Strong genetic structuring in a habitat specialist, the Oxleyan Pygmy Perch *Nannoperca oxleyana*

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This study used allozyme and mitochondrial DNA variation to examine genetic structure in the Oxleyan Pygmy Perch *Nannoperca oxleyana*. This small-bodied freshwater fish has a very restricted distribution occurring only in some small coastal streams in south-east Queensland and northern New South Wales. It was expected that subpopulations may contain little genetic variation and be highly differentiated from one another. The results, based on allozyme and mitochondrial DNA control region variation were in agreement with these expectations. Allozyme variation was very low overall, with only one locus showing variation at most sites. The high differentiation was because a different locus tended to be polymorphic at each site. Mitochondrial variation within sites was also low, but some sites had unique haplotypes. The patterns of similarity among mitochondrial DNA haplotypes were not as expected from geographical proximity alone. In particular, although some northern sites had unique haplotypes, four sites spread along 200 km of coastline were remarkably similar, sharing the same common haplotype at similar frequencies. We suggest that these four streams may have had a confluence relatively recently, possibly when sea levels were lower, 8000–10 000 BP.

Keywords: allozymes, gene flow, genetic structure, mitochondrial DNA, *Nannoperca oxleyana*, Pygmy Perch.

Introduction

The role that current and historical patterns of dispersal have in population genetic structure of natural populations has been assessed for a range of terrestrial and aquatic species (for example, Reeb & Avise, 1990; Templeton *et al.*, 1995). In general, species that have efficient mechanisms for dispersal show limited genetic structuring of their subpopulations, whereas in those species where dispersal is restricted substantial differentiation among subpopulations may occur (Slatkin, 1981). In a review of published studies on marine, anadromous and freshwater fishes, Ward *et al.* (1994) concluded that generally marine fish species exhibit the lowest levels of genetic variation among subpopulations, but the highest levels within subpopulations. In contrast, freshwater species tend to exhibit high levels of structure among subpopulations and low levels of variation within subpopulations. These differences are thought to be largely the result of the differing potential for dispersal among subpopulations of marine and freshwater species. Many freshwater species are restricted to their particular drainage, and dispersal among drainages is likely to be limited by the marine sections separating them. Because the subpopulations are relatively isolated from one another, they will tend to diverge as a result of genetic drift and/or selection. The rate of divergence will be affected by the effective population size and the strength of any selection pressures. Small populations under intense selection would be expected to diverge more quickly than large populations under minimal selection. Thus for those freshwater species that are rare or threatened, that live in specific habitats and usually in small populations, low levels of genetic variation may be expected within populations, whereas substantial differentiation between populations from different drainages may be expected.

The Oxleyan Pygmy Perch *Nannoperca oxleyana* (family Nannopercidae) inhabits small coastal streams on the mainland and islands of south-eastern Queensland and a few localities in northern New South Wales, Australia (Merrick & Schmida, 1984). The Action Plan for Australian Freshwater Fishes (Wager & Jackson, 1993) classified the Pygmy Perch as 'vulnerable' because of its restricted geographical distribution, its absolute dependence on dystrophic waterways of low pH associated with heath vegetation, and low population sizes in many localities. Although *N. oxleyana* is not presently 'endangered', most of its known populations are threatened to some degree by habitat fragmentation, water pollution, collecting for aquarium purposes or by the exotic mosquitofish *Gambusia holbrooki* (Arthington & Marshall, 1996; Kuiter *et al.*, 1996). In developing a recovery plan for the Pygmy Perch (Arthington, 1996), it was considered prudent to determine the genetic structure of the remaining populations and to use this information to maximize the amount of genetic diversity to be maintained through conservation measures.

Many previous studies have used allozyme analysis to assess the levels and patterns of variation among subpopulations of different species (e.g. fish, Meffe & Vrijenhoek, 1988; Crustacea, Hughes *et al.*, 1995; insects, Schmidt *et al.*, 1995). This technique allows inferences to be drawn about the level and pattern of dispersal among subpopulations and their degree of isolation. More recently, variation in mitochondrial DNA has also been used for examining population structure. Mitochondrial DNA is generally maternally inherited in vertebrates and is haploid (Avise, 1991). Thus mitochondrial genes have effective population sizes roughly one quarter those of nuclear genes (Birky *et al.*, 1989). This means that mtDNA is affected more by genetic drift than is nuclear DNA, resulting in a more rapid loss of diversity within small isolated subpopulations and a more rapid rate of differentiation among subpopulations. As a result, the analysis of mtDNA has the potential to be more sensitive than nuclear DNA markers for detecting limited dispersal among subpopulations, although this is not always the case (e.g. Ward & Grewe, 1995 and references therein).

The primary aim of this study was to use variation in both nuclear genes (allozymes) and mtDNA to assess the amount of genetic variation within and among populations of the Pygmy Perch. Given its limited distribution and relatively low densities, we expected low levels of variation within subpopulations from particular drainages and high levels of differentiation among drainages. The second aim was to define the implications of the results for the long-term conservation of *N. oxleyana*.

Materials and methods

Sample collection

Fish were collected from nine sites in south-eastern Queensland, which covered almost all the species' range. We were unable to obtain samples from northern New South Wales as the species is very uncommon there. There were three sites on Moreton Island, one on Fraser Island and five on the adjacent mainland. Precise localities are shown in Fig. 1. The Noosa River sample included fish collected from six different sections of the river. The other samples were all collected from small streams. Seine nets were run within 3 m of stream bank and around macrophyte beds. Where possible 30 fish were sampled from each site. This usually entailed sampling along a substantial stretch of stream, up to 100 m, because fish were not common in most streams. A sample size of 30 individuals provides a 95% chance of detecting at least one copy of each mtDNA haplotype that occurs at 10% or greater frequency in the population and a 95% chance of detecting alleles at allozyme loci that occur at 5% or greater frequency (Schwager *et al.*, 1990). Fish were frozen in liquid nitrogen and transported back to the laboratory where they were stored at $-75°C$ until needed for genetic analysis.

Allozyme analysis

A small sample of muscle tissue was removed from each fish and ground by hand in 0.2 mL of buffer (2.44 g Trizma base, 0.37 g EDTA free acid, 5.36 g NH_4Cl, 19.8 g glucose, 20 mL 0.022 M $NaNO_3$, in 1 L of H_2O). Samples were centrifuged at 16 800 g for 20 min in a Sorvall RC5B refrigerated centrifuge operating at 5°C. Electrophoresis was performed on cellulose acetate plates (Titan III, Helena Laboratories). A total of 30 enzymes was screened, of which 23 gave clearly interpretable banding patterns. Only six loci were polymorphic. These were phosphoglucoisomerase (PGI, EC no. 5.3.1.9), phosphoglucomutase (PGM, EC no. 5.4.2.2), aspartate aminotransferase (AAT, EC no. 2.6.1.1), lactate dehydrogenase (LDH, EC no. 1.1.1.27), esterase (EST, EC no. 3.1.1.1) and alcohol dehydrogenase (ADH, EC no. 1.1.1.1). Unfortunately not all individuals could be scored for *β-Est* and *Adh*, so that sample sizes differ among loci for some sites (Appendix 1).

Analysis of mitochondrial DNA

Total DNA was extracted, by homogenizing ≈25 mg of white muscle tissue in 350 μL of extraction buffer (0.5 M Tris 8.0; 0.25 M NaCl, 0.025 M EDTA; 0.175 mM SDS) and incubated at 55°C overnight. Proteins and lipids
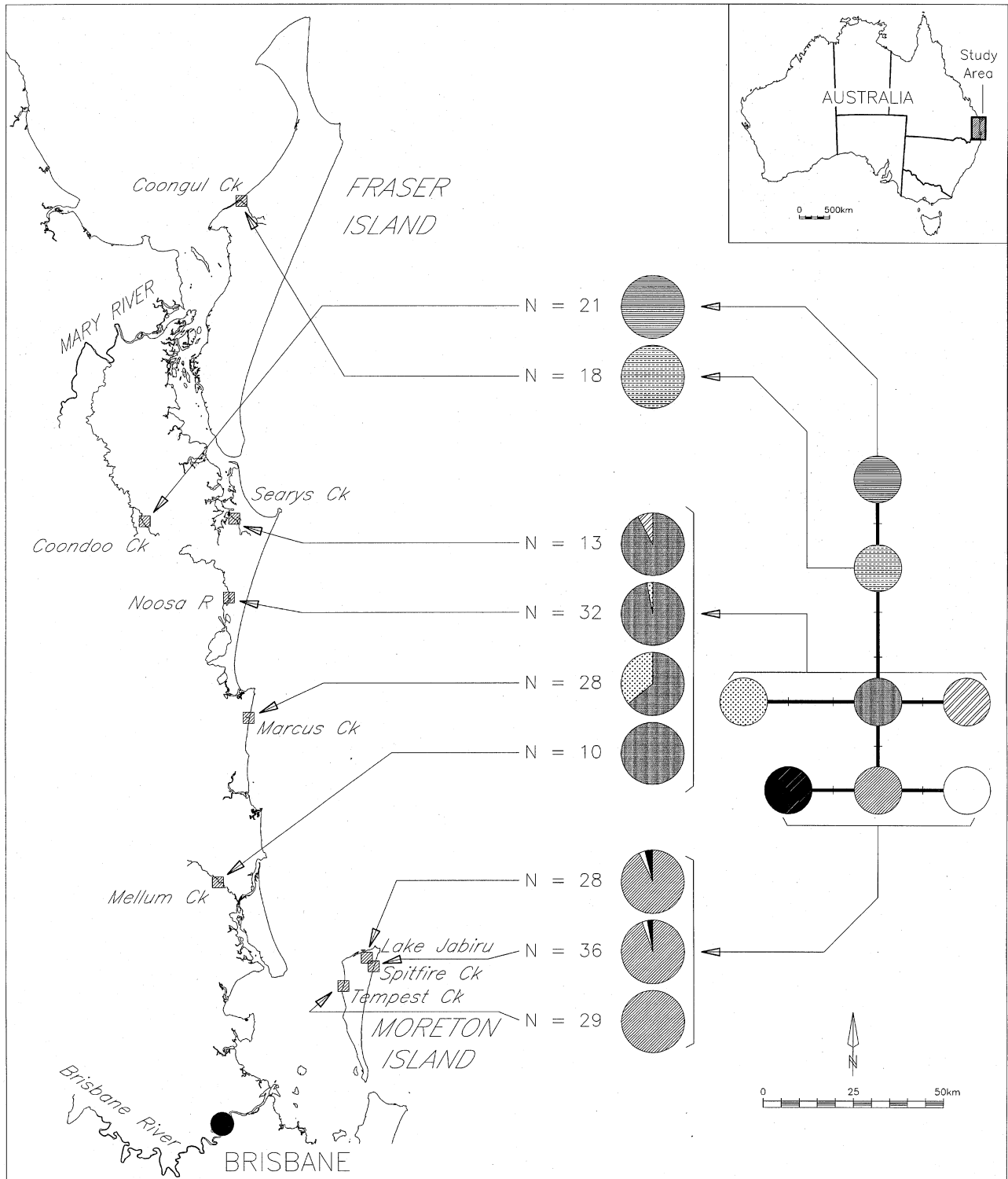
Fig. 1 Map of south-east Queensland coast, showing sampling sites for Pygmy Perch, pie diagrams indicating mtDNA haplotype frequencies at each site and a network indicating relationships between haplotypes.

were removed by sequential extractions with equal volumes of phenol–chloroform–isoamyl alcohol (25 : 24 : 1) and chloroform–isoamylalcohol (24 : 1). DNA was precipitated at 4°C in the presence of isopropanol (1 vol.). Pellets were washed in 1000 μL of ethanol (70%), dried and resuspended in 50 μL of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.5).

Two oligoprimers were used to amplify a 450-bp fragment of the mitochondrial control region. The heavy strand primer (MT16498H; 5′-CCT GAA GTA GGA ACC AGA TG-3′; (Meyer *et al.*, 1990) is located in a conserved region within the control region. The light strand primer (L19; 5′-ACC ACT AGC ACC CAA AGC TA-3′; Bernatchez & Danzmann, 1993) is in the proline tRNA gene which flanks the 3′ end of the control region.

PCR reactions contained 30 nmoles each of dATP, dGTP, dCTP and dTTP (Promega), 1 unit of Taq DNA polymerase (Promega), 2.5 μmoles of MgCl$_2$, 0.5 μmoles of each primer, 5 μL of 10× polymerase reaction buffer (Promega), 0.2 μg of template DNA, adjusted to final volume of 50 μL with ddH$_2$O and overlayed with 30 μL of light mineral oil. Mixtures were then subjected to 35 cycles of: 5 min at 94°C (only on initial cycle), 30 s at 94°C, 30 s at 50°C, 1 min at 72°C, 5 min at 72°C (final cycle only). Samples were stored at 4°C.

Temperature gradient gel electrophoresis combined with heteroduplex analysis (TGGE-HA) was used to screen amplified fragments for variation. Prior to running the TGGE, samples were heteroduplexed with a reference conspecific. Samples contained 10 ng of reference conspecific and sample amplified DNA, 4 M urea and 1× ME (20 mM MOPS, 1 mM EDTA) in a total volume of 5 μL. Each mixture was brought to 94°C for 5 min (for denaturation), then 50°C for 14 min (for heteroduplex formation) and allowed to equilibrate at room temperature for 10 min before being loaded on to the gel. The gel was a 5% denaturing polyacrylamide gel (8 M urea, 2% glycerol, 30 : 0.5 acrylamide:bis in 1× ME buffer). Gels were run through a temperature gradient of 17–52°C at 300 V for 3.5 h.

Gels were silver stained as recommended by the manufacturers (QIAGEN). All samples were run with the same reference individual for heteroduplex analysis. This was then repeated with an individual of a different haplotype, to ensure that gels were being interpreted correctly. No discrepancies were found in assigning haplotypes.

Of the putative haplotypes identified by TGGE-HA, one individual of each haplotype from each site was sequenced. PCR product was cleaned using Quiaquick columns (Quiagen) following the manufacturers specifications. Sequencing reactions were carried out using dye terminator cycle sequencing reactions (Perkin Elmer) according to manufacturers instructions along with ≈30 ng of clean DNA template and 3.2 pmol of primer on an Applied Biosystems 373 automated sequencing machine. Both light and heavy strands were sequenced.

Statistical analysis

For the allozyme data, BIOSYS 1.7 (Swofford & Selander, 1989) was used to calculate allele frequencies, percentage polymorphism and average heterozygosity for each site, and to test for deviations from Hardy–Weinberg equilibrium. Wright's *F*-statistics were used to assess population structure. Further analyses were carried out to examine variation within regions (i.e. among sites on Moreton Island and among mainland sites from Searys Creek South to Mellum Creek) and between regions. The regions were (a) Fraser Island (b) Coondoo Creek (c) Mainland south: Searys Creek, Noosa River, Marcus Creek and Mellum Creek, and (d) Moreton Island: Lake Jabiru, Spitfire Creek and Tempest Creek. Coondoo Creek was analysed as a separate region because it enters the sea via the Mary River, a long way further north than Searys Creek.

Haplotype diversity was calculated for each subpopulation (Nei, 1987). A minimum-spanning network of the evolutionary relationships among mtDNA haplotypes was constructed with the assistance of a program supplied by L. Excoffier. The groups of closely related haplotypes defined by the network determined our groupings for the subsequent population genetic analyses. The degree of population subdivision was estimated using a nested Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992). Groups for the analysis were the same as for the allozyme analysis. This technique partitions variation according to sampling design and yields Φ-statistics which are analogues of Weir & Cockerham's (1984) F_{ST} estimator, θ, when haplotype frequencies only are used in the analysis. AMOVAs were performed both on haplotype frequencies alone and using the sequence divergence among haplotypes assuming a Tamura & Nei (1993) gamma model of evolution (gamma parameter = 0.5) to correct for unequal nucleotide substitution among sites. Φ-statistics were tested for significance using 1000 random permutations of the original data (Schneider *et al.*, 1997).

Results

Allozymes

F_{IS} values were all nonsignificant, with the exception of *Pgm-1* in the Noosa River (Table 1). This could have occurred because this sample was a combination of samples from separate sections of the river. The levels of

Table 1 F_{IS} values for each locus for each site for six polymorphic allozyme loci in Pygmy Perch

Site	Pgi-1	Pgm-1	Aat-1	Ldh-1	b-Est-1	Adh-1
Coondoo Creek	–	–	–	–	0.188	–
Coongul Creek	−0.077	–	–	–	–	–
Searys Creek	–	0.019	–	–	–	–
Noosa River	−0.046	0.393*	–	–	–	–
Marcus Creek	–	–	–	–	–	–
Mellum Creek	–	–	–	–	−0.333	–
Spitfire Creek	–	–	−0.029	–	–	−0.052
Lake Jabiru	–	–	−0.018	–	–	−0.143
Tempest Creek	−0.067	–	−0.068	−0.160	–	0.095

*$P < 0.05$.

Table 2 Variation at six polymorphic allozyme loci in nine populations of the Pygmy Perch

Site	Mean sample size per locus	Mean number of alleles per locus	% loci polymorphic	Heterozygosity (expected HWE)
Coondoo Creek	27.5	1.2	16.7	0.029
Coongul Creek	21.0	1.2	16.7	0.024
Searys Creek	17.0	1.2	16.7	0.059
Noosa River	32.0	1.3	16.7	0.044
Marcus Creek	30.0	1.0	0.0	0.000
Mellum Creek	10.0	1.2	16.7	0.083
Spitfire Creek	29.8	1.3	16.7	0.088
Lake Jabiru	27.5	1.3	16.7	0.077
Tempest Creek	23.2	1.7	66.7	0.217

Table 3 Pairwise F_{ST} values for Pygmy Perch based on allele frequencies at six polymorphic loci

	Coondoo	Coongul	Searys	Noosa	Marcus	Mellum	Spitfire	Jabiru	Tempest
Coondoo	–								
Coongul	0.083	–							
Searys	0.207	0.059	–						
Noosa	0.131	0.118	0.141	–					
Marcus	0.066	0.042	0.162	0.185	–				
Mellum	0.066	0.301	0.198	0.150	0.198	–			
Spitfire	0.368	0.416	0.306	0.314	0.609	0.263	–		
Jabiru	0.216	0.253	0.212	0.202	0.407	0.184	0.009	–	
Tempest	0.289	0.273	0.262	0.291	0.325	0.225	0.088	0.125	–

variation within populations were very low (Table 2), with 1.1 alleles per locus, percentage polymorphism between 0.0 and 14.8% and an average heterozygosity of 0.016. In most populations, only a single locus was polymorphic, but different loci were polymorphic in different populations. This resulted in an F_{ST} value of 0.211, i.e. 21.1% of the total variation was among subpopulations. Lake Jabiru and Spitfire Creek were very similar, as may be expected because they are currently connected within a drainage system (Table 3). The variation among sites on Moreton Island was less than among sites on the mainland, but was still highly significant at three loci (Table 4, $P < 0.001$).

A total of seven mitochondrial DNA haplotypes was identified (Fig. 1). The variation within populations was again low. Eight of the nine populations were either totally monomorphic or were dominated by a single haplotype. Only one population, from Marcus Creek on the mainland, showed a second haplotype at a frequency of more than 0.25. The haplotype diversity within populations was also low, ranging from 0.00 to 0.47 (Table 5). Differentiation among populations was

Table 4 F_{ST} values for Pygmy Perch based on six allozyme loci showing variation among all sites and between regions

	Pgi-1	Pgm-1	Aat-1	Ldh-1	b-Est-1	Adh-1	Mean ± SD
Total	0.034***	0.170***	0.280***	0.123***	0.154***	0.241***	0.211 ± 0.031
Within Moreton	0.051***	–	0.270***	0.131***	–	0.000	0.061 + 0.127
Mainland south	0.017	0.146***	–	–	0.285***	–	0.145 ± 0.041
Between regions	0.023***	0.062***	0.101***	0.030***	0.076***	0.295***	0.185 ± 0.087

***$P < 0.001$.

Table 5 Haplotype diversity of Pygmy Perch in mitochondrial control region at each site

Site	Haplotype diversity
Coondoo Creek	0.000
Coongul Creek	0.000
Searys Creek	0.154 ± 0.126
Noosa River	0.062 ± 0.058
Marcus Creek	0.476 ± 0.067
Mellum Creek	0.000
Spitfire Creek	0.109 ± 0.070
Lake Jabiru	0.204 ± 0.098
Tempest Creek	0.000

Table 6 Results of AMOVA, showing variation in mitochondrial control region of Pygmy Perch within and among regions

Source of variation	Analysis on haplotype frequency only	Analysis on haplotype frequency and sequence divergence
F_{SC}	0.201***	0.251***
F_{ST}	0.875***	0.908***
F_{CT}	0.844***	0.877***

F_{ST}, sites relative to total variation; F_{SC}, sites within region; F_{CT}, regions relative to total variation.
***$P < 0.001$.

extremely high, with an F_{ST} of 0.87, i.e. 87% of the total variation was between subpopulations. The AMOVA based on haplotype frequency indicated that the genetic variation was much greater among the broad geographical groupings than within them (Table 6). The result was very similar when sequence divergence was included. The three subpopulations from Moreton Island were very similar, all sharing the same haplotype at high frequencies (Fig. 1). The pairwise F_{ST} analysis indicated that they were not significantly different (Table 5, $P > 0.05$). The other populations that appeared very similar were those found in coastal streams from Searys Creek South to Mellum Creek. Again the same haplotype was most common in all these sites, although some pairs of sites did differ significantly in frequency of haplotypes (Fig. 1, Table 7). The samples from Coondoo Creek in the Mary River catchment and Coongul Creek on Fraser Island were different from each other and from all other samples, each being fixed for a unique haplotype.

Although the network illustrating relationships among the seven haplotypes is very shallow, with only four or five base pair substitutions separating the most divergent haplotypes, there is still a clear geographical pattern (Fig. 1). All three haplotypes on Moreton Island form a single clade. The haplotype that is common in coastal streams up to Searys Creek is central to the network and each of the other haplotypes found in these streams differ from it by only a single base pair substitution. The Fraser

Table 7 Pairwise F_{ST} values for Pygmy Perch based on mtDNA haplotype frequencies

	Coondoo	Coongul	Searys	Noosa	Marcus	Mellum	Spitfire	Jabiru	Tempest
Coondoo	–								
Coongul	1.000	–							
Searys	0.934	0.941	–						
Noosa	0.959	0.962	0.000 NS	–					
Marcus	0.721	0.735	0.184*	0.276	–				
Mellum	1.000	1.000	0.022 NS	0.000 NS	0.224*	–			
Spitfire	0.927	0.931	0.878	0.913	0.724	0.915	–		
Jabiru	0.878	0.884	0.813	0.871	0.660	0.853	0.000 NS	–	
Tempest	1.000	1.000	0.952	0.967	0.765	1.000	0.007 NS	0.051 NS	–

All comparisons are significant ($P < 0.001$) unless shown.
*$P < 0.05$

Island haplotype also differs from the common one by a single base pair and the Coondoo haplotype is one further base pair removed again.

Discussion

The results from the analysis of allozyme variation suggest very low levels of heterozygosity within sub-populations. These levels are low even for freshwater fish, which according to Ward *et al.* (1994) have average heterozygosity levels around 0.046. Of the 49 species reported in their summary, only three species had levels lower than reported here for *N. oxleyana*. Ward *et al.* (1994) report heterozygosity levels of 0.052 for ana-dromous fishes and 0.059 for fully marine species. Our result is not surprising given the degree of isolation of *N. oxleyana* populations, tending to accentuate the effects of genetic drift, whereas any loss of variation resulting from drift in more connected populations will tend to be counteracted by gene flow. Surprisingly the Tempest Creek population had the highest level of heterozygosity, even though it was one of the smallest streams sampled.

Similarly low levels of intrapopulation diversity were seen in the mtDNA. This is more difficult to compare with other published work, because results depend on the particular fragment of the mitochondrial genome that is being analysed. The control region is recorded as having the greatest amount of variation in most species (Avise, 1991) but even different parts of that region may vary in the amount of variation within populations (Faber & Stepien, 1997). Chenoweth & Hughes (1997) examined variation in the same section of the mito-chondrial genome in the Australian bass (*Macquaria novomaculeata*), a catadromous species. Much higher levels of variation were found within populations (within population diversity values of 0.587). The same fragment has also been examined in the Barramundi (*Lates calcarifer*), a catadromous species thought to migrate at least between adjacent river systems. Ex-tremely high levels of variation were found (Chenoweth *et al.*, 1998). These differences are not unexpected as both the bass and the barramundi are likely to have much larger effective population sizes than the Pygmy Perch, which is restricted to particular freshwater habitats, occurs mainly in low densities and cannot disperse through salt water (Kuiter *et al.*, 1996).

The levels of variation among populations of *N. oxleyana* are also quite high. Twenty-one percent of the allozyme variation and 87% of the control region variation was distributed among subpopulations. These results support the idea that dispersal among popula-tions is extremely limited. The higher levels of differen-tiation for mtDNA were not unexpected, as mtDNA is

expected to differentiate at a faster rate than nuclear DNA, because of its maternal inheritance and haploid nature and thus lower effective population size (Birky *et al.*, 1989).

On the basis of the allozyme data alone, it could be suggested that the only two subpopulations likely to exchange individuals currently are Lake Jabiru and Spitfire Creek. This is not surprising, as these two sites are currently connected via a swampy drainage system. Significant differences in allele frequencies are seen between all other pairs of sites suggesting no or very limited dispersal of fish between them. This may seem an obvious result as the subpopulations came from differ-ent streams. However, during flood conditions it was considered possible that some areas of coastline would be fresh, thus allowing the possibility of dispersal between nearby streams.

The mtDNA results suggest a similar pattern, particularly with respect to Mary River and more southern coastal rivers, and the fixed differences between island and mainland sites indicate no recent dispersal of fish between them. The similarities among the four streams from Searys south to Mellum Creek could be interpreted either as the result of some current gene flow between these sites or the retention of variation arising from relatively recent connection of these rivers. The first interpretation seems unlikely, given the sensitivity of this species to brackish or saline water (Kuiter *et al.*, 1996) and the fact that the allozyme data indicate limited gene flow. The other alternative, that these rivers have been connected relatively recently is entirely possible. Sea level changes over the late Pleistocene have been extensive (Chappell & Shackleton, 1986) and as recently as 8000 years ago, the coastline would have extended out past both Fraser and Moreton Island. Our data could indicate that at this time, these four streams had a confluence before reaching the sea. Although no published studies exist on this area, information from the Queensland De-partment of Minerals and Energy (A. Stevens, pers. comm.) indicates the possibility that the Brisbane River, which currently flows out through Moreton Bay, may have flowed a long way further north before entering the sea. All mainland streams south of Fraser Island may have flowed into the Brisbane River prior to entering the sea and thus formed part of the same drainage. This suggestion is purely speculative and, because of the high wave action on that particular stretch of coastline, even seismic profiling is unlikely to reveal the positions of past drainages (A. Stevens, pers. comm.).

That the three haplotypes on Moreton Island form part of the same clade suggests that at least the two rare ones have arisen since these sites became separated from

the mainland streams. Although Moreton Island was attached to the mainland as recently as 8000 BP, evidence from the mtDNA data suggests that the Moreton Island streams may not have connected with easterly flowing streams along the Sunshine Coast at that time, as indicated by the greater similarity of Moreton Island haplotypes to each other than to any mainland haplotypes.

Similarly, mitochondrial DNA data from Fraser Island and Coondoo Creek suggest that these populations have been separated from the other mainland populations for longer than the other mainland populations have been isolated from each other, because they have no haplotypes in common and appear to form a separate clade. This is not surprising as geomorphological evidence suggests that a ridge separates the Mary and southern drainages (Bridges *et al.*, 1990). The larger differences between the two northern sites, compared to the four other mainland sites, also suggest that in recent times, Searys Creek, which flows into Tin Can Bay, has flowed out to the south, rather than north of Fraser Island, via the Mary River (Fig. 1).

The evolutionary sequence resulting in the present-day patterns can be inferred from the congruence of the network with geographical distribution. The most likely scenario is that the present haplotypes evolved from a common ancestor, probably the central haplotype, that was widely distributed. The base substitutions probably occurred once the populations had become isolated from one another, possibly with the Coondoo Creek haplotype evolving later from the Fraser Island one. The alternative, that all haplotypes were present at the time of isolation and different ones have become dominant (or fixed) since, is less likely because the geographical concordance with the network would not be expected.

Interestingly, a recent study of genetic variation in a freshwater shrimp, which inhabits the same coastal heath streams as the Pygmy Perch, has indicated very similar patterns (Woolschot, 1997). Allozyme variation within and between populations from mainland streams shows that subpopulations from the Noosa River right down to rivers opposite Moreton Island are very similar in allozyme frequencies, but highly significantly different from those flowing into Coondoo Creek in the Mary River drainage (F_{ST} values = 0.65).

As the geomorphological evidence for river confluence is inconclusive, we plan to examine a number of other freshwater species from this region, that may be expected to have experienced the same historical events of isolation and connection. Similar patterns of genetic similarity would be expected, if the dispersal abilities and recent histories of the species coincide.

The implications of these results for the long-term conservation of *N. oxleyana* are significant. Most of the genetic diversity in the species is found between subpopulations from isolated localities rather than within subpopulations. Therefore, to maximize the preservation of genetic diversity as many subpopulations as possible should be maintained. In addition the large differences between subpopulations indicate that they have been isolated from one another for a considerable period of time. This should be taken into account if any restocking from other subpopulations or from stock bred in aquaria is to be considered. Finally, it is clear that the dispersal abilities of this species are very limited and that isolated subpopulations, once extinct are likely to remain so.

A number of fish and crustaceans have distributions and habitat requirements similar to *N. oxleyana* in south-east Queensland. If they cannot disperse among streams at flood time, as seems to be the case of *N. oxleyana*, it seems likely that the subpopulations in particular streams will be genetically differentiated to a similar extent. We are currently examining two additional heathland fish species, a threatened species and a common widespread species. Both are restricted to fresh water and occur in the same geographical area as *N. oxleyana*. This may shed light on whether the strong structure in *N. oxleyana* is largely a result of small population sizes or whether the limits to dispersal alone are more likely to have led to this pattern.

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Appendix 1

Allele frequencies at six polymorphic allozyme loci in the Pygmy Perch

Locus	Coondoo	Coongul	Searys	Noosa	Marcus	Mellum	Spitfire	Jabiru	Tempest
Pgi-1									
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063
B	1.000	0.929	1.000	0.956	1.000	1.000	1.000	1.000	0.938
C	0.000	0.000	0.000	0.044	0.000	0.000	0.000	0.000	0.000
D	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(N)	29	21	17	34	30	10	31	29	24
Pgm-1									
A	0.000	0.000	0.235	0.000	0.000	0.000	0.000	0.000	0.000
B	1.000	1.000	0.765	0.824	1.000	1.000	1.000	1.000	1.000
C	0.000	0.000	0.000	0.176	0.000	0.000	0.000	0.000	0.000
(N)	29	21	17	34	30	10	31	29	24
Aat-1									
A	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.017	0.345
B	1.000	1.000	1.000	1.000	1.000	1.000	0.972	0.983	0.655
(N)	29	21	17	34	30	10	36	29	29
Ldh-1									
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.138
B	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.862
(N)	29	21	17	34	30	10	36	29	29
β-Est-1									
A	0.879	1.000	1.000	1.000	1.000	0.750	1.000	1.000	1.000
B	0.121	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000
(N)	29	21	17	34	30	10	26	21	14
Adh-1									
A	0.000	0.000	0.000	0.000	0.000	0.000	0.342	0.250	0.368
B	1.000	1.000	1.000	1.000	1.000	1.000	0.658	0.750	0.632
(N)	20	21	17	22	30	10	19	28	19