Application of molecular tools for managing wild genetic resources in Australian freshwater crustaceans

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Abstract

Major threats to the conservation of aquatic genetic diversity are the fragmentation of populations by building dams and weirs and extraction of water for irrigation, the degradation of aquatic habitat and the movements of water and animals between drainage systems. This paper presents molecular data on the Australian freshwater prawn Macrobrachium australiense to demonstrate the use of molecular markers in the conservation and management of genetic diversity in this species. First, mitochondrial DNA sequence data is presented to test hypotheses concerning historical drainage structure. Comparisons are made across four major drainage basins, the Gulf of Carpentaria, Lake Eyre, the Murray Darling and coastal southeast Queensland. The risks of moving animals between these basins are discussed in the light of evidence from another freshwater shrimp, Paratya australiensis. Second, mitochondrial sequence data and allozymes are used to compare patterns of connectivity within each of these basins, each situated in a different landscape type. Third, microsatellite markers are used to assess movement between adjacent waterholes in a single river system. Finally, the potential for using genetic data to identify aquatic bioregions is assessed, first based on M. australiense data and then on other crustacean and fish species sampled across the same regions.

Keywords: wild genetic resources management; freshwater crustaceans

1. Introduction

As the world’s population grows, the need for clean freshwater is likely to become more acute. Of the world’s freshwater, 68.9% is locked up in glaciers, 30.8% in groundwater and only 0.3% is actually available for human and environmental use (UNEP, 2002). Currently 50% of the renewable water is used annually (UNEP, 2002). Australia is one of the driest continents, harbouring only a single ‘big river’. This is the Murray-Darling River system and it is already strongly impacted by human use (Nilsson et al., 2005). Currently, freshwater ecosystems are experiencing extinctions at a higher rate than any other ecosystem (Abell, 2002). In North America, freshwater mussels, crustaceans, fishes and amphibians are
going extinct at the rate of 4% per decade (Ricciardi and Rasmussen, 1999).

In order to manage and conserve as much freshwater biodiversity as possible, it is vital that the current diversity and its distribution are understood. As well, the processes that maintain this diversity need to be understood and maintained where possible. Examples of such processes are migration and dispersal, reproductive strategies and adaptation. In particular, it is important to identify populations or groups of populations that have evolved in isolation for significant evolutionary time, because these are likely to harbour unique genetic, biological and behavioural diversity.

Major threats to freshwater biodiversity include habitat degradation, such as removal of riparian vegetation and pollution, habitat fragmentation, such as the building of dams and weirs and drought, resulting in increased need for water extraction from natural waterways. In addition, translocations and inter-basin water transfers may have significant impacts, especially if they result in mixing of populations that have been evolving independently (Moran, 2002).

Recently, molecular markers have been used to address many questions concerning the identification of freshwater diversity and the processes maintaining it. For example, molecular markers can be used to identify local populations that are evolutionarily isolated from others of their species (i.e. evolutionarily significant units or ESU’s, sensu Moritz, 1994), to determine whether local population extinctions are likely to be recolonised by natural dispersal (Cook et al., 2007) and at what scale this recolonisation is likely (Hughes, 2007). In addition, molecular markers can be used to predict the risks involved with translocations and inter-basin water transfers.

Macrobrachium australiense is a freshwater prawn that is widespread throughout inland and coastal waterways of Australia. While the entire life-cycle occurs in freshwater, it has been recorded as being tolerant of up to 17.5 ppt salinity in laboratory conditions (Short, 2004). Its habitat ranges from coastal creeks in eastern Australia to highly turbid inland waterholes (Short, 2004). In this paper, we will use M. australiense as a model to demonstrate how molecular markers can contribute to answering the following questions:

- How isolated are populations in different drainages?
- How isolated are populations in different subcatchments within drainages and what are the major barriers to dispersal in this species?
- How much dispersal occurs between waterholes within a river?
- Are the patterns identified in M. australiense reflected in other obligate aquatic species?
- How can the answers to these questions assist in managing aquatic biodiversity?

2. How isolated are populations in different drainages?

M. australiense have been sampled from four main areas or drainage basins of eastern Australia, the Lake Eyre Basin, the Gulf of Carpentaria,
the northern part of the Murray-Darling basin and coastal south-east Queensland (Figure 1). Two studies used a fragment of the mitochondrial cytochrome oxidase I gene to compare populations in the Lake Eyre Basin with those in the Murray-Darling (Cook et al., 2002; Carini and Hughes, 2004). Populations in the two basins were clearly differentiated, with each containing a monophyletic clade of haplotypes, more closely related to each other than to those in the other basin. Carini and Hughes (2004) then used molecular clock estimates to calculate the likely time that populations in the two basins had been isolated from one another. The estimate was 995,000 years ago, in the middle of the Pleistocene. Hence, the molecular data suggests that these prawns in the two basins have been evolving independently for about a million years. Interestingly, independent estimates of when these two drainages were last connected put the time as much earlier, around 5 million years ago (T. Flottmann, pers. comm.).

Figure 1: Map of Australia, showing the four major drainage basins referred to in this paper: Lake Eyre, Gulf of Carpentaria, Murray-Darling and South East Queensland

A more recent study used the same mitochondrial gene to compare populations of *M. australiense* in the Lake Eyre basin with those in the Gulf of Carpentaria basin (Masci et al., in review). Again, populations in the two drainages were monophyletic, with a clear distinction between the two drainages. The timing since separation was again placed in the Pleistocene,
this time at 1.2 million years. This suggests again that populations of the prawn have been connected during the Pleistocene, even though geological estimates suggest that the drainages have not been connected for at least 5 million years (Coventry et al., 1985). This seems to suggest the possibility of massive floods in inland Australia, which may have connected populations of this and presumably other freshwater species.

*M. australiense* also occurs in coastal streams in eastern Australia. A study by Sharma et al. (in review), also using mitochondrial COI, showed that there was significant isolation between populations from groups of coastal streams on a very small scale in south-east Queensland. Populations at the northern extent of the ‘Sunshine Coast’ (see Figure 1) were quite divergent from those in the south. This was surprising, given the relatively small scale of this study of only a few hundred kilometres. Populations at the northern end of the study area were estimated to have been isolated from the southern populations since the early Pleistocene, 1.6 million years ago.

To summarise, it appears that major drainage basins in inland Australia have been isolated since some time in the Pleistocene. In addition, some populations in coastal streams have also been isolated, and it is likely that further study will identify more isolated populations when work is extended along the east coast.

3. **What are the implications of these findings for managers?**

The findings reported above suggest that populations of *M. australiense* in the four areas so far examined have been evolving independently for the last million or so years. In itself, this indicates that unique genetic diversity is contained in each of the major basins, and, as such, should be preserved. One management action that is likely to threaten this diversity is the movement of water between basins. These ‘inter-basin water transfers’ or IBT’s, are being considered as potential ways to ensure adequate water for drinking and agricultural use in Australia. If organisms are transferred between basins when the water is transferred, there is the potential to mix populations of *M. australiense* (and other freshwater species in the same systems) that have been evolving separately over at least a million years.

The outcomes of such mixing are largely unknown. In theory, there are four possible outcomes:

1. The population introduced to the new area may not survive and persist.
2. Both the populations may persist, with no interbreeding between them.
3. The two populations may hybridise, thus losing the unique genetic combinations of each.
4. The introduced population may send the resident population extinct.

A recent study of another freshwater crustacean in south-east Queensland showed clear evidence that the latter outcome is entirely possible. This study reported the result of translocating a population of the atyid shrimp *Paratya australiensis* between two sub-catchments of the Brisbane River in south-east Queensland (Hughes et al., 2003). At the time of
the translocation itself, the two populations were thought to be conspecific, differing only at a few allozyme loci (Hancock and Hughes, 1999). However, it was later discovered that the two populations had likely been isolated from one another for about 3 million years (Hurwood et al., 2003). The outcome was the complete local extinction of the resident population within only eight generations. The introduced genotype took over the stream up to two kilometres upstream and about 500 meters downstream.

This example indicates the possible risks of moving water (and thus individual organisms) between catchments. This is a major concern, particularly as so little is known of freshwater biodiversity. For example, a study, using molecular markers in the Sydney Catchment Area in New South Wales identified multiple un-described species in all invertebrate groups examined, which included freshwater crayfish, freshwater shrimps, mayflies, caddis flies (Baker et al., 2003) and coleopterans (Wheatley, 2003).

4. How much dispersal occurs among sub-catchments within major drainage basins and what are the major barriers to dispersal?

Molecular markers have also been used to examine dispersal of *M. australiense* within drainage basins in the Lake Eyre Basin, the Murray Darling, the Gulf of Carpentaria and the coastal creeks of south-east Queensland (Table 1). These studies use the idea that where dispersal among sites is widespread, then they will be genetically homogeneous, due to mixing of individuals and genes among populations (Slatkin, 1987). Alternatively, when dispersal among populations is limited, then the genetic composition of populations will diverge, because of different selection pressures and random processes (Slatkin, 1987). These studies calculated $F_{ST}$ values, to infer the levels of dispersal among rivers within these basins. $F_{ST}$ estimates the proportion of the total genetic variation that is between populations. An $F_{ST}$ of zero implies that all the variation is within populations and there is no variation among populations. This would indicate widespread dispersal. An $F_{ST}$ of one indicates that all variation is between or among populations. Any $F_{ST}$ above about 0.25 indicates extremely limited dispersal between populations (Hartl, 1980). Examination of Table 1 shows extremely high $F_{ST}$ values between rivers within the Gulf of Carpentaria, Lake Eyre and southeast Queensland, but non significant $F_{ST}$ values among sub-catchments of the Darling River. The rivers within the Gulf of Carpentaria and south-east Queensland are separated by marine conditions, so this result suggests that, despite the ability of the species to survive in brackish water in laboratory conditions (Short, 2004); marine conditions are a major barrier to dispersal in this species. Sub-catchments of the Lake Eyre Basin are connected via Lake Eyre. This lake is mostly dry, but on the rare occasions when it does fill with water, the water is highly saline (Ruello, 1976). Therefore, again, it appears that saline conditions restrict dispersal of this species. The rivers of the Darling are also only connected on a seasonal basis, consisting of a series of isolated waterholes for most of the
year. However, the non-significant $F_{ST}$ values in the Darling suggest that *M. australiense* are able to move between river systems during flood times - or at least, they have done relatively recently.

### Table 1: $F_{ST}$ values between rivers for each of four drainage basins

<table>
<thead>
<tr>
<th>Drainage basin</th>
<th>$F_{ST}$ value (Allozymes)</th>
<th>$F_{ST}$ value (mtDNA)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulf of Carpentaria</td>
<td>-</td>
<td>0.289***</td>
<td>Masci et al. (in review)</td>
</tr>
<tr>
<td>Lake Eyre Basin</td>
<td>0.437***</td>
<td>0.411***</td>
<td>Cook et al. (2002); Carini and Hughes (2004)</td>
</tr>
<tr>
<td>Murray-Darling</td>
<td>0.023 n.s.</td>
<td>0.048 n.s.</td>
<td>Cook et al. (2002); Carini and Hughes (2004)</td>
</tr>
<tr>
<td>Coastal South East Queensland</td>
<td>0.043***</td>
<td>0.817***</td>
<td>Sharma and Hughes (in review)</td>
</tr>
</tbody>
</table>

*** $P < 0.001$

These results imply that each sub-catchment probably contains a separate genetic stock or management unit (*sensu* Moritz, 1994). In other words, with the exception of the Murray-Darling Basin, any management actions should be approached at the sub-catchment scale.

### 5. How much dispersal occurs between waterholes within a river?

The previous analyses were concerned with patterns of connectivity at large (between drainage basins) and medium (between sub-catchments within drainage basins) spatial scales. In addition, the patterns observed reflected limitations in dispersal over evolutionary timescales. An additional question is how much do individual *M. australiense* move between waterholes within a single river?

One of the assumptions on which the inference of dispersal from $F_{ST}$ values is based is that populations have been behaving in a particular way for long enough for them to have reached equilibrium between gene flow (dispersal) and genetic drift. This is not always the case (Chenoweth and Hughes, 2003) and so other methods have been developed that concentrate on estimating current levels of dispersal among populations without having to rely on this assumption. These methods use more sensitive molecular markers such as microsatellites, which have higher mutation rates than mitochondrial DNA or coding nuclear DNA, and use the genotype of individuals at a number of loci to estimate the probability that they have come from each of a number of sampled populations.

*M. australiense* was sampled from 10 waterholes along the Moonie River, a tributary of the Darling River. These waterholes are isolated from one another for most of the year. However, most years there is sufficient rain for the river to flow for short periods, so we were interested to know whether the prawns made use of this hydrological connectivity to move between waterholes. Two methods
were applied to the question. In the first, we assigned individual prawns to the waterhole to which they were allocated the highest probability, based on their multi-locus microsatellite genotype using the program Geneclass (Piry et al., 2004). Figure 2 shows that most individuals were allocated to the waterhole in which they were captured, although in each pool, there were individuals that were more likely to have come from another pool that had been sampled, or in some cases from a pool that had not been sampled. The problem with this method is that no statistical significance is attached to the assignment of individuals to populations. The other method we used identified those individuals that had a less than 5% chance of having been born in the population from which they were sampled (Figure 3). These individuals are therefore assigned as immigrants. In the Moonie study, all pools except the most upstream and the most downstream had between 5 and 20% of individuals that were labelled immigrants. This result implies that the rare connectivity of pools each year does offer an opportunity for these prawns to disperse between them.

Figure 2: Results of assignment testing study, showing:
  
a) location of the Moonie River;
  b) sampling sites along the Moonie River;
  c) number of individuals caught in each pool and the pools to which they were assigned.
Figure 3: Results of estimation of immigrants and residents in each of 10 pools along the Moonie River

These techniques will allow us to assess the impacts of building new dams and weirs on movement patterns, as well as assessing the relative importance of upstream versus downstream movement. In addition, it will allow us to predict which sites are likely to be quickly recolonised following local extinctions. For example, based on the data presented in Figure 3, the upstream and downstream sites in this study appear to be made up totally of individuals that have recruited from within their own pool, whereas the other pools all contain some immigrants. This suggests that these intermediate pools would be less seriously affected by temporary extinctions or pool drying.

6. Are the patterns identified in *M. australiense* reflected in other obligate aquatic species?

The analysis of patterns of genetic diversity in *M. australiense* across major drainage basins suggests that populations in each of the major drainage basins represent significant unique genetic diversity, and that efforts should be made to avoid mixing them, or mixing the water, which may contain larvae or eggs. A question that arises is, are the patterns identified in *M. australiense* similar for other species? If so, then it may be possible to develop a system to identify aquatic bioregions, based on molecular data. Translocations and inter-basin water transfers should not occur between
these regions, but less of a risk is posed by movements of water and organisms within them.

As mentioned earlier, a number of aquatic species that span the Lake Eyre and Murray-Darling basins appear to have been connected as recently as the Pleistocene. However, the patterns vary between species. The Australian smelt *Retropinna semoni* (Hughes and Hillyer, 2006) and a freshwater mussel *Velesunio ambiguous* Species C (Hughes et al., 2004) show a pattern very similar to *M. australiense*, with monophyletic clades on either side of the drainage divide, and a timing of separation of early to mid-Pleistocene. Three other species, a freshwater crayfish *Cherax destructor* (Hughes et al., 2003), an eel-tailed catfish *Neosalarias hortilii* (Huey et al., 2006) and a bony bream *Nematochisma erebi* (Hughes and Hillyer, 2006) all show evidence of more recent connections and no significant divergence between clades on either side of the divide, even sharing of haplotypes in the case of the bony bream.

To date, other than *M. australiense*, the only species that has been examined across the Lake Eyre/Gulf of Carpentaria divide is the bony bream. As found between the Lake Eyre and the Murray-Darling, a much more recent connection is suggested for this species than for *M. australiense*, and again there are haplotypes shared across the divide (Masci et al., in review). Clearly more species need to be examined in this region.

As shown above, *M. australiense* shows two distinct clades at the northern and southern extremes of the Sunshine coast area. Genetic divergence between north and south have also been reported for the oxleyan pygmy perch *Nannoperca oxleyana* (Pleistocene, Hughes et al., 1999), the ornate rainbowfish *Rhadinocentrus ornatus* (Miocene, Page et al., 2004), a freshwater shrimp *Caridina indicinacta* Species B (Pleistocene, Page and Hughes, 2007) and a freshwater crayfish *Cherax dispar* (Miocene, Bentley et al., in prep.). In contrast, another group of species were effectively genetically homogeneous in the region (*Caridina indicinacta* Species D, Page and Hughes, 2007; *Macrobrachium tolmerum*, Sharma and Hughes, in review; and the empire gudgeon *Hypseleotris compressa*, Sharma, 2007). Each of the latter three species is thought to have some tolerance for brackish or saline conditions, probably explaining the lack of genetic structure in them.

In summary, the patterns identified in *M. australiense* do appear to be reflected in some, but not all freshwater species. The differences between species probably reflect differences in life-history and dispersal abilities, such that those species with limited dispersal abilities, obligate restriction to freshwater and small population sizes are those most likely to show high levels of divergence between drainages, or even between sub-catchments within drainages. As such, these species should make the best indicators of bioregions for aquatic genetic diversity. Certainly *M. australiense*, a widespread species across northern, central and eastern Australia, appears to be a good surrogate for defining areas likely to contain unique aquatic genetic diversity.

Currently, the Queensland Government is proposing to build a new dam at the northern end of the Sunshine Coast, on the Mary River, and to transfer water south for use in
Brisbane. Our analysis of aquatic genetic diversity in the region would indicate that by doing this, there is a significant risk of mixing populations that have been evolving independently from as long ago as the Miocene. As demonstrated in the translocation example, the possible consequences of this mixing are the loss of unique genetic diversity. Only a handful of species have been studied in this level of detail, but we would predict that hundreds of other obligate freshwater species with limited dispersal abilities will show patterns similar to those identified here for fish and crustaceans and will therefore be at risk if the proposed water transfers go ahead.

Whiting et al. (2000) proposed using freshwater crayfish to identify aquatic bioregions in Australia. Unlike our approach, they based their categorisation on species or genetic diversity, allowing also for areas containing unique endemic diversity. Their approach was therefore more focused on maximum diversity, rather than on maintaining evolutionary distinctiveness as is proposed here. Interestingly, they did not identify the possibility of more than one bioregion in the south-east Queensland region.

7. Conclusion

Using *M. australiense* as a model, we can make some recommendations about the management of freshwater genetic diversity. Translocations and inter-basin water transfers should not occur between any of the four major basins examined in this study. Translocations and inter-basin transfers should not occur between northern and southern regions of the Sunshine Coast, or between the Mary River and the Brisbane River until further assessment of the distribution of genetic diversity can be made. Any such actions are likely to result in loss of genetic diversity.

Local extinctions in inland areas are likely to be recolonised quickly, as long as potential source populations exist within the same catchment and the whole catchment has not dried up or been degraded. Local extinctions in coastal creeks are unlikely to be recolonised naturally, as they are separated by marine saline conditions, which pose a barrier for *M. australiense* and many other species. This conclusion is particularly significant because this area of south-east Queensland is rapidly being developed for housing and tourism operations.

The potential to define aquatic bioregions based on the distribution of molecular genetic diversity looks promising. We should focus our efforts on those species that have limited dispersal potential, are restricted to freshwater, and to which both terrestrial and saline conditions pose significant barriers. Finally, species with small population sizes may be the best indicators as they are likely to diverge more quickly when catchments become isolated.

References


and mitochondrial DNA. Heredity 90, 64-70.