetic differentiation among populations was moderately high with an overall

fixation index (F_{st}) of 0.1569 ($P < 0.0001$). All population pair-wise F_{st} comparisons (Table 4) were significant ($P \leq 0.05$) before and after sequential Bonferroni correction with the exception of the Hawaii populations comparison; $F_{st} = 0.0026$ ($P = 0.2745$). The highest differentiation among populations based on both the $\delta\mu^2$ distance and F_{st} (Table 4) was observed between the Israel and Texas populations ($\delta\mu^2 = 41.1985$, $F_{st} = 0.3520$). The Hawaii populations exhibited the greatest level of similarity ($\delta\mu^2 = 0.1512$, $F_{st} = 0.0026$). The MDS plot revealed two separate population groups or clusters (Fig. 2). The first group included the two Hawaii populations and the Myanmar and India-cultured populations. Within the first group, the Hawaii populations were closely associated as were the Myanmar and India-cultured populations. The second group consisted of the Kentucky, Mississippi and Texas populations with the Kentucky and Mississippi closely associated. The locations of the India-wild and Israel populations illustrate the dissimilarity of these populations to the others with the Israel population exhibiting the greatest differentiation.

Analysis of contributions to diversity (Table 5) revealed that population contributions to total genetic diversity were partitioned disproportionately with the Israel population exhibiting the greatest contribution; $C_t = 0.008$ and the Kentucky and Texas populations equally exhibiting the lowest contribution; $C_t = -0.010$. Contributions to total allelic richness were highest for the India-cultured and Myanmar populations; $C_{rt} = 0.076$ and 0.070, respectively, and lowest for the Texas and Kentucky populations; $C_{rt} = -0.028$ and -0.027 respectively. Contributions

Table 4 Matrix of pair-wise comparisons of F_{st} (above diagonal) and $\delta\mu^2$ (below diagonal) for nine populations of *Macrobrachium rosenbergii*

Population	Hawaii-1	Hawaii-2	India-C	India-W	Israel	Kentucky	Mississippi	Myanmar	Texas
Hawaii-1		0.0026	**0.0610**	**0.1808**	**0.2623**	**0.1570**	**0.1618**	**0.0567**	**0.1854**
Hawaii-2	0.1512		**0.0547**	**0.1724**	**0.2593**	**0.1484**	**0.1513**	**0.0502**	**0.1769**
India-C	1.1552	0.9552		**0.1419**	**0.2029**	**0.1401**	**0.1320**	**0.0126**	**0.1695**
India-W	12.5481	12.6409	12.8043		**0.3092**	**0.2163**	**0.2256**	**0.1112**	**0.2449**
Israel	21.1193	21.9744	29.1836	15.5560		**0.3169**	**0.3226**	**0.1902**	**0.3520**
Kentucky	6.7302	5.9443	2.6530	25.9859	41.1767		**0.0443**	**0.1189**	**0.0169**
Mississippi	10.6741	11.0307	6.6571	27.9914	38.2518	3.5380		**0.1126**	**0.0534**
Myanmar	0.5128	1.4565	1.0572	8.5065	19.7537	7.5329	8.6392		**0.1419**
Texas	4.4461	4.5733	1.2135	23.5085	41.1985	1.2232	3.5340	4.5476	

Values in bold indicate significance at the $\alpha = 0.05$ level after sequential Bonferroni adjustment.

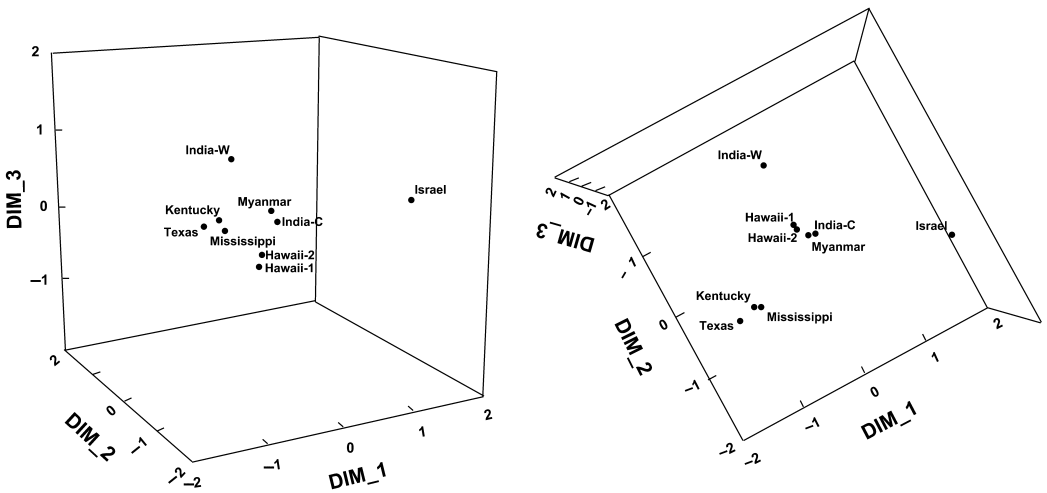

Figure 2 Multidimensional scaling plot illustrating genetic differentiation among nine populations of *Macrobrachium rosenbergii* based on F_{st}.

Table 5 Mean values for contributions to genetic diversity for nine populations of *Macrobrachium rosenbergii* at five microsatellite loci, where G_{st} = relative divergence of the *k*th population from the other populations; C_t = the contribution of the *k*th population to total diversity; C_s = the contribution to total diversity based on *k*'s diversity; C_d = the contribution of the *k*th population to total diversity due to *k*'s allelic divergence or uniqueness; C_{rt} = the contribution of the *k*th population to total allelic richness; C_{rs} = the contribution to total allelic richness based on *k*'s allelic richness; C_{rd} = the contribution of the *k*th population to total allelic richness due to *k*'s allelic divergence or uniqueness

Population	G_{st}	C_t	C_s	C_d	C_{rt}	C_{rs}	C_{rd}
Hawaii-1	0.134	0.001	0.008	−0.007	0.002	0.006	−0.004
Hawaii-2	0.127	0.000	0.009	−0.009	−0.010	0.008	−0.018
India-cultured	0.116	0.007	0.019	−0.012	0.076	0.064	0.012
India-wild	0.198	0.005	−0.007	0.012	0.003	−0.035	0.038
Israel	0.275	0.008	−0.027	0.035	0.019	−0.045	0.064
Kentucky	0.152	−0.010	−0.006	−0.004	−0.027	−0.023	−0.004
Mississippi	0.157	−0.006	−0.004	−0.002	−0.023	−0.025	0.003
Myanmar	0.102	0.005	0.021	−0.016	0.070	0.080	−0.010
Texas	0.176	−0.010	−0.012	0.003	−0.028	−0.030	0.002

to total diversity based on intrinsic diversity were highest for the Myanmar and India-cultured populations; $C_s = 0.021$ and 0.019, respectively, and lowest for the Israel population; $C_s = -0.027$. Similarly, contributions to total allelic richness based on intrinsic allelic richness were also highest for the Myanmar and India-cultured populations; $C_{rs} = 0.080$ and 0.064, respectively, and lowest for the Israel population; $C_{rs} = -0.045$. Conversely, contributions to total diversity based on uniqueness were highest for the Israel an India-wild populations; $C_d = 0.035$ and 0.012 respectively. Similarly, contributions to total allelic richness based on uniqueness were also highest for the Israel and India-wild populations; $C_{rd} = 0.064$ and 0.038 respectively.

Discussion

A major focus of the current study was to estimate relative levels of genetic variation present in some available GFP strains that would be useful for stock improvement programmes or intraspecific hybridization. Of the populations evaluated, the Myanmar and India-cultured populations would be the greatest resources of genetic diversity based on these populations possessing the highest values of allelic richness and expected heterozygosity. Also, the Myanmar and India-cultured populations exhibited the highest contributions to total diversity and total allelic richness based on intrinsic diversity as well as the highest number private alleles and estimates of private allelic richness. The Hawaii populations also possess relatively high levels of within population diversity; however, these populations did not exhibit substantial differentiation from the other populations evaluated in the present study.

The India-wild, Kentucky, Mississippi and Texas populations offer in contrast relatively poor resources as these populations exhibited substantial reductions in within population diversity. Although contributions to diversity based on uniqueness were high in the India-wild population, this population along with the Kentucky, Texas and Mississippi populations did not possess any private alleles.

Although the lowest within population diversity was observed for the Israel population; this population appears to contain some unique attributes. The Israel population possesses private alleles, and private allelic richness was estimated at four loci. Contributions based on uniqueness were high in the Israel population and this population also exhibited the highest relative divergence based on G_{st}, $\delta\mu^2$ and F_{st}. In addition, a large excess of heterozygotes ($F_{is} = -0.225$, $P < 0.0001$) along with low numbers of alleles at all loci indicate that only a small number of families contributed to the sample. Addiitonal investigation of the Israel population could therefore be beneficial to determine the value of this population as a resource for increasing diversity in a synthetic line.

A major outcome of this study is recognition that diversity in all cultured populations was low when compared with that present in the Myanmar and India-cultured populations. Reductions in microsatellite diversity in cultured populations has been observed in many other aquatic species including giant tiger prawn *Penaeus monodon* (Xu,

Primavera, de la Pena, Pettit, Belak & Alcivar-Warren 2001), Atlantic salmon *Salmo salar* (Norris, Bradley & Cunningham 1999; Skaala, Hoyheim, Glover & Dahle 2004), Pacific abalone *Haliotis discus hannai* (Li, Park, Endo & Kijima 2004), Atlantic cod *Gadus morhua* (Pampoulie, Jörundsdóttir, Steinarsson, Pétursdóttir, Stefánsson & Daníelsdóttir 2006), sea trout *Salmo trutta* (Was & Wenne 2002) and Japanese flounder *Paralichthys olivaceus* (Sekino, Hara & Taniguchi 2002).

In contrast, a study by Chareontawee *et al.* (2007) that evaluated microsatellite diversity in five hatchery and two wild populations of freshwater prawns in Thailand reported no significant differences in levels of diversity between hatchery and wild populations. Mean estimates of allelic richness (9.87 ± 5.09) and expected heterozygosity (0.74 ± 0.18) were considered to be relatively high levels of genetic variation by the authors. Results of this study, however, show levels of diversity in some wild (Myanmar) and even cultured (India) populations can be significantly higher than those reported in Thailand.

Although wild populations are usually expected to exhibit higher genetic variation than cultured stocks, here very high diversity was recorded in the India-cultured population when compared with the wild sample from this region. A possible explanation for this finding may be the result of a stock enhancement initiative. In 2000, producers in Andhra Pradesh state suspected that genetic diversity declined in cultured stocks; affecting productivity. As a result, new broodstock were collected from a variety of natural sources across India and cultured post-larval prawns (PL's) were imported from Thailand (C. Mohanakumaran Nair, pers. comm.; Kerala Agricultural University, Kerala, India);likely increasing diversity in this population to the current level. Although diversity was high in the India-cultured population, significant deficiency of heterozygotes was detected. It is possible that this is a result of null alleles at loci *Mbr*-1 and *UVC* 819 or increased homozygosity of high frequency alleles, as the most prevalent homozygous genotypes at these loci in the India-cultured population contain the highest frequency alleles.

In contrast, the India-wild population showed a surprisingly low genetic variation. A number of factors could have contributed to this result, including over exploitation of wild broodstock and/or PL's as well as environmental degradation. Alternatively, wild GFP populations in the Mahanadi River may naturally have relatively low levels of diversity. Divu *et al.* (2008) evaluated two wild populations in India (Kerala & Karnataka) and reported levels of diversity similar to those found in the India-wild population evaluated in this study.

A second major focus of this study was to develop a better understanding of the levels and patterns of genetic diversity that remain in cultured GFP stocks in the United States. Of the cultured US populations examined herein, the Hawaii populations had significantly higher levels of within population diversity than did the three continental populations (Mississippi, Kentucky and Texas). Significant departures from Hardy-Weinberg equilibrium were not observed in the Hawaii populations; however, the continental populations all showed significant evidence for disequilibrium. The significantly positive overall estimates of F_{is} in the Mississippi and Kentucky populations suggest the occurrence of moderate levels of inbreeding in these populations; whereas the significantly negative estimate of F_{is} in the Texas population reflects a small number of parents contributing to the sample.

In the United States, commercial hatcheries in temperate climates (Mississippi, Kentucky and Texas) obtain broodstock in the fall and hold reproductive animals indoors in temperature-controlled environments (Daniels, Cavalli & Smullen 2000). Typically, broodstock are obtained only once per year and from only a single location. This practice greatly increases the chance of recruiting closely related individuals; thus, increasing the probability of inbreeding. Also, this practice greatly increases the chance of recruiting a subset of the genetic pool. In contrast, Hawaii has a tropical climate that permits year round availability of broodstock from ponds (Fast & Leung 2003). Thus, culture practices in Hawaii may have less of an impact on diversity by maintaining a continuous production cycle and avoiding yearly bottlenecks when broodstock are sourced.

Differentiation between the Hawaii populations and the continental US populations is likely due to successive founder effect. Prawns were first introduced to the continental United States from Hawaii in the 1960s (Ling & Costello 1979). Currently, commercial hatcheries stocks in the continental United States are derived from two sources. During the 1970s, the Texas population was established from PL's obtained from both Blue Lobster Farm (Madera, CA, USA) and Puerto Rico (C. Upstrom, pers. comm.; Aquaculture of Texas,

Weatherford, TX, USA). Later, the Texas stock was sourced to establish the Mississippi and Kentucky populations. Blue Lobster Farm was one of the original locations in the continental United States to receive PL's from Hawaii during the 1960s. Likewise, GFP in Puerto Rico were first introduced from Hawaii in 1975 (Lacroix, Glude, Thomas & Le Menn 1994). Due to founder effect, it is likely that individuals used to establish the Blue Lobster Farm and Puerto Rico stocks only represented a portion of the total Hawaii gene pool. Similarly, individuals that established the Texas stock may have only represented a portion of the Blue Lobster Farm and Puerto Rico gene pools. Over time, the Hawaii and continental populations may have diverged as a result of sequential bottlenecks and random genotypic frequency shifts.

It is obvious that genetic variation estimated from neutral markers like microsatellites do not directly correspond with strain productivity. Additional experiments should evaluate GFP from different origins under the same production conditions.

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