High levels of genetic structure in the Australian freshwater fish, *Ambassis macleayi*

Joel A. Huey¹

Griffith University, Australian Rivers Institute, Griffith School of Environment, 170 Kessels Road, Nathan,
Queensland, Australia 4111

Andrew M. Baker²

Queensland University of Technology, School of Natural Resource Sciences, 2 George Street, Brisbane,
Queensland, Australia 4001

Jane M. Hughes³

Griffith University, Australian Rivers Institute, Griffith School of Environment, 170 Kessels Road, Nathan,
Queensland, Australia 4111

Abstract. The genetic structure of the freshwater fish *Ambassis macleayi* Castelnau 1878 was explored across the tropical catchments of the Gulf of Carpentaria Basin, northern Australia. The Gulf of Carpentaria provides a unique opportunity to explore simultaneously contemporary and historical gene flow resulting from unique climatic patterns and historical connectivity among catchments via a freshwater lake that existed during lower sea levels. The control region of the mtDNA genome and 4 microsatellite loci were used to detect significant genetic structure among and within catchments. Within catchments, genetic structure suggested that dispersal of *A. macleayi* is restricted, despite high levels of connectivity during summer monsoonal events. Among catchments, divergence appeared to be deeper than what would be predicted based on the last opportunity for connectivity via the lake of Carpentaria (~10,000 years before present [ybp]). Overall, these results have important implications for *A. macleayi* and other members of this genus. If individuals are not proficient dispersers, recolonization after disturbance will be limited. Because of historical isolation among catchments, each catchment harbors unique genetic diversity that should be conserved independently.

Key words: gene flow, genetic structure, Lake Carpentaria, *Ambassis macleayi*, freshwater fish.

The long-term viability of a species is partly determined by its resilience to disturbance. This pattern is especially true for riverine species because populations inhabit highly variable environments that are subject to floods, droughts, glaciers, and human impacts (Lake 2003, Magoulick and Kobza 2003). For freshwater fish and other taxa unable to resist disturbance, dispersal among populations is a common strategy for maintaining population viability at the landscape scale (e.g., Labbe and Fausch 2000). This strategy maintains genetic diversity in populations, facilitates the spread of advantageous alleles, and allows species to recolonize extirpated regions

(Vrijenhoek 1998, Moran 2002). For freshwater fishes, one might expect populations to exchange high numbers of migrants, subsequently homogenizing gene frequencies and generating panmixia at large spatial scales (Huey et al. 2006, Hughes and Hillyer 2006, So et al. 2006). However, the opposite is often observed. Many species of freshwater fish exhibit strong to moderate genetic structure, indicative of restricted gene flow and limited dispersal among populations (Leclerc et al. 2008, Pereira et al. 2009).

Restricted gene flow is often observed in freshwater species because of the isolating nature of riverine systems (Ward et al. 1994, de Woody and Avise 2000, Hughes 2007). Within catchments, in-stream barriers to dispersal, such as waterfalls, dam walls, habitat heterogeneity, dried river reaches, and steep catchment gradients, act to restrict gene flow among

¹ E-mail addresses: j.huey@griffith.edu.au

² am.baker@qut.edu.au

³ jane.hughes@griffith.edu.au

FIG. 1. Lake Carpentaria, Australia. The dark lines represent current coastline and rivers, and the dotted lines represent the -120-m sea level contour with projected river channels (adapted from Voris 2000).

populations (Amoros and Bornette 2002). Among catchments, restricted gene flow is often caused by the inability of individuals to traverse catchment boundaries or to disperse through marine environments between the mouths of exorheic drainages (McGlashan and Hughes 2002, Masci et al. 2008).

Genetic structure among catchments often reflects predictions made under the Death Valley zoogeographic model, whereby populations are totally isolated (no migration) and, consequently, travel down unique evolutionary paths (Meffe and Vrijenhoek 1988). Populations in separate catchments often exhibit deep genetic divergences that reflect historical isolation. This pattern contrasts with genetic structure within catchments that often exhibits restricted gene flow caused by the dendritic nature of riverine systems (Meffe and Vrijenhoek 1988). Identifying the role of these isolating events in driving genetic divergence among populations is central to understanding the nature of within-species genetic variation and is valuable when managing wild fish populations (Meffe and Vrijenhoek 1988, Vrijenhoek 1998, Avise 2000).

Genetic structure among aquatic populations in Australia's Gulf of Carpentaria Basin has been studied for only a few species (Macaranas et al. 1995, McGuigan et al. 2000, de Bruyn et al. 2004, Baker et al. 2008, Huey et al. 2008, Masci et al. 2008), despite its obvious value for exploring the effects of landscape, historical, and biological processes on gene flow and genetic structure in wild populations of freshwater fish. Historically, catchments in the Gulf of Carpentaria Basin and the southern flowing catch-

ments of Papua New Guinea were connected via Lake Carpentaria, a large inland lake that is now a marine gulf (Torgersen et al. 1983, 1988, Chivas et al. 2001) (Fig. 1). Chivas et al. (2001) suggested that this lake existed until ~ 9700 years before present (ybp), when rising sea levels inundated the lake and generated the conditions seen today in the Gulf of Carpentaria. Other estimates indicate that marine waters first entered Lake Carpentaria 12,200 and 10,800 ybp (Reeves et al. 2007, 2008). Many freshwater species have distributions across the Gulf of Carpentaria in both northern Australia and southern Papua New Guinea, a pattern suggesting that Lake Carpentaria historically was a conduit for gene flow for some species. The shift from a freshwater lake to a marine gulf is predicted to be a major driver of genetic structure among populations and has been invoked to explain genetic structure for some crustacean species in the region (de Bruyn et al. 2004, Baker et al. 2008).

One species found across this distribution, *Ambassis macleayi* Castelnau 1878 (Macleay's Glassfish), provides an ideal opportunity to explore the landscape and historical processes important in determining genetic structure in wild fish populations. Studies of this species from the Alligator River and aquarium observations suggest that most recruitment occurs during the dryer winter months (Kennard 1995, Pusey et al. 2004). During this dry period, most surface water is retained in isolated waterholes that become hydrologically connected during the wet monsoon period over summer (Perry 1964, Perry et al. 1964, Twidale 1964a, b). Because connectivity is lost in the dry season, new recruits have fewer opportunities to disperse to other populations, possibly leading to low migration among populations and divergence in gene frequencies.

The control region of mitochondrial deoxyribonucleic acid (mtDNA) and microsatellites were used to investigate the genetic structure of populations of *A. macleayi* among and within catchments in the Gulf of Carpentaria Basin. We predicted that populations of *A. macleayi* would exhibit significant genetic structure among populations within catchments (restricted gene flow) because of the breeding behavior of this species. We also predicted that populations of *A. macleayi* would be highly structured among catchments because of the isolation of catchments by the Gulf of Carpentaria $\sim 10,000$ ybp.

Methods

Study area and sampling

Sites were sampled across the Gulf of Carpentaria Basin during 2 trips (September 2004, August 2005).

TABLE 1. Microsatellite loci developed and used in our study.

Locus	Primer sequence (5'-3')	Microsatellite repeat motif
AMB14	F CCCGCAATTCACATGAGAAGC R CATGGCCAGATTGTCCTTACG	(CA) ₁₁
AMB16	F CTGCCTCTGTGGTGTGTTGAAGC R GATCCATCCAGTCTGCCCTCG	(CA) ₉ AG(CA) ₅
AMB21	F CTGCAGATCAAAGCGAGAACG R GAAGAGGATGAAGGAATGCCG	(CA) ₁₂
AMB22	F CAGAGAAAATCCTCAAATCCCC R CTTCTCTTTGAGCTGTGGCAGG	(TG) ₁₂

5 min, followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 30 s, with a final extension of 72°C for 5 min. The product was then held at 4°C until subsequent analysis. Screening populations involved running denatured PCR product and GeneScan™ 350 TAMRA™ (Applied Biosystems, Scoresby, Australia) size marker through a 5% denaturing acrylamide gel using a GelScan 2000× (Corbett Research, Sydney, Australia). A 1:1 PCR product:dye (5 mg of bromophenol blue, 50 mL of formamide, 100 µL of 0.5 M ethylenediaminetetraacetic acid [EDTA]) mixture was made and denatured by holding the product at 95°C for 5 min and then applying ice. This mixture was then run at 1200 V and scored by eye and with ONE-SCAN software (version 2.03; Scanalytics Inc., Rockville, Maryland). After identification of size, each sample was recorded for use in statistical analysis.

Statistical analysis

Mitochondrial sequences were aligned by eye and with SEQUENCHER (version 4.11; Gene Codes Corporation, Ann Arbor, Michigan). The gene diversity and nucleotide diversity at each site were calculated using ARLEQUIN (version 3.1; Excoffier et al. 2005). The evolutionary relationships between haplotypes were ascertained with TCS (version 1.21; Clement et al. 2000), with 95% parsimoniously plausible branch connections between haplotypes.

For the microsatellite data set, sources of scoring and sampling error were investigated using MICRO-CHECKER (van Oosterhout et al. 2004). MICRO-CHECKER generates expected homozygote and heterozygote allele size frequencies and uses Hardy-Weinberg Equilibrium theory (HWE) to detect deviations from expectations (van Oosterhout et al. 2004). These deviations are then investigated and used as evidence for null alleles, stutter bands, and long allele dropout. Deviations from HWE were tested at each sampled population and locus with exact tests with 10,000 dememorization steps (ARLEQUIN) (Levene 1949, Guo and Thompson 1992). In conjunction with

HWE, F_{IS} values were calculated using GENEPOP (Raymond and Rousset 1995). Average gene diversity across loci was calculated (ARLEQUIN).

Evidence for recent reductions in the effective population sizes of populations was sought in the microsatellite data set and analyzed with the program BOTTLENECK (Piry et al. 1999). BOTTLENECK uses the total number of alleles to estimate expected heterozygosity based upon 3 mutation models, the infinite alleles model (IAM), the 2-phase model (TPM), and the stepwise mutation model (SMM). Then, BOTTLENECK statistically tests the relationship between observed and expected heterozygosity using a Sign test, Wilcoxon sign-rank test, or a standardized differences test with each locus as a replicate (Piry et al. 1999). For our study, the Wilcoxon sign-rank test was used because it provides higher power than the other tests and requires as few as 4 polymorphic loci and 15 individuals (Cornuet and Luikart 1996, Piry et al. 1999). BOTTLENECK also uses a qualitative approach when identifying bottlenecks. Luikart et al. (1998) used simulations to show that the mode of the allele frequency distribution shifted if populations had recently (a few generations) experienced a population bottleneck. In our study, BOTTLENECK was used to calculate the allele frequency distribution and to identify mode shifts qualitatively.

Analysis of Molecular Variance (AMOVA) was used to partition the genetic variation hierarchically among catchments and among sites within catchments (ARLEQUIN). For the mtDNA data set, fixation values were calculated using Φ_{ST} , an F_{ST} analogue that uses sequence divergence among haplotypes when partitioning variation (Excoffier et al. 1992). For the microsatellite data set, the F_{ST} analogue θ was used (Weir and Cockerham 1984). For the microsatellite data set, locus-by-locus AMOVAs also were done. Pairwise F_{ST} tables were calculated for both markers (ARLEQUIN).

For both genetic markers, Mantel tests were calculated to test for significant relationships between geographic and genetic distances (ARLEQUIN). A

significant correlation between geographic and genetic distance can be suggestive of isolation by distance, whereby adjacent populations exchange more migrants than distant populations (Wright 1943). In all cases, Slatkin's Linearized F_{ST} (ARLEQUIN) was used ($F_{ST}/[1 - F_{ST}]$) (Slatkin 1995). These tests were done across the entire sampling area and in each catchment individually. Mantel tests were calculated separately for stream distance and overland distance (derived using Google Earth™) in an attempt to identify the relative importance of floodplain vs channel movement. Stream distances between catchments in the Gulf of Carpentaria Basin were calculated via the Gulf of Carpentaria (along the present-day coastline).

The program IM (Hey and Nielsen 2004) was used to estimate the time since divergence between populations in different catchments. IM uses Bayesian techniques and Markov Chain Monte Carlo (MCMC) algorithms to estimate simultaneously the constant effective population sizes for 2 populations (θ_1 , θ_2), the ancestral constant effective population size prior to the population split (θ_A), gene flow rates per gene copy per generation (m_1 , m_2), and the number of generations ago that the populations split (t) as a function of the mutation rate (μ). Contemporary migration among catchments is unlikely because of the marine Gulf of Carpentaria, so it was assumed that the migration rate between catchments would be 0. Prior distributions for the parameters were specific to each pairwise comparison, and multiple runs of each comparison were made to ensure that the best estimate of divergence was obtained. For each comparison, 10 million steps were used in the chain with a burn-in of 100,000 steps. Once the posterior distribution was obtained,

the bin that yielded the highest residence time was used as the point estimate. An estimate of credibility was obtained by taking the 95% intervals from the posterior distribution.

Only mtDNA was used for the IM analysis because estimates of all parameters are scaled by mutation rate, which is unknown for microsatellite loci. To convert the estimate of mutational time since population divergence (t) to years (t), we applied the equation used in Hey and Nielsen (2004), $t = t\mu$ (or, $t = t/\mu$), where μ is the number of mutations per locus per generation. The mutation rate was derived from a divergence rate of 3.6% per million generations (Donaldson and Wilson 1999). Members of the genus *Ambassis* become sexually mature in 1 y (Pusey et al. 2004), so estimates of generations since population divergence were converted to years since population divergence.

Results

Sampling of *A. macleayi* across the Gulf of Carpentaria Basin yielded 140 individuals, taken from 9 populations and 3 catchments (Nicholson, $n = 72$; Leichhardt, $n = 88$; Norman, $n = 10$) (Fig. 2). Unfortunately, *A. macleayi* was not captured in the Flinders, Gilbert, Staaten, and Mitchell catchments. Using SEQUENCHER, 396-bp fragment of the mtDNA control region was aligned and was used for further analysis (no indels were detected). Screening of the control region revealed only 4 haplotypes (Genbank Accession numbers: GU201600–GU201603, haplotypes A–D), each restricted to a single drainage (Fig. 2). Each site contained only a single haplotype, so haplotype and nucleotide diversity were 0 for each population. The evolutionary relationship between haplotypes suggested that 3 of the 4 haplotypes were all 1 bp

TABLE 2. Gene diversity and F_{IS} at each locus, across populations, based on microsatellites. For F_{IS} values, * indicates that the population significantly deviates from Hardy–Weinberg equilibrium (HWE) at that locus ($p < 0.05$) and mono indicates that the locus was monomorphic in that population.

Catchment	Site	Gene diversity	Microsatellites			
			F_{IS}			
			AMB14	AMB16	AMB21	AMB22
Nicholson	GD1	0.52	0.21	−0.04	0.02	−0.14
	AG	0.57	0.10	Mono	0.44*	−0.04
	KFC	0.42	−0.10	Mono	−0.09	0.34*
Leichhardt	LD	0.22	0.22*	Mono	−0.04	0.17
	MO	0.29	−0.13	Mono	Mono	0.08
	AU	0.46	0.05	−0.17	−0.08	−0.03
	FL	0.53	0.14	−0.02	0.25	0.11
	NA	0.58	0.08	−0.40	−0.06	−0.02
Norman	IF	0.57	0.64	0.18	−0.30	−0.06*

TABLE 3. Results from Analysis of Molecular Variance. Fixation indices that significantly deviate from 0 are marked. KFC = Kingfisher Camp site on the Nicholson River. * = $p < 0.05$, ** = $p < 0.01$.

Source	Control region		Microsatellites	
	% variation	Φ_{ST}	% variation	F_{ST}
Among all catchments	51.14	0.51*	14.08	0.14**
Among populations within catchments	48.86	1.00**	16.01	0.19**
Within populations	0.00	1.00**	69.90	0.30**
Among populations within the Leichhardt catchment		—		0.13**
Among all catchments (no KFC)	100.00	1.00**	20.78	0.21**
Among populations within catchments (no KFC)	0.00	0.00	8.75	0.11**
Within populations (no KFC)	0.00	1.00**	70.47	0.30**

divergent from an extinct or unsampled haplotype (Fig. 2 inset). The haplotype from 1 site (Kingfisher Camp [KFC]) on the Nicholson River was 5 bp divergent from the closest extant haplotype.

Screening of microsatellite loci revealed 7, 5, 14, and 15 alleles across all populations at loci AMB14, 16, 21, and 22, respectively. MICRO-CHECKER identified some evidence for null alleles (3 of 36 tests; data not shown). Four of the tests for HWE revealed populations that deviated significantly from HWE at a locus (Table 2). Three of these populations suffered from heterozygote deficiency (AG at AMB21, KFC at AMB22, and LD at AMB14). Population IF suffered from heterozygote excess at locus AMB22. Microsatellite gene diversity ranged from 0.22 to 0.58. Microsatellite data revealed no quantitative evidence for a recent population bottleneck (data not shown). The mode of the allele distribution for 1 population, FL, was shifted.

AMOVA was done on 2 separate data sets. The first included site KFC in the Nicholson catchment, which was divergent at the mtDNA control region. The second excluded that site (Table 3). Both mtDNA and microsatellite loci showed significant genetic structure among catchments (mtDNA $\Phi_{CT} = 0.51$, microsatellite $F_{CT} = 0.14$). Both markers also showed

genetic structure among populations within catchments (mtDNA $\Phi_{SC} = 1.00$, microsatellite $F_{SC} = 0.19$). mtDNA did not show genetic structure among or within catchments when site KFC was removed from the data set (mtDNA $\Phi_{SC} = 0.00$), but microsatellites showed significant genetic structure among populations within catchments even after site KFC was removed from the data set ($F_{ST} = 0.11$). All microsatellite loci had similar F_{ST} values at each hierarchical level (locus-by-locus AMOVA; Table 4). Pairwise F_{ST} tests based on microsatellite loci were significant for 33 of 36 comparisons (Table 5).

Mantel tests were not done on mtDNA data because of the extremely low levels of diversity. Only 2 significant correlations were found in the microsatellite data set. Genetic and geographic distance (stream and overland distances) were highly positively correlated among all sites (stream distance: $r = 0.65$, $p = 0.004$; overland distance: $r = 0.61$, $p < 0.001$). Neither stream nor overland distance were correlated with genetic distance within the Leichhardt catchment (stream distance: $r = 0.52$, $p = 0.089$; overland distance: $r = 0.58$, $p = 0.091$) (Fig. 3).

Control region mtDNA was used to calculate point estimates of the time since population divergence, t , for each pairwise comparison between catchments.

TABLE 4. Results from locus-by-locus Analysis of Molecular Variance. Fixation indices that deviate significantly from 0 are marked. KFC = Kingfisher Camp site on the Nicholson River. * = $p < 0.05$, ** = $p < 0.01$.

Locus	Among catchments		Within catchments		Within populations	
	% variation	F_{CT}	% variation	F_{SC}	% variation	F_{ST}
AMB14	-2.56	-0.03	30.06	0.29**	72.50	0.28**
AMB16	-2.98	-0.03	17.26	0.17*	85.72	0.14**
AMB21	27.44	0.27*	11.23	0.15**	61.34	0.39**
AMB22	15.75	0.16*	9.03	0.11**	75.22	0.25**
AMB14 (no KFC)	22.57	0.23*	7.89	0.10**	69.53	0.30**
AMB16 (no KFC)	-6.17	-0.06	18.55	0.17**	87.62	0.12**
AMB21 (no KFC)	24.63	0.25*	11.86	0.16**	63.51	0.36**
AMB22 (no KFC)	18.43	0.18	5.35	0.07**	76.22	0.24**

TABLE 5. Pairwise F_{ST} values based on microsatellite data. Fixation indices that deviate significantly from 0 are marked. * = $p < 0.05$. See Fig. 1 for site codes and locations.

	AU	NA	LD	FL	MO	GD1	KFC	AG	IF
AU	0.000								
NA	-0.012	0.000							
LD	0.043	0.192*	0.000						
FL	0.075*	0.060*	0.180*	0.000					
MO	0.038	0.169*	0.066*	0.152*	0.000				
GD1	0.254*	0.161*	0.420*	0.139*	0.358*	0.000			
KFC	0.335*	0.286*	0.438*	0.211*	0.401*	0.318*	0.000		
AG	0.261*	0.196*	0.465*	0.115*	0.363*	0.083*	0.218*	0.000	
IF	0.263*	0.224*	0.448*	0.203*	0.318*	0.225*	0.352*	0.164*	0.000

The Nicholson catchment was divided into 2 sub-catchments, the Nicholson River proper (represented by site KFC) and the Gregory River (represented by sites AG and GD1). This action generated 6 pairwise combinations (Table 6) and revealed 2 groups of estimates. Estimates for the group that included the Nicholson River ranged from 266.5 to 378.8 thousand ybp, and estimates for the other group ranged from 109.4 to 120.6 thousand ybp.

Discussion

Genetic diversity

Four of the 36 tests for HWE deviated significantly from expectations. Significant tests were not consistent across loci or populations, and MICRO-CHECKER showed limited evidence for null alleles or mis-scored data. Therefore, it is unlikely that deviations

from HWE were the product of selection, population specific processes (e.g., the Wahlund effect), poor gel scoring, or PCR-based errors. Control-region variation was extremely low, and a single haplotype was fixed at each site. In addition, a single haplotype was fixed at all sites in the Leichhardt catchment. This result is unusual for the control region, with other studies typically revealing higher genetic variation at this region (Stepien and Faber 1998, Hughes et al. 1999, Krieg et al. 2000, Ikeda et al. 2003, Takagi et al. 2006, Huey et al. 2008).

Low genetic diversity can be caused by selection or population processes, such as historical bottlenecks and low effective population size (Nei et al. 1975). The control region of the mtDNA genome is noncoding, so it is assumed to be free of selective constraint (Ballard and Kreitman 1995, Ballard and Dean 2001). However, because the mtDNA genome is not subjected to

FIG. 3. Scatterplot correlating genetic distance (Slatkin's Linearized F_{ST}) and geographic distance (km) using stream and straight-line distances within the Leichhardt catchment. Lines represent the line of best fit through the data points.

TABLE 6. Results of IM analysis (Hey and Nielsen 2004) showing the maximum likelihood point estimate (MLE) of the time since population divergence (t) and the 95% credibility intervals (CI).

Comparison	MLE t	0.025 CI	0.975 CI
Nicholson vs Gregory	361,953	70,146	934,343
Nicholson vs Leichhardt	378,788	70,146	934,343
Nicolson vs Norman	266,554	53,311	765,993
Gregory vs Leichhardt	109,428	30,864	485,410
Gregory vs Norman	115,039	30,864	491,021
Leichhardt vs Norman	120,651	30,864	491,021

recombination, selection on genes elsewhere on the genome will affect all other genes. This process, known as genetic hitchhiking (Maynard Smith and Haigh 1974), might explain the observed low diversity in the mtDNA data set. Unfortunately, neutrality tests, which can identify selection and bottlenecks in the mtDNA data set (Tajima 1989a, b, Fu 1997), were not done because of a lack of diversity within sites. Therefore, it is difficult to say whether selection can explain the low diversity in the mtDNA data set.

Population bottlenecks typically affect most loci across the genome, and therefore, can be verified by investigating different loci (Piry et al. 1999). Microsatellite loci revealed no substantial evidence for a population bottleneck, a result suggesting that a sudden reduction in population size does not explain the low variation at the control region of the mtDNA genome. However, the program BOTTLENECK suffers from low power when few loci are used (Cornuet and Luikart 1996, Luikart and Cornuet 1998).

Gene flow and genetic structure within catchments

Unfortunately, multiple sites within catchments were obtained only for the Leichhardt (5 sites) and Nicholson (3 sites) catchments. Nevertheless, significant genetic structure among populations within catchments was detected with microsatellite loci ($F_{SC} = 0.19$). Furthermore, this pattern was retained after removing the highly divergent KFC site in the Nicholson catchment ($F_{SC} = 0.11$). This pattern was not caused by data from only a few sites; 7 of 10 pairwise F_{ST} values in the Leichhardt catchment (the only catchment with adequate spatial sampling) were significant. This result indicates that gene flow among populations of *A. macleayi* is restricted within the Leichhardt catchment, despite monsoonal events that cause widespread flooding and hydrological connectivity across this catchment.

The breeding behavior of *A. macleayi* is a likely explanation for low levels of gene flow among

populations within catchments. Studies of *A. macleayi* suggest that most recruitment occurs in the dry winter months (Kennard 1995, Pusey et al. 2004) when hydrological inputs are minimal and individuals residing in disconnected refugia would be unable to move between adjacent populations. Therefore, populations would be isolated when population sizes were at their maximum (i.e., post-spawning). When connectivity returned, populations would have decreased in size because of mortality in the new generation. Low levels of gene flow among populations within catchments also contradicts the suggestion that *A. macleayi* migrates upstream during the early wet season in response to changes in habitat availability (Bishop et al. 1995) because migration of this nature would reduce genetic divergence among populations.

Our suggestion that breeding behavior, rather than physical barriers, is responsible for restricting gene flow among populations in *A. macleayi* is supported by studies of other Australian freshwater fishes that have different breeding strategies. For example, low levels of genetic structure have been observed among populations within catchments in studies of *Neosilurus hyrtlui*, *Porochilus argenteus*, and *Nematalosa erebi* in the Lake Eyre Basin (Huey et al. 2006, 2008, Hughes and Hillyer 2006). For these species, peak spawning occurs during the wet summer months, thereby increasing the likelihood that recruitment will occur during periods of summer flow and high connectivity.

If populations of *A. macleayi* adhere to the stepping-stone model of genetic structure, our detection of restricted gene flow among subpopulations suggests that adjacent subpopulations should be more genetically similar than distant subpopulations (isolation by distance; Wright 1943). Mantel tests correlating genetic and geographic distance within the Leichhardt catchment were not significant and did not support this hypothesis. Despite the nonsignificant Mantel test results, the scatterplot comparing genetic and geographic distance suggests some weak evidence for an isolation by distance effect. If populations remain in migration-drift equilibrium, populations might soon exhibit a significant correlation between genetic and geographic distance. Migration-drift equilibrium is attained when the antagonistic forces of genetic drift and gene flow are maintained at steady rates for an extended period of time and strike a balance whereby F_{ST} does not increase with time (Boileau et al. 1992, Slatkin 1993, Efremov 2004).

Closer inspection of the scatterplot correlating genetic and geographic distance in the Leichhardt

catchment (Fig. 3) revealed another possible explanation for the lack of evidence for isolation by distance. Three pairwise comparisons (Augustus Downs [AU] with Moondarra Dam [MO], East Leichhardt Dam [LD], and Nardoo [NA]) had much less genetic divergence than predicted by the line of best fit through the scatterplot. The population from AD might be the product of a recent colonization event and might not be in migration–drift equilibrium with other populations in the catchment. As such, the AD population would not be expected to exhibit isolation by distance. Instead, pairwise comparisons would show less divergence than expected, as observed in our data set. Reanalysis with this population removed was not pursued because only 4 populations would have remained in the data set.

Historical connectivity among catchments

Excluding the highly divergent site at KFC in the Nicholson catchment, populations in catchments in the Gulf of Carpentaria were each fixed for a single unique haplotype, with 2 bp separating each haplotype. The lack of shared haplotypes among populations in separate catchments indicates allopatric fragmentation and total isolation among catchments because even low levels of gene flow would cause some sharing of haplotypes. Furthermore, the lack of shared haplotypes suggests an old vicariant event because genetic drift has had time to remove shared haplotypes from populations. Microsatellite data supported this finding and revealed significant structure among catchments (all sites: $F_{CT} = 0.14$, KFC removed: $F_{CT} = 0.21$).

Overall, the phylogeographic history of this locus appears to be simple. Each haplotype is found in a single catchment without evidence for long distance colonizations, range expansions, and other complex historical events. This result contrasts markedly with the findings of Cook and Hughes (2010), in which a complex gene tree for a freshwater species across northern Australia suggests a complex phylogeographic history dominated by combinations of long-distance dispersal, historical retention of haplotypes, vicariance, and range expansions.

Pairwise estimates of time since population divergence could be split into 2 groups, those involving the divergent population in the Nicholson River (KFC), and the rest. Point estimates between the Nicholson River and the rest of the Gulf of Carpentaria Basin ranged from 266 to 378 thousand ybp. Other point estimates ranged from 109 to 120 thousand ybp. The 95% credibility intervals were all older than the predicted time that Lake Carpentaria became saline,

~10 thousand ybp (Chivas et al. 2001, Reeves et al. 2007, 2008). Therefore, populations of *A. macleayi* in each catchment probably became isolated much earlier, possibly because of a different historical event.

The observation of highly restricted gene flow among populations within catchments provides a possible explanation for the disparity between estimates of time since divergence among catchments and the estimate for the most recent connectivity event when Lake Carpentaria was fresh. Because populations of *A. macleayi* currently exhibit high genetic structure among populations within catchments, it is likely that populations in separate catchments had already begun to diverge when Lake Carpentaria was fresh. For this species, Lake Carpentaria was rarely used as a conduit for gene flow among catchments, despite available hydrological connectivity. The distribution of this species, from northern Australia to the trans-Fly region of Papua New Guinea (Pusey et al. 2004), indicates that perhaps Lake Carpentaria was responsible for rare dispersal events, although probably much longer than 10,000 ybp.

Another explanation for the disparity between estimates of time since divergence among catchments and the estimate for the most recent connectivity event is the appropriateness of the mutation rate used to calibrate the estimate. Some authors have suggested that using rates derived from interspecific divergence to calibrate estimates for intraspecific divergence might be inappropriate (Ho et al. 2005, Ho and Larson 2006; but see Emerson 2007). For example, estimates of the divergence rate of the control region based on recently diverged populations are much higher than the rates used in our study (23.3%/million y, BurrIDGE et al. 2006 vs 3.2%/million y, Donaldson and Wilson 1999). If the faster rate were used, estimates of time since population divergence might overlap with the estimate for the most recent connectivity among catchments via the Lake of Carpentaria.

The divergence between the Kingfisher Camp site and the rest of the Nicholson catchment clearly is large considering that these sites are all found within a single contemporary, hydrologically connected catchment. It is important to note that the population at this site was declared highly divergent based on microsatellite loci (pairwise F_{ST} : range = 0.211–0.438, mean = 0.320). Thus, this divergence probably is not an anomaly unique to the mtDNA data set. Drainage rearrangements frequently are invoked to explain higher levels of divergence among fish populations within catchments than among catchments (McGlashan and Hughes 2000, 2001, Waters and Wallis 2000,

Poissant et al. 2005). Drainage rearrangements can be caused by river capture when a river steals the headwaters of another via unequal rates of erosion or uplift or when rearrangement of river deltas causes rivers to coalesce before they reach the ocean (Bishop 1995, Waters and Wallis 2000, Hughes et al. 2009). In the case of the Nicholson catchment, the Nicholson and Gregory rivers might have historically reached the Gulf/Lake Carpentaria independently, and populations in each would have been diverging in isolation. Then, after populations in each catchment had diverged, drainage rearrangements could have caused the rivers to merge before reaching the Gulf of Carpentaria, and restricted gene flow could have allowed each river to remain fixed for different haplotypes.

In another study of genetic structure in freshwater fish populations from this region, Masci et al. (2008) sampled mtDNA in *Nematalosa erebi* from the divergent KFC site. They found that the population at the KFC site was not deeply divergent from populations in other catchments in the Gulf of Carpentaria Basin. Population genetic studies on *N. erebi* suggest that it is a more proficient disperser than *A. macleayi* (Hughes and Hillyer 2006, Masci et al. 2008). Thus, the historical signature might have been lost because of recent gene flow. Alternatively, the result from the study by Masci et al. (2008) might have arisen from a rare, intercatchment dispersal event during an intermittent freshwater connection from a more divergent catchment west of the Nicholson catchment. This hypothesis has been proposed for a similarly confusing result for a freshwater crayfish, *Cherax quadricarinatus*, in this region (Baker et al. 2008).

Results from previous studies in the Gulf of Carpentaria Basin highlight the role of Lake Carpentaria in driving genetic structure in freshwater taxa (de Bruyn et al. 2004, Baker et al. 2008). In 3 studies of freshwater crustaceans (*Macrobrachium rosenbergii*, *Macrobrachium australiensis*, and *C. quadricarinatus*), mtDNA sequences showed shallow divergences among catchments, with many haplotypes shared between sites in distant catchments (de Bruyn et al. 2004, Baker et al. 2008, Masci et al. 2008). The same result also was found in 2 freshwater fishes (*Neosilurus hyrtlui* and *N. erebi*; Huey et al. 2008, Masci et al. 2008).

The results of these studies differ from ours in which genetic divergence among catchments was pronounced with no shared haplotypes and 2 bp separating haplotypes in each catchment. In the only study to date divergences between catchments that historically flowed into Lake Carpentaria, Huey et al. (2008) estimated that most populations of *N. hyrtlui* in

separate catchments began diverging before Lake Carpentaria became saline. Thus, despite showing shallow divergences between catchments, the taxa described above also might have begun to diverge before the lake became saline. Alternatively, the lake might have last become saline earlier than predicted by Chivas et al. (2001). For example, Unmack (2001) suggested that it is improbable that Lake Carpentaria provided an effective migration route within the last 10,000 y.

Overall, our study revealed high genetic structure among populations, both within and among catchments. This result has 2 important implications: 1) if populations of *A. macleayi* were to become locally extinct, many generations might be required for individuals from extant populations to recolonize extirpated regions; and 2) if *A. macleayi* were to be lost totally from a catchment, valuable and unique genetic divergence would be lost, and human intervention would be required for the species to recolonize the catchment.

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