

Genetic diversity in wild stocks of the giant freshwater prawn (*Macrobrachium rosenbergii*): Implications for aquaculture and conservation

P.B. Mather and M. de Bruyn

Abstract

The giant freshwater prawn (*Macrobrachium rosenbergii*) is cultured widely around the world but little is known about the levels and patterns of genetic diversity in either wild or cultured stocks. Studies have suggested that genetic diversity may be relatively low in some cultured stocks due to the history of how they were founded and subsequent exposure to repeated population bottlenecks in hatcheries. In contrast, wild stocks have an extensive distribution that extends from southern Asia across Southeast (SE) Asia to the Pacific region. Therefore, wild stocks could be an important resource for genetic improvement of culture stocks in the future. Understanding the extent and patterns of genetic diversity in wild giant freshwater prawn stocks will assist decisions about the direction future breeding programs may take. Wild stock genetic diversity was examined using a 472 base-pair segment of the 16S rRNA gene in 18 wild populations collected from across the natural range of the species. Two major clades ("eastern" and "western") were identified either side of Huxley's line, with a minimum divergence of 6.2 per cent, which implies separation since the Miocene period (5-10 MYA). While divergence estimates within major clades was small (maximum 0.9 per cent), evidence was also found for population structuring at a lower spatial scale. This will be examined more intensively with a faster evolving mtDNA gene in the future.

Introduction

Freshwater aquaculture has expanded rapidly in the Asia-Pacific region over the last 30 years, both in terms of the size of the industry and the diversity of species that are cultured. While marine penaeid prawns remain the major crustacean group used in culture in the region, freshwater prawns of the genus *Macrobrachium* have seen a dramatic increase in production. The giant freshwater prawn (*M. rosenbergii*) is the largest species in the genus (Fig. 1) and is by far the most important culture species. In Asia, harvesting of wild stocks also forms a significant industry and production from the wild increased from 5 246 t in 1984 to approximately 130 000 t in 1998, with a value exceeding US \$800 million (FAO 2000). Countries producing the largest volume of *M. rosenbergii* (1998 figures) include China (48 per cent), Bangladesh (37 per cent) and Thailand (6 per cent). While Asia accounts for more than 98 per cent of global production, culture of this species has expanded rapidly both within Asia and more widely in regions far removed from the species'

natural distribution (e.g. Brazil) (FAO 2000) and it is now cultured in at least 43 countries across five continents.

The natural distribution of *M. rosenbergii* extends from Pakistan in the west to southern Vietnam in the east, across SE Asia, and south to northern Australia, Papua New Guinea, and some Pacific and Indian Ocean Islands. *M. rosenbergii* larvae require brackish water for survival and early development (New and Singholka 1985). Fully mature females migrate from freshwater to estuarine areas to spawn, where free-swimming larvae hatch from eggs attached to the female's abdomen. The larvae metamorphose into post-larvae after three to six weeks and then migrate upstream towards freshwater. While there has been little effort to determine if *M. rosenbergii* is capable of marine dispersal, larvae have been raised to post-larvae stage in 100 per cent artificial seawater, suggesting that the species may be capable of at least limited marine dispersal (Smith et al. 1976; Sandifer and Smith 1979).

M. rosenbergii has been cultured in SE Asia

using modern aquaculture techniques since the early 1960s. Early work by Fujimura (1966) at the Anuenue Fisheries Research Center in Hawaii resulted in the development of the successful mass-rearing techniques employed today for commercial-scale hatchery production of post-larvae. Brood stock from Malaysia were introduced to the Center in 1965, but consisted of only 12 individuals (Hedgecock et al. 1979). Later, brood stock from Hawaii (mostly) and SE Asia were introduced into many regions where *M. rosenbergii* was not indigenous, including



Fig. 1. *Macrobrachium rosenbergii*, the giant freshwater prawn

North and South America, Africa, Europe and parts of Asia (most notably China and Taiwan), to initiate culture industries (New 2000). Thus, initial brood stock numbers for many new culture industries were very small and were most likely sourced from only a limited area of the species' natural range. Even in countries where wild stocks occur naturally and are still healthy, little attention has been paid to genetic diversity levels in cultured stocks. While the level of inbreeding in cultured stocks has not been examined to date, it is likely that it may be quite high.

In the early 1990s, commercial stocks in Taiwan experienced productivity declines from 16 000 t to just 7 665 t, which was attributed to inbreeding depression effects. Corresponding declines in production have been described in countries within the species' natural range (e.g. Thailand) and this is believed to result from brood stock commonly being sourced from grow-out ponds rather than from the wild, causing high levels of inbreeding over time (New 2000). Brood stock are also commonly selected on the basis of readiness to spawn, which may lead to a loss of performance as the practice exerts an indirect negative effect on weight-at-harvest. Choosing brood stock for readiness to spawn can result in the smallest females being used, which may lead to a substantial reduction in mean size across generations. This phenomenon was suspected in the hatchery stock at the Anuenue Research Center in the 1980s (New 1995). Thus, while the genetic attributes of cultured stocks worldwide are essentially unknown, many factors are in play that suggest that genetic diversity may be low and declining.

Some wild stocks of *M. rosenbergii* have also seen rapid declines in recent years, largely as a result of over-harvesting, habitat-loss and increased pollution, particularly in SE Asia. Declines have been recorded in Bangladesh, India, Indonesia, Malaysia, the Philippines and Thailand (New et al. 2000), and the species is now believed to be extinct in Singapore, largely as a result of pollution and loss of natural habitat (Ng 1997). Wild stocks can provide an immediate resource for addressing genetic diversity problems in cultured stocks, and consequently require conservation. To this end, the patterns and extent of genetic diversity that are present in wild stocks need to be adequately documented so that we can identify which stocks may carry unique genetic attributes and prioritize conservation efforts.

M. rosenbergii systematics

Systematic studies of *Macrobrachium* species in the past have mainly been based on analyses of external morphological traits. While a single *M. rosenbergii* species is recognized currently across the natural range, two forms of *M. rosenbergii* ("eastern" and "western") have been described independently based on external morphology (De Man 1879; Johnson 1973). Morphological traits, however, have been shown to be strongly influenced by the environment in some *Macrobrachium* species, and may not be indicative of underlying genetic divergence (Dimmock et al. 2002). In addition, allozyme variation in many decapod crustacean species tends to be highly conservative and may not be representative of true molecular levels of divergence. Lindenfelser (1984) analyzed morphometric and allozyme data, and concluded that the boundary for "eastern" and "western" *M. rosenbergii* forms corresponds approximately with Wallace's Line that dissects Indonesia. Further, allozyme and morphological studies of wild *M. rosenbergii* stocks by Malecha and co-workers (1977, 1987 and Hedgecock et al. 1979) recognized three "races": an eastern, a western and an Australian "race".

Mating trials were carried out between geographic "races", with the conclusion that reproductive barriers to gene exchange were not evident among different "races" as

most crosses were fertile (Sarver et al. 1979; Malecha 1987). A recent re-analysis of the mating trial data has suggested, however, that mating between individuals from widely separated geographic regions may result in reduced larval survival. In contrast, crosses among mainland Asian wild stocks from different sources (Thailand and Malaysia) have produced evidence for hybrid vigor (heterosis) despite the fact that they belong to the same geographical "race" (Dobkin and Bailey 1979). So, there is circumstantial evidence that *M. rosenbergii* as currently recognized could be polytypic, both regionally and within regions. If true, this has important implications for both conservation of wild genetic resources and also for potential future directions for the culture industry.

Whilst it is surprising that no molecular analyses (DNA-marker studies) have been undertaken on this species to date, the technique has proven very powerful and informative in other decapods where biochemical markers have proven conservative. Variation in the 16S ribosomal RNA mitochondrial DNA (mtDNA) gene has proven useful for addressing evolutionary relationships at both the inter- and intra-specific level in a number of major crustacean groups (Sarver et al. 1998; Crandall et al. 1999; Tong et al. 2000; Wetzler 2001). Thus, patterns of variation in mtDNA among wild stocks of *M. rosenbergii* can provide an independent and informative



Fig. 2. Locations sampled for *Macrobrachium rosenbergii*

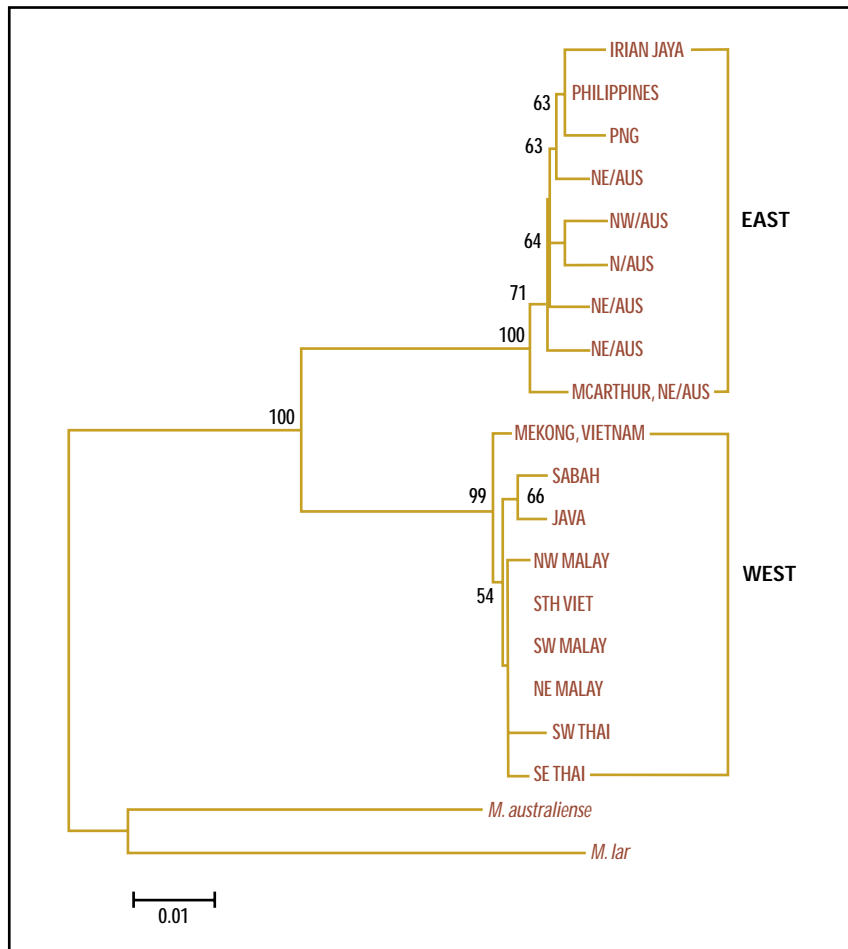


Fig. 3. Neighbour-joining distance tree of the relationships between *Macrobrachium rosenbergii* 16S rRNA haplotypes. Bootstrap values (percentages) are shown for nodes with support >50%

appear to have been shaped by eustatic sea-level change, resulting from climatic change in the past, which may have facilitated or restricted gene flow among populations. Within the eastern clade, a surprisingly low degree of divergence (1-2bp) was observed between the Philippines haplotype and all other eastern "race" haplotypes (Papua New Guinea, Irian Jaya and Australia) analyzed. As the Philippines and Australian/New Guinea landmasses have been geographically distant for at least the past 10 million years (Hall 1996), this suggests that some marine dispersal of *M. rosenbergii*, perhaps via larval dispersal, has taken place. The topology of the neighbour-joining tree (Fig. 3) indicates that within the eastern clade, the McArthur River haplotype from the Gulf of Carpentaria, Australia, is ancestral to all other eastern haplotypes identified. Furthermore, it would appear that gene-flow has occurred from a southerly (Australian) to northerly (PNG, Irian Jaya, Philippines) direction. There is also evidence for some finer-scale genetic structuring within Australia, with the northern and north-western stocks genetically differentiated from Gulf of Carpentaria and Cape York stocks in north-eastern Australia (Fig. 3).

Within the western clade, the Mekong River haplotype appears to be ancestral to all others. The Java and Sabah (Borneo) haplotypes are genetically similar but distinct from all other mainland Asian haplotypes (Fig. 3), which are all identical, with the exception of the Kraburi River haplotype from south-western Thailand. The authors have hypothesized elsewhere that this may be the result of past genetic admixture of stocks that are today geographically discrete, as all of these locations were once connected via extensive river drainage basins (Siam or Malacca Straits River Systems; Voris 2000) that covered much of the Sunda Shelf during Pleistocene low sea-level stands (de Bruyn et al. In press). Ongoing gene-flow between these sites, however, could also result in the observed genetic pattern and this will be further investigated using COI population-level analysis.

Whether the "eastern" and "western" forms of *M. rosenbergii* actually represent two distinct species or sub-species is open to debate. However, the levels of divergence identified here do have important implications for both the aquaculture industry and for conservation of wild stocks. A detailed understanding of the molecular relationships among wild stocks should provide a foundation for future genetic improvement of the species in culture via

assessment of the significance of natural genetic diversity.

16S rRNA diversity in wild *M. rosenbergii* stocks

We, therefore, set out to document genetic diversity in wild *M. rosenbergii* stocks from across the natural range from southern Asia, to SE Asia and into the Asia-Pacific region. Molecular genetic analysis of a 472 base-pair segment of the 16S rRNA mtDNA gene, sampled from individuals representing 18 wild populations of *M. rosenbergii*, supports previous allozyme and morphological work that has identified two distinct forms of *M. rosenbergii* (De Man 1879; Johnson 1973; Lindenfelser 1984). The boundary between the two major clades corresponds with Huxley's extension (Huxley 1868) of Wallace's Line (Fig. 2), a well-known biogeographical break that runs through Indonesia. The level of divergence present between "eastern" and "western" forms of

M. rosenbergii identified in this study is in the order of 6.2 per cent for the 16S mtDNA gene (Fig. 3), a level of divergence which is further supported by preliminary COI results indicating divergence levels approaching 15 to 16 per cent for the same stocks. COI evolves more rapidly than 16S RNA, and after calibration of the different rates of evolution of the two molecules, estimates of divergence time since populations shared a common ancestor are similar. The extensive 16S mtDNA divergence between "eastern" and "western" forms indicates they have been separated since at least Miocene times (5-10 MYA; de Bruyn et al. In press). These divergence levels exceed interspecific 16S divergence levels between several penaeid prawn species (Tong et al. 2000), and some freshwater crayfish species (Grandjean et al. 2002).

Divergence estimates among populations within each of the two major clades are relatively small (maximum 0.9 per cent) and

selective "hybridization" of discrete stocks that may show heterosis (hybrid vigor). Similarly, this study has identified genetically distinct populations that should be the focus of increased conservation effort, as this genetic diversity can provide an important resource for the development of sustainable breeding programs in the future. Ongoing work will extend this initial data in a number of ways. Firstly, sampling effort will be increased to include a number of additional sites throughout *M. rosenbergii*'s natural range, and population sample sizes increased. Secondly, COI mtDNA and microsatellite variation (a suite of microsatellite primers are presently being developed in our lab) will be utilized to quantify levels of genetic divergence between populations and to estimate relative levels of genetic variation within and among populations for management purposes. Finally, existing *M. rosenbergii* culture stocks will be sampled, allowing for comparisons of genetic diversity levels to be made between wild and domesticated stocks. This will allow us to estimate the relative levels of inbreeding that may be present in these stocks and suggest approaches for developing improved culture strains in the future.

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P.B. Mather and **M. de Bruyn** are from the School of Natural Resources Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Queensland 4001, Australia.
E-mail: p.mather@qut.edu.au