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Genetic structure of *Melanotaenia australis* at local and regional scales in the east Kimberley, Western Australia

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The Kimberley region of Western Australia possesses a poorly studied freshwater fish fauna with high endemism in an aquatic landscape subject to monsoonal floods and dry season isolation. In the first population genetic study of freshwater fish in this region, the authors tested the effects of geographic barriers on genetic structure at multiple spatial scales in east Kimberley populations of the western rainbowfish, *Melanotaenia australis*, the most widespread and abundant species in the region. Based on allozyme comparisons, hierarchical analysis of F_{ST} revealed increasing genetic subdivision with spatial scale. Minimal genetic structure within creeklines demonstrated that wet season dispersal, rather than dry season isolation, determines genetic structure at small scales. At the scale of sub-catchments, a pattern of isolation by distance along creeklines was evident. Genetic subdivision between adjacent river systems was greater between rivers separated by a plateau than by lowlands. This implies greater connectivity of populations in lowland areas and may explain the greater similarity of the east Kimberly freshwater fish fauna with lowlands to the east than with the more rugged regions to the west. Similarly, greater connectivity between lowland populations may account for the on-average larger distribution of lowland Melanotaeniids.

Key words: allozymes; freshwater fish; genetic subdivision; Kimberley; *Melanotaenia*; western rainbowfish.

INTRODUCTION

For species tied to fresh water for their entire life cycle, the physical structure of freshwater systems and their limitations to dispersal play a critical role in shaping patterns of population genetics, distribution and ultimately speciation. Freshwater habitat provides a contrast between the opportunities for connectivity at broad scales with often fine-scale genetic variation because of geographic barriers within watercourses. A stream hierarchy model has been proposed, where the pattern of genetic variation between populations reflects the hierarchical branching structure of river systems (Meffe & Vrijenhoek,

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1988). While this model has been supported in some cases, departures have been detected because of historical drainage rearrangements (Hurwood & Hughes, 1998; McGlashan & Hughes, 2001; Burridge *et al.*, 2007; Thacker *et al.*, 2007) and within-stream barriers such as waterfalls (Currens *et al.*, 1990; McGlashan & Hughes, 2000; Costello *et al.*, 2003; Wofford *et al.*, 2005) and man-made dams (Leclerc *et al.*, 2008). It is likely that each geographical region will exhibit its own unique effects on population genetic structure through a combination of local geological, hydrological and ecological processes.

Inferring the effects of physical barriers on the connectivity of fish populations is important for resolving evolutionary and ecological processes. In Australia, biogeographic analyses have established the dominant role of drainage boundaries and isolation through aridity in controlling the distribution and speciation in freshwater fish (Unmack, 2001). Phylogeographic and population genetic approaches are widely employed to understand the fine-scale processes underpinning these broader patterns. While regional scale phylogeographic studies have been concentrated on the Great Dividing Range and the inland waters of eastern Australia, it is evident that the degree of conformity to the stream hierarchy model reflects a combination of the ecology of the species and the geology of the area under investigation.

The Great Dividing Range is a major barrier between the coastal and the inland freshwater faunas (Unmack, 2001). Studies of several species from the eastern slopes of the Great Dividing Range have revealed movement of haplotypes between drainages, most likely through drainage rearrangement (Hurwood & Hughes, 1998; McGlashan & Hughes, 2001; McGlashan *et al.*, 2001). Similarly, movement of haplotypes has been detected across the range (Thacker *et al.*, 2007). Investigations of desert species have revealed large genetic differences between drainages but small yet significant differences within drainages (Hughes & Hillyer, 2006). The freshwater fish of the tropical north coast of Australia remain poorly studied. The river systems of this region contain coastal floodplains but are well defined further inland. Watercourses are subjected to regular, intense wet season flooding and dry season drought. The combination of these characteristics suggests that these river systems will impose constraints on the movements and population genetics of the freshwater fauna that are unique on the Australian continent.

The Kimberley region is the most remote part of Australia's north coast. In the western and central Kimberley, river systems run along well-defined courses, often through gorges dissecting the bedrock that are unlikely to be subject to drainage rearrangements by the mechanisms described by Bishop (1995). The eastern Kimberley has extensive lowlands subject to flooding but with well-defined river systems further inland. The region contains a similarly high diversity of species compared with other tropical regions in Australia (Unmack, 2001) but is noteworthy for its high level of endemism and outlying populations of more eastern species (Allen & Leggett, 1990; Unmack, 2001). The effects of the Kimberley environment on the ecology and genetics of freshwater fish and the processes responsible for the high degree of endemism remain unexamined. The present study of population genetics of a widespread species subject to a variety of geographic barriers will provide the first step in understanding these processes.

The western rainbowfish, *Melanotaenia australis* Castelnau is one of the most common and widespread freshwater fish on Australia's north coast. It ranges from the Ashburton River in the Pilbara region of Western Australia (Merrick & Schmida, 1984) to the Adelaide River in the Northern Territory (Bishop *et al.*, 2001). Rainbowfishes (Melanotaeniidae) are an abundant and diverse family of obligate freshwater fishes native to tropical Australasia (Allen & Cross, 1982; Allen *et al.*, 2002), with 67 described taxa (McGuigan, 2001; Allen *et al.*, 2002). The Melanotaeniidae is characterized by local endemism, with many species restricted to single drainage systems, especially in New Guinea (Allen & Cross, 1982; Allen, 1991). Despite a larger area of river systems and greater longitudinal and latitudinal range of the family, comparatively few species occur in Australia (Allen & Cross, 1982; Allen *et al.*, 2000, 2002). *Melanotaenia* is the most species-rich genus and contains numerous species with restricted or fragmented distributions (Allen, 1991). Given the high levels of local endemism in *Melanotaenia* and the Kimberley freshwater fish fauna, the large distribution of *M. australis* is contrary to expectation.

High local endemism in Australian freshwater fishes is strongly associated with prevalent geographic barriers limiting dispersal (Unmack, 2001). Conversely, the pattern of local endemism in *Melanotaenia* occurs despite several attributes of this genus that suggest a high dispersal capability compared with many other Australian freshwater fish. Females are highly fecund (Ivantsoff *et al.*, 1988; Pusey *et al.*, 2001), and both sexes reach maturity rapidly (Bishop *et al.*, 2001). The lowland *Melanotaenia* of Northern Australia spawn year round, with peak activity during the wet season (Larson & Martin, 1989; Bishop *et al.*, 2001). Coinciding with the peak in reproductive activity, populations in low-lying areas undertake extensive lateral and upstream migrations (Larson & Martin, 1989; Bishop *et al.*, 1995). Based on these attributes, it can be predicted that genetic structure will be relatively weak within drainages but pronounced between drainages. The phylogeographic study of *Rhadinocentrus ornatus* Regan, confirmed the potential for evolution of genetically distinct lineages in neighbouring river systems (Page *et al.*, 2004). However, the study of Hurwood & Hughes (2001) on *Melanotaenia splendida* Peters, revealed dispersal between isolated headwaters of adjoining drainages.

By investigating a widespread species subject to a variety of geographic barriers, the authors aim to determine the genetic structure imposed by Kimberley freshwater habitats on freshwater fish at multiple spatial scales. Given that the river environments can impose similar population genetic structure on sympatric species (Tibbets & Dowling, 1996), these results may be applicable to other Kimberley freshwater fish. Furthermore, a detailed study of the population genetics of a *Melanotaenia* may offer insight into the mechanisms behind the radiation of this genus.

MATERIALS AND METHODS

STUDY SITE

Investigation of smaller scale patterns of genetic subdivision was undertaken in the headwaters of the Chamberlain River, at Kachana Station in the east Kimberley

(16°30′; 129°47′). Outlying sites were sampled at the Dunham River (16°10′; 128°22′), the Keep River (15°24′; 129°04′) and a minor tributary of the Keep River (15°24′; 129°04′) *c.* 400 m from the Keep River site (Fig. 1). Although Kachana Station contains the headwaters of both the Chamberlain and the Dunham Rivers, they run unlinked until they enter salt water at Cambridge Gulf. The Keep River serves as a further outlier, which joins the ocean 100 km east of Cambridge Gulf. The topography of inland areas is characterized by river systems dissecting ancient plateaus, while coastal areas are of comparatively low topographic relief.

The creeklines at Kachana Station typically consist of pools 1–2 m in depth, separated by stretches of dry or shallow creekline unsuitable for *M. australis*. Pools are linked each year from monsoonal cyclonic rainfall events that result in brief but torrential flows. Creeklines are sufficiently steep sided that water flows rapidly rather than flooding surrounding areas. The distribution of these pools generally becomes increasingly fragmented further upstream. Cockatoo Creek has only recently become a permanent watercourse, as a result of revegetation work commencing in 1994, combined with a sequence of wet years.

SAMPLING DESIGN AND SPECIMEN COLLECTION

Sampling was undertaken in late May 2004, early in the dry season. Twenty sites were selected, so that four spatial scales were sampled: pools within creeklines, creeklines within sub-catchments, sub-catchments within a river system and between river systems (Fig. 1). Six creeklines were sampled from within two sub-catchments at Kachana Station. Where possible, three pools >500 m apart were sampled per creekline. A single site in the northern sub-catchment was located at the crest of an escarpment, separated from adjacent populations by a series of waterfalls up to 15 m high (Kachana Escarpment site). This provided an indication of the effectiveness of major barriers within creeklines in creating genetic structuring. Subdivision at a regional scale was tested with sites at the Dunham and Keep Rivers. A maximum of 50 fish per pool were collected using a 10 × 1 m seine with 6 mm mesh.

ALLOZYME ELECTROPHORESIS

Upon capture, specimens were stored in liquid nitrogen before being transferred to a −80° C freezer. In previous studies, allozymes have shown a suitable level of genetic variation for investigations of population genetics of freshwater fish (Shaw *et al.*, 1994; Tibbets & Dowling, 1996; Hughes *et al.*, 1999) and were selected as the molecular marker. Genetic variation was examined using horizontal starch-gel electrophoresis. Twelve enzymes representing 16 gene loci were examined as detailed in Table I. Five loci had readily scorable variation and were examined in all populations: glucose-6-phosphate isomerase (*GPI-1*), leucyl-proline peptidase (*PEP-LP*), phosphoglucomutase (*PGM*), phosphogluconate dehydrogenase (*PGD*) and superoxide dismutase (*SOD*).

ANALYSIS OF ALLOZYME DATA

Genetic analysis was conducted using GENEPOP (version 3.4; Raymond & Rousset, 1995). All loci at all sites were tested for conformity to Hardy–Weinberg equilibrium using exact tests. Genetic subdivision between sites was measured using Wright's (1978) F_{ST}. F_{ST} was averaged across all loci using the method of Weir & Cockerham (1984) and tested for significance differentiation between populations using log-likelihood-based exact tests (Goudet *et al.*, 1996). F_{ST} was analysed in a hierarchical fashion based on spatial scale. Initially, F_{ST} was calculated within each creekline. Samples within each creekline were then pooled for analysis of the subdivision between creeklines within each sub-catchment. As the analysis proceeded to higher spatial scales, pooling was conducted so that equal numbers of individuals were included from the replicates at the smaller

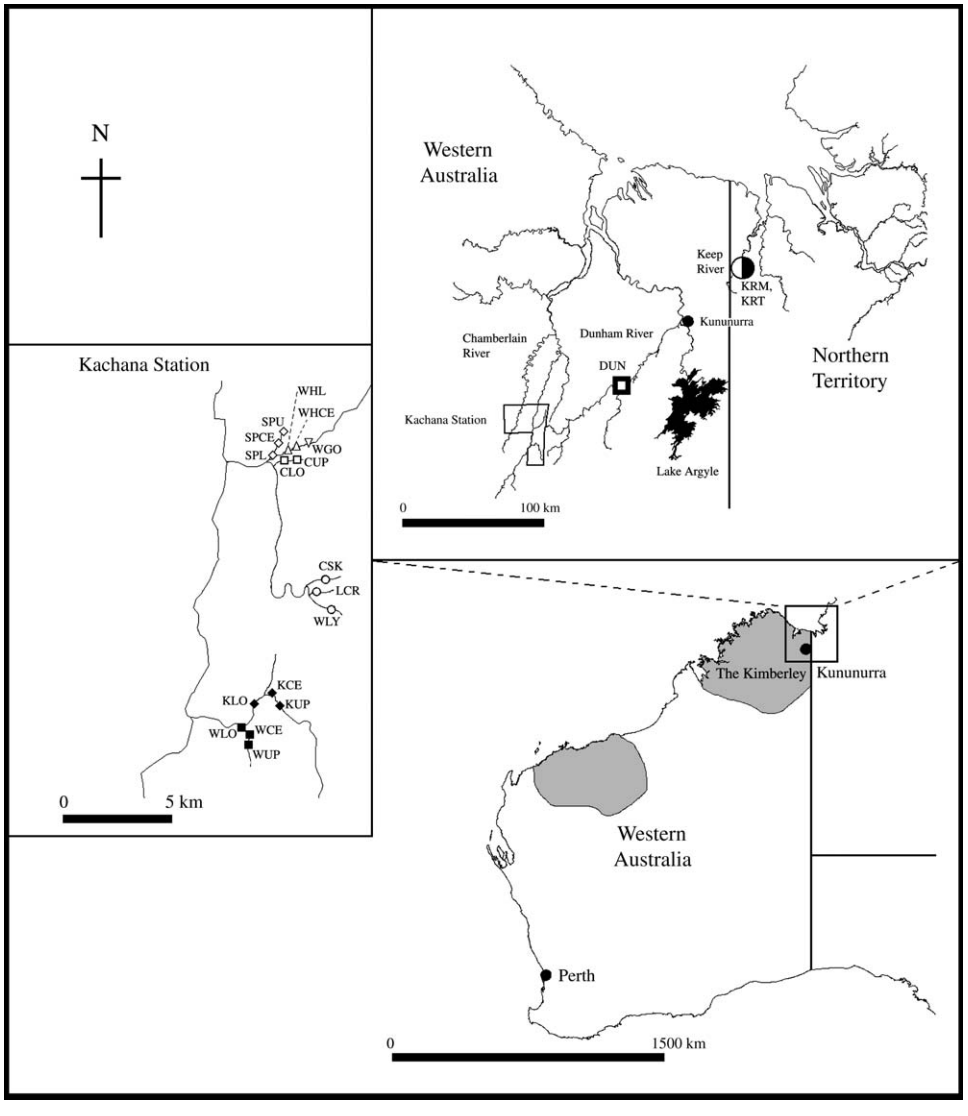

FIG. 1. Layout of sites where *Melanotaenia australis* was collected. Within Kachana Station, the open symbols represent the northern sub-catchment and the closed symbols represent the southern sub-catchment. Shaded area is the Western Australian distribution of *M. australis*. ◇, Sandpit; △, Wanjamia; ▽, Escarpment; □, Cockatoo; ○, Central branch; ◆, Kachana Creek; ■, Weiner; ◑, Keep River sites; ◘, Dunham River sites. CLO, Cockatoo Creek lower Gorge; CSK, Cleanskin Creek; CUP, Cockatoo Creek upper; DUN, Dunham River; KCE, Kachana Creek central; KLO, Kachana Creek lower; KRM, Keep River, main channel; KRT, Keep River, tributary; KUP, Kachana Creek upper; LCR, Lee Creek; SPCE, Sandpit Creek central; SPL, Sandpit Creek Lower; SPU, Sandpit Creek upper; WCE, Weiner Creek central; WGO, Wanjamia; WHCE, Wanjamia Creek central; WHL, Wanjamia Creek lower; WLO, Weiner Creek lower; WLY, Wallaby Creek; WUP, Weiner Creek upper.

TABLE I. Enzymes assayed for polymorphism for a population genetic study of *Melanotaenia australis* in the east Kimberley. Loci in boldface were used in the population genetic study. Tissue used: L, liver; M, muscle. Variation: M, monomorphic; P, polymorphic

Enzyme	Locus	Buffer	Tissue used	Variation
Adenylate kinase (EC 2.7.4.3)	*AK*	TC6	M, L	M
Aspartate aminotransferase (EC 2.6.1.1)	*AAT-1*	TC8	M	P
	AAT-2		M	M
Creatine kinase (EC 2.7.3.2)	*CK*	TC6	M, L	M
Glucose-6-phosphate isomerase (EC 5.3.1.9)	***GPI-1***	LiOH	L	P
	GPI-2		L	M
Isocitrate dehydrogenase (EC 1.1.1.42)	*IDH-1*	TM	L	P
	IDH-2		M	M
Lactate dehydrogenase (EC 1.1.1.27)	*LDH-1*	TEB	L, M	M
Malate dehydrogenase (EC 1.1.1.37)	*MDH-1*	TC8	L, M	M
	MDH-2		L, M	M
Leucyl-proline peptidase (3.4.-.-)	***PEP-LP***	LiOH	L	P
Valyl-leucine peptidase (3.4.-.-)	*PEP-VL*	LiOH	L	P
Phosphoglucomutase (EC 5.4.2.2)	***PGM***	TM	L, M	P
Phosphogluconate dehydrogenase (EC 1.1.1.44)	***PGD***	TM	L, M	P
Superoxide dismutase (EC 1.15.1.1)	***SOD***	TEB	L, M	P

spatial scale. This avoids biasing the values of F_{ST}, should differences in allele frequencies exist at the smaller spatial scales. For each calculation of F_{ST}, an estimate of the variance was made using the jackknife procedure (Reynolds *et al.*, 1983). Multidimensional scaling (MDS) of the matrix of pair-wise F_{ST}, calculated in SYSTAT 11, was used to show the pattern of genetic subdivision in two dimensions.

Values of pair-wise F_{ST} were used to test for the effect of isolation by distance at small spatial scales. Distances were calculated from a 1:50 000 topographic map scanned into ArcView 3.4. The distances between sites were measured as direct distances and as indirect distances along creeklines. The significance of this correlation of genetic distance and geographic distance was determined using Mantel's (1967) test in GENEPOP (randomizations = 1000). The strength of the relationships provided evidence of the relative importance of within-stream *v.* between-stream dispersal in creating the pattern of genetic structure. Geographically outlying sites were not included because the magnitude of the geographic distance could mask the relationship at smaller scales. Pair-wise F_{ST} values were calculated for outlying sites to compare the effects of different geographic barriers on genetic subdivision.

Allelic diversity tends to be reduced during periods of low effective population size (genetic bottlenecks), making it a useful indicator of restricted gene flow between neighbouring populations. Levels of allelic diversity, measured as expected heterozygosity (Nei, 1978), were investigated as a further test for isolation within creeklines. To test for restricted gene flow in the most upstream pools, a paired Student's *t*-test was conducted on allelic diversity between the most downstream and the upstream pools of each creekline.

The effect of pool size on allelic diversity was tested using regression analysis in Statview 4.0. A positive relationship would be evidence of restricted gene flow because gene flow is insufficient to prevent loss of variation because of small population size in the smaller pools. Prior to the analysis of pool size and allelic diversity, the level of allelic diversity was standardized by dividing the observed allelic diversity by the average for

each sub-catchment, giving each sub-catchment a mean of 1. This removes the effect that historical processes may have had on the background level of allelic diversity. This analysis was performed for all pools and for upstream pools only.

RESULTS

ALLOZYMES

After sequential Bonferroni correction ($P < 0{\cdot}05/120$) (Rice, 1989), there were no departures from Hardy–Weinberg expectations. Within the east Kimberley, differences in allelic frequency were pronounced between the Kachana Station and the Keep and Dunham River sites at three loci (Table II). Differences in allelic frequency at the *SOD* locus between the Kachana Station and the Keep and Dunham Rivers were considerable. Similarly, the common *PGD*c* allele at the Keep and Dunham Rivers was absent from the Kachana populations, while the Dunham and Keep River populations lacked *PGM*a*, which was widespread within Kachana Station. With the exception of the *SOD* locus, allelic frequencies of the most abundant allele showed no obvious pattern within Kachana Station. For *SOD*, the average (±S.E.) frequency of the most abundant allele in the northern sub-catchment (Central, Cockatoo, Wanjamia and Sandpit Creeks) was $0{\cdot}95 \pm 0{\cdot}01$ compared with $0{\cdot}72 \pm 0{\cdot}04$ in the southern sub-catchment (Weiner and Kachana Creeks). The frequency of this allele at the escarpment site (0·64) was more similar to those in the southern sub-catchment. Uncommon alleles were shared widely between populations (Table II).

Hierarchical analysis of F_{ST} demonstrated pronounced genetic subdivision between river systems within the east Kimberley (Table III). When subdivision between rivers was considered on a pair-wise basis, the river systems separated by an escarpment (Chamberlain and Dunham) rather by lowlands (Dunham and Keep) exhibited much higher genetic subdivision (Table IV).

Within Kachana Station, F_{ST} was generally low, but significant genetic subdivision occurred at all spatial scales (Table III). At the finest scale of within creeklines, subdivision was low but significant in one of six occasions at $P < 0{\cdot}05$ (Table III). Values of F_{ST} were also low in comparisons between creeklines, and significant differentiation was found only in the southern sub-catchment ($P < 0{\cdot}05$). F_{ST} was considerably higher between sub-catchments, largely because of a major difference in allelic frequencies at the *SOD* locus (Table II). The waterfall in Wanjamia Creek had the most pronounced effect on the extent of genetic subdivision within Kachana Station. The value of F_{ST} across this barrier was three times as high as that between sub-catchments (Table IV). The low stress (0·095) and high R^2 value (0·996) demonstrate that the MDS provided a close approximation of the distances in the original F_{ST} matrix and that the plot explains a large proportion of the variance. The MDS plot illustrated the closer relationship of sites within sub-catchments and the outlying nature of the escarpment site (Fig. 2). Despite its location in the northern sub-catchment, the escarpment population was more similar to those in the southern sub-catchment.

The escarpment site was excluded from the analysis of isolation by distance because of the genetic distinctiveness (Table II) and physical separation of this

TABLE II. Allelic frequencies, allelic diversity (A) and standardized allelic diversity (AS) for populations of *Melanotaenia australis*. *n*, number of individuals sampled. CLO, Cockatoo Creek lower Gorge; CSK, Cleanskin Creek; CUP, Cockatoo Creek upper; DUN, Dunham River; KCE, Kachana Creek central; KLO, Kachana Creek lower; KRM, Keep River, main channel; KRT, Keep River, tributary; KUP, Kachana Creek upper; LCR, Lee Creek; SPCE, Sandpit Creek central; SPL, Sandpit Creek Lower; SPU, Sandpit Creek upper; WCE, Weiner Creek central; WGO, Wanjamia; WHCE, Wanjamia Creek central; WHL, Wanjamia Creek lower; WLO, Weiner Creek lower; WLY, Wallaby Creek; WUP, Weiner Creek upper

Locus		KRM	KRT	DUN	WLO	WCE	WUP	KUP	KCE	KLO	CLO	CUP	WHCE	WHL	WGO	SPU	SPCE	SPL	CSK	LCR	WLY
GPI-1	*a*	0·92	0·81	0·69	0·79	0·75	0·69	0·65	0·71	0·77	0·79	0·79	0·77	0·74	0·89	0·67	0·81	0·78	0·76	0·81	0·72
	b	0·08	0·19	0·29	0·21	0·25	0·31	0·35	0·29	0·23	0·21	0·21	0·22	0·26	0·11	0·32	0·19	0·22	0·24	0·19	0·28
PEP-LP	*a*	0	0	0	0·03	0	0	0	0·01	0·01	0	0	0	0	0	0	0	0	0	0	0·01
	b	0·42	0·41	0·54	0·29	0·42	0·26	0·35	0·10	0·26	0·29	0·17	0·47	0·29	0·26	0·27	0·32	0·26	0·27	0·33	0·28
	c	0·58	0·59	0·46	0·68	0·58	0·74	0·65	0·80	0·73	0·71	0·82	0·52	0·71	0·74	0·73	0·67	0·73	0·73	0·67	0·71
PGD	*a*	0·84	0·74	0·64	1·00	1·00	0·99	0·99	0·99	1·00	0·99	0·99	0·97	0·98	1·00	1·00	0·95	0·98	0·98	1·00	0·98
	b	0·02	0	0·04	0	0	0·01	0·01	0	0	0·01	0·01	0·02	0·03	0	0	0·05	0·02	0·02	0	0·02
	c	0·13	0·26	0·32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	d	0	0	0	0	0	0	0	0·01	0	0	0	0	0	0	0	0	0	0	0	0
	e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PGM	*a*	0	0	0	0·05	0·03	0·01	0·01	0·01	0·01	0	0	0	0	0·02	0	0	0	0	0	0
	b	1	1	0·92	0·82	0·75	0·84	0·85	0·86	0·98	0·89	0·94	0·89	0·86	0·98	0·84	0·80	0·87	0·86	0·87	0·79
	c	0	0	0·08	0·13	0·22	0·15	0·14	0·13	0·01	0·11	0·06	0·11	0·14	0	0·16	0·2	0·13	0·14	0·13	0·21
SOD	*a*	0	0·01	0·18	0·79	0·80	0·84	0·59	0·64	0·68	0·96	0·95	0·90	0·95	0·64	0·98	0·94	0·91	0·98	0·94	0·99
	b	1	0·99	0·82	0·21	0·20	0·16	0·41	0·36	0·32	0·04	0·05	0·1	0·05	0·36	0·02	0·06	0·09	0·02	0·06	0·01
A		0·19	0·24	0·38	0·29	0·32	0·28	0·34	0·30	0·25	0·21	0·17	0·26	0·24	0·22	0·23	0·26	0·24	0·22	0·22	0·25
A_S		—	—	—	0·97	1·09	0·95	1·15	1·00	0·85	0·92	0·76	1·12	1·04	—	1·02	1·12	1·03	0·96	0·95	1·08
n		45	48	44	39	32	40	40	40	44	40	40	40	40	44	44	40	45	44	43	41

TABLE III. Hierarchical analysis of F_{ST} with spatial scale of east Kimberley populations of *Melanotaenia australis*. Small-scale investigations took place in the headwaters of the Chamberlain River within Kachana Station. Outlying sites were sampled at the Dunham and Keep Rivers. F_{ST} was averaged across loci using the method of Weir & Cockerham (1984) and tested for significance using the method of Goudet *et al.* (1996): $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$

	Southern Creeks		Northern Creeks				Within sub-cathcments				
Locus	Weiner	Kachana	Central	Cockatoo	Wanjamia	Sandpit	South	North	Sub-cathcments	Rivers	Regions
GPI-1	0·004	0·007	0·000	−0·011	−0·008	0·013	0·003	−0·004	0·003	0·034***	0·122***
PEP-LP	0·014	0·017	−0·010	0·020	0·060*	−0·010	−0·003	0·007*	−0·002	0·046***	—
PGD	−0·002	−0·002	0·000	−0·013	−0·019	0·016	0·000	−0·002	0·009**	0·352***	0·762***
PGM	0·002	0·042**	0·002	0·002	−0·008	−0·003	0·035**	0	0·000	0·062***	0·242***
SOD	0·000	−0·004	0·008	−0·010	0·004	0·010	0·048**	0	0·180***	0·708***	0·027
Mean	0·004	0·014*	−0·003	0·003	0·019	0·002	0·017**	0·001	0·035***	0·335***	0·472***
S.D.	0·003	0·004	0·003	0·006	0·014	0·004	0·008	0·002	0·021	0·134	0·272

TABLE IV. Genetic subdivision of *Melanotaenia australis* associated with differing geographic barriers in the east Kimberley. Genetic subdivision measured using pair-wise F_{ST} ± S.D. All F_{ST} values are significant at the $P < 0{\cdot}001$ level of significance

Locations	Barrier	Direct distance (km)	Indirect distance (km)	F_{ST}
Keep River–Chamberlain River	Plateau + lowlands	180	380	0·376 ± 0·161
Keep River–Dunham River	Lowlands	113	395	0·060 ± 0·015
Chamberlain River–Dunham River	Plateau + lowlands	70	360	0·301 ± 0·102
North Kachana–South Kachana	Adjacent sub-catchments	11	25	0·035 ± 0·021
Wanjamia lower–Wanjamia escarpment	Waterfall	1·3	1·4	0·089 ± 0·032

site. Within Kachana (excluding the escarpment population), pair-wise F_{ST} was positively associated with direct and indirect (creekline) distance, with indirect distance proving the more accurate model (direct distance $P < 0{\cdot}005$, $R^2 = 0{\cdot}123$; indirect distance $P < 0{\cdot}005$, $R^2 = 0{\cdot}250$).

Allelic diversity showed no significant difference between upstream and downstream pools [lower mean = 0·24 ± 0·01 (S.E.), upper mean = 0·26 ± 0·03, d.f. = 4, $P > 0{\cdot}05$]. Standardized allelic diversity was not significantly related to pool size (all pools: $R = 0{\cdot}17$, $R^2 = 0{\cdot}03$, d.f. = 15, $F = 0{\cdot}46$, $P = 0{\cdot}51$; upstream pools: $R = 0{\cdot}54$, $R^2 = 0{\cdot}29$, d.f. = 7, $F = 2{\cdot}43$, $P = 0{\cdot}17$).

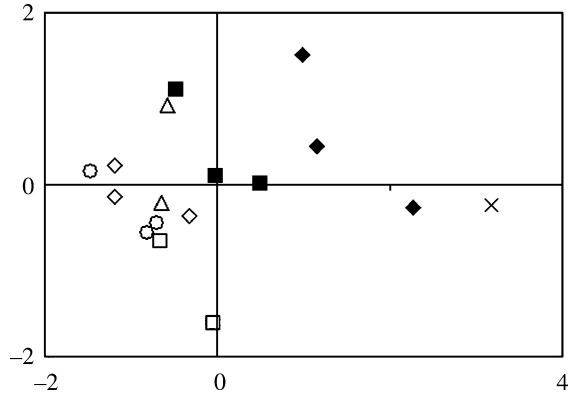

FIG. 2. Multidimensional scaling plot of genetic subdivision (pair-wise F_{ST}) of *Melanotaenia australis* within Kachana Station (stress = 0·095, $R^2 = 0{\cdot}966$). Closed symbols are sites in the northern sub-catchment, open symbols are sites in the southern sub-catchment. ◇, Sandpit; △, Wanjamia; ×, Escarpment; □, Cockatoo; ○, Central branch; ◆, Kachana; ■, Weiner.

DISCUSSION

POPULATION GENETICS IN THE KIMBERLEY

Based on the physical structure of their environment, genetic subdivision in freshwater fish is expected to increase in a hierarchical fashion, reflecting the branching pattern of the drainage system (Meffe & Vrijenhoek, 1988). This prediction has been supported in several studies (Shaw *et al.*, 1994; Tibbets & Dowling, 1996; Hughes *et al.*, 1999; Johnson, 2001). In contrast, studies undertaken in Australia's Great Dividing Range have revealed cases of dispersal across geological divides through drainage rearrangement, leading to extensive genetic variation within contemporary drainages (Hurwood & Hughes, 1998, 2001; McGlashan & Hughes, 2001; Thacker *et al.*, 2007). The east Kimberley populations of *M. australis* conform to the hierarchical model predicted by Meffe & Vrijenhoek (1988). Genetic subdivision is generally not significant at small spatial scales, low but significant between adjacent sub-catchments and considerable between adjacent rivers. This hierarchical pattern of genetic structure was attributable to isolation by distance, with distance along creeklines being the superior model. The conformity to the stream hierarchy model reflects the geological stability of the study region and the paucity of within-stream barriers at Kachana Station. Among other conditions, drainage rearrangement requires a geologically active environment or rivers capable of lateral movement (Bishop, 1995). The Kimberley does not meet either of these criteria, and consequently, a hierarchical pattern of genetic structure is likely to hold in many Kimberley species.

This is the first study of the population genetics of a freshwater species in the Kimberley. The pattern of genetic subdivision within Kachana Station demonstrates the effect of these watercourses on the genetic structure of populations. The almost complete absence of genetic subdivision within creeklines indicates that wet season dispersal, rather than dry season isolation of populations, is the dominant force underlying patterns of genetic structure at the scale of creeklines. While pools are separated for much of the year by sections of dry creekbed, dispersal across patches of unsuitable habitat must be extensive. This is supported by the observation that *M. australis* has been able to recolonize Cockatoo Creek after it recommenced flowing in response to revegetation work. In contrast, flooding in the headwaters of drainages appears to have a minimal role in creating dispersal between creeklines. In these regions, wet season runoff flows rapidly down watercourses, rather than flooding expansive areas.

The relative importance of geographic barriers in creating genetic subdivision provides some insight into patterns of distribution and speciation in Kimberley freshwater fish. Despite similar geographic distances between the river systems, F_{ST} between the Chamberlain and the Dunham River systems is high (0·301) compared with that between the Dunham and the Keep rivers (0·060). This suggests the importance of the plateaus separating the Dunham and Chamberlain sites in creating subdivision compared with the lowlands separating the Dunham and Keep Rivers. Low levels of genetic subdivision between adjacent lowland sites imply greater connectivity between populations and suitable habitat and offer an

explanation for the east Kimberley fish fauna having more in common with low-lying sites to the east than with the more rugged northern and western Kimberley (Unmack, 2001).

Based on the effect of plateaus in creating genetic subdivision detected in the present study, the ancient plateaus of the northern and central Kimberley are predicted to cause highly subdivided species through isolation between adjacent river systems. The strong isolation may account for the restricted distributions of numerous species of freshwater fish in the Kimberley (Allen *et al.*, 2002) and the high level of endemism in the region (Allen & Leggett, 1990; Allen *et al.*, 2002). Given the common effect of geography on creating genetic subdivision (Tibbets & Dowling, 1996), it is anticipated that other freshwater species will show extensive genetic subdivision within the Kimberley. Such subdivision may well be larger than that encountered in *M. australis* because this species is considered to have relatively high dispersal ability compared with co-occurring species (Ivantsoff *et al.*, 1988; Larson & Martin, 1989; Bishop *et al.*, 1995). Of particular interest would be genetic analysis of freshwater fish from the eastern and western parts of the north Kimberley, given the biogeographic division in freshwater fish fauna identified by Unmack (2001). As has occurred in several other genetic studies of Australian fish, this may reveal species of freshwater fauna yet to be recognized on the basis of morphology (Crowley *et al.*, 1986; Jerry & Woodland, 1997; McGuigan, 2001; Bostock *et al.*, 2006).

IMPLICATIONS FOR POPULATION GENETICS IN FRESHWATER FISHES

Analysis of allelic diversity and the distribution of allele frequencies in *M. australis* provided no evidence of consistent isolation of upstream populations. Despite a patchy distribution of pools in the upper reaches of creeklines, allelic diversity was not lower compared with downstream sites. Furthermore, smaller pools showed no evidence of a reduction in allelic diversity through small effective population size. This confirms evidence from the analysis of genetic subdivision that dispersal, even between upstream pools, is a regular occurrence. Some previous studies have reported reduced levels of heterozygosity in upstream pools (Ryman & Stahl, 1981; Shaw *et al.*, 1994; McGlashan *et al.*, 2001; Wofford *et al.*, 2005) because of the comparatively small population size and within-stream barriers. Comparison of the data with these results suggests that reduced allelic diversity as a result of isolation is enhanced in populations subjected to prominent within-stream barriers.

Although the escarpment population in this study showed no reduction in allelic diversity, the waterfall was associated with significant genetic subdivision when compared with subdivision within the remainder of Kachana Station. This supports several examples of the importance of within-stream barriers in creating genetic subdivision (Currens *et al.*, 1990; Hurwood & Hughes, 1998; McGlashan & Hughes, 2000; Matsubara *et al.*, 2001; Costello *et al.*, 2003; Leclerc *et al.*, 2008). Differences in allele frequency across the waterfall may assist in maintaining genetic diversity further down the creek through one-way gene flow (Hänfling & Weetman, 2006). Thus, while movement

between peripheral pools results in low genetic subdivision and normal levels of diversity, major barriers can have significant effects at local scales.

SPECIATION IN *MELANOTAENIA*

Examining the population genetics of a widespread species affords the opportunity to investigate the role of a variety of geographic barriers in creating genetic divergence between populations and their role in speciation. The Melanotaeniidae occur at a range of altitudes and distances from the coast (Allen, 1991; Allen *et al.*, 2002), necessitating the investigation of both coastal and inland geographic barriers. In the east Kimberley, *M. australis* exhibited low levels of genetic subdivision between drainages separated by coastal lowlands compared with plateaus. Such an effect may explain the pattern where distributions of *Melanotaenia* inhabiting lowland areas are on average considerably larger (Allen, 1991). Despite melanotaeniids possessing characteristics associated with high dispersal ability (Ivantsoff *et al.*, 1988; Larson & Martin, 1989; Bishop *et al.*, 1995), dispersal in upstream species must be considerably restricted between drainages, resulting in poor ability for species to colonize new areas or recolonize after a local extinction. Restricted dispersal may also lead to allopatric speciation between geographically close but effectively reproductively isolated populations.

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References

Allen, G. R. (1991). *Field Guide to the Fishes of New Guinea*. Papua New Guinea: Publication No. **9**. Madang: Christensen Research Institute.

Allen, G. R. & Cross, N. J. (1982). *Rainbow Fishes of Australia and Papua New Guinea*. Sydney: Angus and Robertson.

Allen, G. R. & Leggett, R. (1990). A collection of freshwater fishes from the Kimberley region of Western Australia. *Records of the Western Australian Museum* **14,** 527–545.

Allen, G. R., Hortle, K. G. & Renyaan, S. J. (2000). *Freshwater Fishes of the Timika Region New Guinea*. Timika: PT Freeport Indonesia.

Allen, G. R., Midgley, S. H. & Allen, M. (2002). *Field Guide to the Freshwater Fishes of Australia*. Perth: Western Australian Museum.

Bishop, P. (1995). Drainage rearrangement by river capture, beheading and diversion. *Progress in Physical Geography* **19,** 449–473.

Bishop, K. A., Pidgeon, R. W. J. & Walden, D. J. (1995). Studies on fish movement dynamics in a tropical floodplain river: prerequisites for a procedure to monitor the impacts of mining. *Australian Journal of Ecology* **20,** 81–107.

Bishop, K. A., Allen, S. A., Pollard, D. A. & Cook, M. G. (2001). Ecological studies on the freshwater fishes of the Alligator Rivers Region, Northern Territory: autecology. *Supervising Scientist Report* **145**. Canberra: Environment Australia.

Bostock, B. M., Adams, M., Laurenson, L. J. B. & Austin, C. M. (2006). The molecular systematics of *Leioptherapon unicolour* (Gunther, 1859): testing for cryptic speciation in Australia's most widespread freshwater fish. *Biological Journal of the Linnean Society* **87,** 537–552.

Burridge, C. P., Craw, D. & Waters, J. M. (2007). An empirical test of freshwater vicariance via river capture. *Molecular Ecology* **16,** 1883–1895.

Costello, A. B., Down, T. E., Pollard, S. M., Pacas, C. J. & Taylor, E. B. (2003). The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in Bull trout, *Salvelinus confluentus*. *Evolution* **57,** 328–344.

Crowley, L. E. L. M., Ivantsoff, I. & Allen, G. R. (1986). Taxonomic position of the two crimson spotted rainbowfish *Melanotaenia duboulayi* and *Melanotaenia fluvatalis* (Pisces: Melanotaeniidae), from eastern Australia, with special reference to their early life history stages. *Australian Journal of Marine and Freshwater Research* **37,** 385–398.

Currens, K. P., Schreck, C. B. & Li, H. W. (1990). Allozyme and morphological divergence of rainbow trout (*Oncorhyncus mykiss*) above and below waterfalls in the Deschutes River, Oregon. *Copeia* **1990,** 730–746.

Goudet, J., Raymond, M., De Meeüs, T. & Rousset, F. (1996). Testing differentiation in diploid populations. *Genetics* **144,** 1933–1940.

Hänfling, B. & Weetman, D. (2006). Concordant genetic estimators of migration reveal anthropogenically enhanced source-sink population structure in the River Sculpin, *Cottus gobio*. *Genetics* **173,** 1487–1501.

Hughes, J. M. & Hillyer, M. J. (2006). Mitochondrial DNA and allozymes reveal high dispersal abilities and historical movement across drainage boundaries in two species of freshwater fishes from inland rivers in Queensland, Australia. *Journal of Fish Biology* **68,** 270–291.

Hughes, J. M., Ponniah, M., Hurwood, D., Chenoweth, S. & Arthington, A. (1999). Strong genetic structuring in a habitat specialist, the Oxleyan Pygmy Perch *Nannoperca oxleyana*. *Heredity* **83,** 5–14.

Hurwood, D. A. & Hughes, J. M. (1998). Phylogeography of the freshwater fish, *Mogurnda adspersa*, in streams of northeastern Queensland, Australia: evidence for altered drainage patterns. *Molecular Ecology* **7,** 1507–1517.

Hurwood, D. A. & Hughes, J. M. (2001). Historical interdrainage dispersal of eastern rainbowfish from the Atherton Tableland, north-eastern Australia. *Journal of Fish Biology* **58,** 1125–1136.

Ivantsoff, W., Crowley, L. E. L. M., Howe, E. & Semple, G. (1988). *Biology and Early Development of Eight Fish Species from the Alligator Rivers Region. Technical Memorandum* **22**. Canberra: Australian Government Publishing Service.

Jerry, D. R. & Woodland, D. J. (1997). Electrophoretic evidence for the presence of the undescribed 'Bellinger' catfish (*Tandanus* sp.) (Teleostei: Plotosidae) in four New South Wales mid-northern coastal rivers. *Marine and Freshwater Research* **48,** 235–240.

Johnson, J. B. (2001). Hierarchical organisation of genetic variation in the Costa Rican live bearing fish *Brachyrhaphis rhabdophora* (Poecilidea). *Biological Journal of the Linnean Society* **72,** 519–527.

Larson, H. K. & Martin, K. C. (1989). *Freshwater Fishes of the Northern Territory*. Darwin: Northern Territory Museum of Arts and Sciences.

Leclerc, E., Mailhot, Y., Mingelbier, M. & Bernatchez, L. (2008). The landscape genetics of yellow perch (*Perca flavescens*) in a large fluvial ecosystem. *Molecular Ecology* **17,** 1702–1717.

Mantel, N. (1967). The detection of disease clustering and a generalised regression approach. *Cancer Research* **27,** 209–220.

Matsubara, H., Sakai, H. & Akihisa, I. (2001). A river metapopulation structure of a Japenese freshwater goby, *Odontobutis obscura*, deduced from allozyme genetic indices. *Environmental Biology of Fishes* **61,** 285–294.

McGlashan, D. J. & Hughes, J. M. (2000). Reconciling patterns of genetic variation with stream structure, earth history and biology in the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae). *Molecular Ecology* **9,** 1737–1751.

McGlashan, D. J. & Hughes, J. M. (2001). Low levels of genetic differentiation among populations of the freshwater fish *Hypseleotris compressa* (Gobiidae: Eleotridinae): implications for its biology, population connectivity and history. *Heredity* **86,** 222–233.
McGlashan, D. J., Hughes, J. M. & Bunn, S. E. (2001). Within-drainage population genetic structure of the freshwater fish *Pseudomugil signifer* (Pseudomugilidae) in northern Australia. *Canadian Journal of Fisheries and Aquatic Sciences* **58,** 1842–1852.
McGuigan, K. L. (2001). An addition to the rainbowfish (Melanotaeniidae) fauna of north Queensland. *Memoirs of the Queensland Museum* **46,** 647–655.
Meffe, G. K. & Vrijenhoek, R. C. (1988). Conservation genetics in the management of desert fishes. *Conservation Biology* **2,** 157–169.
Merrick, J. R. & Schmida, G. E. (1984). *Australian Freshwater Fishes: Biology and Management*. Netley: Griffin Press Limited.
Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89,** 583–590.
Page, T. J., Sharmam, S. & Hughes, J. M. (2004). Deep phylogenetic structure has conservation implications for ornate rainbowfish (Melanotaeniidae: *Rhadinocentrus ornatus*). *Marine and Freshwater Research* **55,** 165–172.
Pusey, B. J., Arthington, A. H., Bird, J. R. & Close, P. G. (2001). Reproduction in three species of rainbowfish (Melanotaeniidae) from rainforest streams in northern Queensland, Australia. *Ecology of Freshwater Fish* **10,** 75–87.
Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86,** 248–249.
Reynolds, J., Weir, B. S. & Cockerham, C. C. (1983). Estimation of the coancestory coefficient: basis for a short term genetic distance. *Genetics* **105,** 767–779.
Rice, W. R. (1989). Analysing tables of statistical tests. *Evolution* **43,** 223–225.
Richardson, B. J., Baverstock, P. R. & Adams, M. (1986). *Allozyme Electrophoresis*. Sydney: Academic Press.
Ryman, N. & Stahl, G. (1981). Genetic perspectives of the identification and conservation of Scandanavian stocks of fish. *Canadian Journal of Fisheries and Aquatics Sciences* **38,** 1562–1575.
Shaw, P. W., Carvalho, G. R., Magurran, A. E. & Seghers, B. H. (1994). Factors affecting the distribution of genetic variability in the guppy, *Poecilia reticulata*. *Journal of Fish Biology* **45,** 875–888.
Thacker, C. E., Unmack, P. J., Matsu, L. & Rifenbark, N. (2007). Comparative phylogeography of five sympatric *Hypseleotris* species (Teleostei: Eleotridae) in south-eastern Australia reveals a complex pattern of drainage basin exchanges with little congruence across species. *Journal of Biogeography* **34,** 1518–1533.
Tibbets, C. A. & Dowling, T. E. (1996). Effects if intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae). *Evolution* **50,** 1280–1292.
Unmack, P. J. (2001). Biogeography of Australian freshwater fishes. *Journal of Biogeography* **28,** 1053–1089.
Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38,** 1358–1370.
Wofford, J. E. B., Gresswell, R. E. & Banks, M. A. (2005). Influence of barriers to movement on within-watershed genetic variation of coastal cutthroat trout. *Ecological Applications* **15,** 628–637.
Wright, S. (1978). *Evolution and the Genetics of Populations: Variability Within and Among Natural Populations*, **4**. Chicago: The University of Chicago Press.

