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Genetic and Morphological Differences between Populations of the Western Minnow, *Galaxias occidentalis*, from Two River Systems in South-western Australia

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Abstract. Allozyme electrophoresis was used to examine patterns of genetic differentiation in the western minnow, *Galaxias occidentalis*, from the North Dandalup and Canning Rivers in south-western Australia. Two distinct genetic forms of this species were identified in these rivers and both forms occur sympatrically in samples from two sites. A significant deficit of heterozygotes and a non-random association of alleles among loci was observed in these two samples. The distribution of one of the genetic forms extended from the headwater streams in the Darling Range to the transition zone between the ranges and the Swan Coastal Plain. With the exception of one population, the distribution of the other form extended from the transition zone to the Swan Coastal Plain. The exception was a population that was caught from a site in the ranges but grouped with the populations from the coastal plain. Discriminant analyses of meristic and morphological data were undertaken to determine if morphological differences exist between the two identified genetic groups. All individuals were correctly classified by the analyses of samples from each catchment separately and of the two sympatric populations separately. However, the discriminant analysis of individuals from all sites correctly classified only 85·7% of the Darling Range genetic group and 79·4% of the Swan Coastal Plain genetic group. Further research is required to clarify the taxonomic status of these two genetic forms of *G. occidentalis*.

Introduction

The western minnow, *Galaxias occidentalis* Ogilby (Teleostei:Galaxiidae), is one of the most abundant species of native freshwater fish in south-western Australia and is endemic to the region. It inhabits streams, swamps and lakes over a distance of approximately 500 km, from Two Peoples Bay (34°57′S,118°11′E), near Albany, to Moora (30°39′S,116°00′E), north of Perth (Allen 1982). *G. occidentalis* occurs in the westward flowing rivers that have their headwaters in the Darling Range (Pusey *et al.* 1989). These rivers flow through jarrah forest before descending a steep escarpment and crossing the Swan Coastal Plain. There are considerable physical and biological differences between the stream habitats on the Darling Range and Swan Coastal Plain (Storey *et al.* 1990).

Most species in the family Galaxiidae spend their entire lives in fresh water, but several species, including *G. maculatus* and *G. truttaceus* from eastern Australia, have a marine larval stage (McDowall and Frankenberg 1981; Humphries 1989). It is thought that *G. occidentalis* undergoes an upstream migration to spawn on vegetation in upper reaches (McDowall and Frankenberg 1981; Merrick and Schmida 1984; Pen and Potter 1991). The strongest support for this hypothesis comes from a study by Pen and Potter (1991), who examined gonadosomatic data and seasonal changes in density of *G. occidentalis* from the Collie River, Western Australia. Pen and Potter (1991) found that soon after the tributaries of the river began to flow, the density of *G. occidentalis* in them greatly increased. In addition, the majority of the females caught in these tributaries had gravid, spawning or spent ovaries. From these observations Pen and Potter (1991) concluded that *G. occidentalis* from this river system moves into ephemeral tributaries to breed.

The absence of a marine larval stage in the life history of *G. occidentalis* may limit dispersal between populations from different river systems. The degree of gene exchange among populations of this species may also be limited by environmental factors. For example, physical barriers, such as waterfalls, may limit the degree of gene exchange among populations and may provide opportunities for genetic divergence to occur among populations.

A preliminary study of the genetic structure of *G. occidentalis* revealed significant differences between populations from the Darling Range and Swan Coastal Plain (Edward *et al.*, unpublished data). The current study reports the findings of the preliminary study and extends the work by combining genetic and morphometric analyses, with the specific objectives of assessing *(i)* the extent of genetic differentiation between populations of *G. occidentalis* from

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the Darling Ranges and the Swan Coastal Plain and *(ii)* whether morphological differences exist between the two genetic groups recognized in the electrophoretic study.

Materials and Methods

Field Collections

Samples of *G. occidentalis* were collected from sites in the North Dandalup and Canning Rivers during the period August to October 1987. Fish were collected by seine and hand-nets. The seine (9 mm mesh) was placed across the stream and fish were driven downstream into the net over a 50–100 m reach. Submerged vegetation, logs and large rocks within the reach were swept with hand-nets. Fish were placed on dry ice and were stored at –70°C on return to the laboratory.

Samples were collected from five sites in the North Dandalup River: two on the Darling Range (ND3, ND5), two on the Swan Coastal Plain (ND9, ND10), and one in the transition zone between the range and the coastal plain (ND6) (Fig. 1). Four sites in the Canning River were sampled: three on the Darling Range (CD5, LC1, LC2) and one site on the Swan Coastal Plain (LC7) (Fig. 1). As sites ND9 and ND10 are separated by only a few kilometres and the sizes of these samples were small, these samples were pooled. Similarly, samples from sites LC1 and LC2 in the Canning River were pooled. Site codes are the same as those used by Storey *et al.* (1990).

Electrophoresis

The standard length of each fish was measured with Vernier calipers and a piece of liver and muscle was dissected from each fish. A small portion of each liver and muscle sample was homogenized in a 10% sucrose solution buffered with Tris-HCl (pH 8) containing 0.1% (v/v) mercaptoethanol and 0.1% (w/v) bromophenol blue. The remainders of the tissue samples were stored in individual vials at –70°C and the fish were fixed for three days in 2% formaldehyde and stored in 70% alcohol.

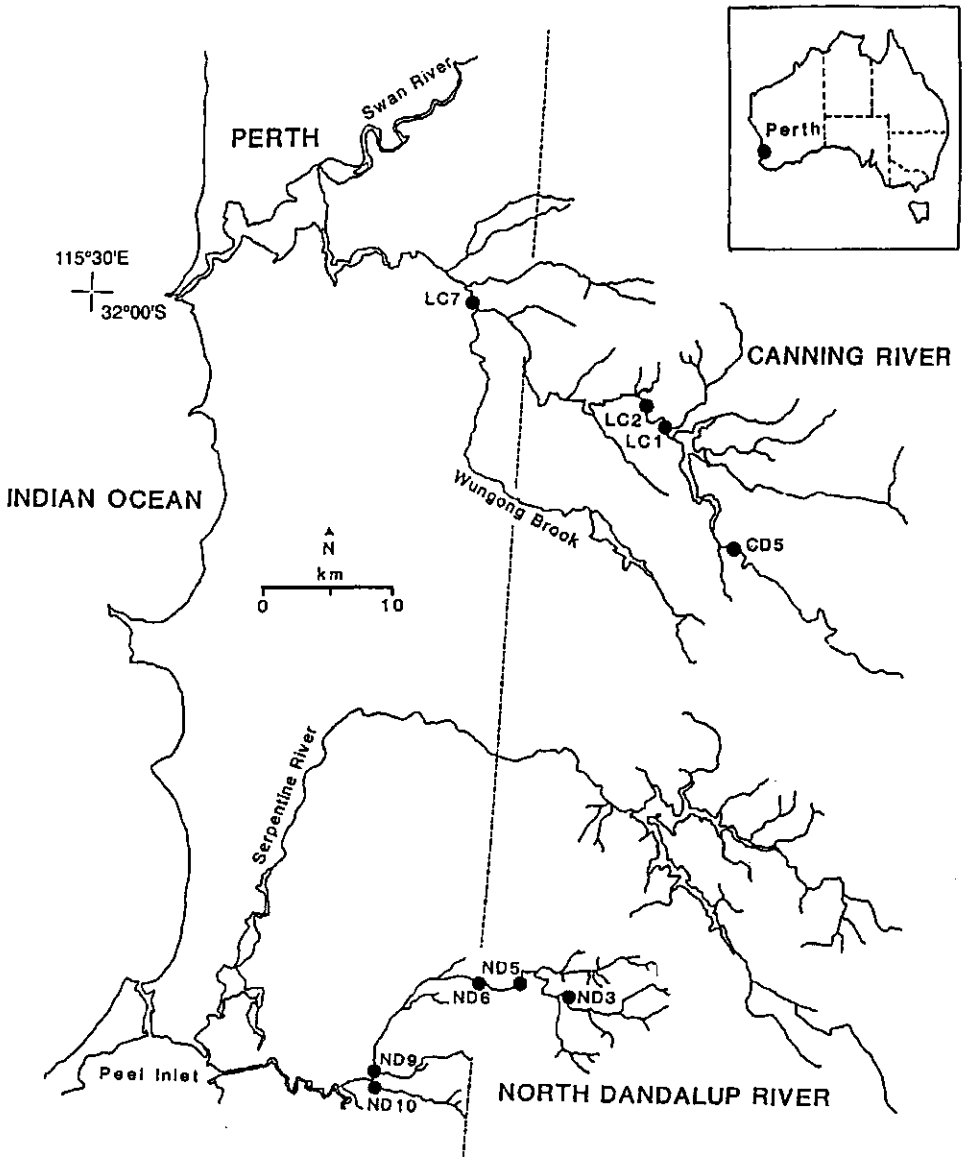

Fig. 1. Location of sampling sites for *Galaxias occidentalis*. The dotted line indicates the western limit of the Darling Range.

Extracts were subjected to electrophoresis in horizontal starch gels (4·8% Sigma and 7·2% BDH starch) by the technique of Brewer (1970). Seventeen enzymes were screened for genetic variation (Table 1), using 11 individuals from each of Samples ND6, LC7, CD5, ND3 and ND9. Four polymorphic enzymes, which could be scored consistently, were chosen for further analysis: glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 5.4.2.2), tripeptide aminopeptidase (identified by using L-leucyl-glycyl-glycine substrate) (PEPB, EC 3.4.-.-), and peptidase-C (identified by using L-valyl-leucine substrate) (PEPC, EC 3.4.-.-). The glycolytic enzymes, GPI and PGM, were subjected to electrophoresis on Tris-maleate buffer (pH 7.4) (Buffer 17 of Shaw and Prasad 1970) and the peptidases, PEPB and PEPC, were run on a lithium hydroxide buffer (Buffer 10 of Shaw and Prasad 1970). Locus nomenclature follows Shaklee *et al.* (1990). Alleles at each locus were labelled alphabetically, in order of decreasing mobility of their respective allozymes.

Genetic Analysis

Allelic frequencies were calculated for each population at each of the four polymorphic loci. Goodness-of-fit χ^2 analyses were used to compare observed genotype frequencies with those expected under Hardy–Weinberg equilibrium. For cases where there were small expected values, χ^2 tests were performed with pooled homozygote and pooled heterozygote classes. Tests were not performed if more than 20% of the cells had expected values less than four. Departures of observed heterozygosity (H_o) from expected heterozygosity (H_e) were expressed as $D = (H_o - H_e)/H_e$. A negative value of D indicates fewer heterozygotes than expected in a randomly breeding population.

Allozymic differentiation among pairs of populations was measured by using Nei's (1978) unbiased genetic distance from the statistical package BIOSYS. Since the estimates of genetic distance were based on data from only four polymorphic loci, they will not be compared with values for other species based on random samples of loci but will be used in this study solely to quantify divergences at the four loci. The genetic distance data were summarized in a cluster analysis using unweighted pair-group analysis (UPGMA; Sneath and Sokal 1973).

Contingency χ^2 tests were used to compare the genetic composition within and among populations. In cases where the tests involved multi-allelic loci and where the expected values of some genotypic classes were small, rare genotypes were pooled. Contingency χ^2 tests were not performed if more than 20% of the cells had expected values less than four.

Analysis of Morphometric and Meristic Data

Morphological measurements and meristic counts were made on all fish from Samples ND3 and ND6, which were the samples shown to contain both of the two different genetic forms of *G. occidentalis*. Several individuals from these samples had been damaged during dissection and were not included in the morphometric analysis. Seven fish from each of the remaining samples (ND5, ND9/10, CD5, LC1/2 and LC7) were randomly selected for morphometric analysis.

Thirteen measurements or counts were recorded for each fish: number of pectoral fin rays, number of anal fin rays, standard length, body depth at anus, length of caudal peduncle, depth of caudal peduncle, distance from posterior edge of anal fin to anus, head length, diameter of eye, length of upper jaw, length of lower jaw, distance from tip of snout to anterior margin of eye, and length of the base of anal fin. All measurements were made with Vernier calipers to the nearest 0.1 mm. The fin ray counts were made under a dissection microscope.

Discriminant analyses were used to determine if there were significant morphological differences between the two genetic groups identified in the electrophoretic study. All 13 morphological variables were used in five separate analyses: *(a)* using all individuals, *(b)* using individuals from the North Dandalup River only, *(c)* using individuals from the Canning River only, *(d)* using individuals from Site ND3 only, and *(e)* using individuals from Site ND6 only. The discriminant analyses were undertaken with the statistical graphics software package STATGRAPHICS.

Table 1. Proteins examined for electrophoretic variation in *G. occidentalis*

Buffer systems shown are those that had the best activity: LiOH, lithium-borate/Tris-citrate; TEB, Tris-borate-EDTA; TC6, Tris-citrate; TM, Tris-maleate-EDTA. Variability of loci: M, monomorphic; P, polymorphic; P?, possibly polymorphic, but not fully resolved

Protein	Protein abbreviation	Enzyme Commission number	No. loci	Buffer	Variability
Alcohol dehydrogenase	ADH	1.1.1.1	1	TM	P?
Adenylate kinase	AK	2.7.4.3	1	TEB	M
Creatine kinase	CK	2.7.3.2	–	–	–
Glutamate dehydrogenase	GLUDH	1.4.1.–	1	TC6	M
Glucose-6-phosphate isomerase	GPI	5.3.1.9	2	TM	1P, 1M
Hexokinase	HK	2.7.1.1	1	TEB	M
Isocitrate dehydrogenase ($NADP^+$)	sIDHP	1.1.1.42	1	TM	M
L-Lactate dehydrogenase	LDH	1.1.1.27	2	TM	1P?, 1M
Malate dehydrogenase	sMDH	1.1.1.37	1	TC6	M
Malic enzyme ($NADP^+$)	sMEP	1.1.1.40	1	TM	M
Mannose-6-phosphate isomerase	MPI	5.3.1.8	1	TC6	M
Tripeptide aminopeptidase	PEPB	3.4.–.–	1	LiOH	P
Peptidase-C	PEPC	3.4.–.–	1	LiOH	P
Peptidase-S	PEPS	3.4.–.–	1	LiOH	P?
Phosphogluconate dehydrogenase	PGDH	1.1.1.44	1	TM	P?
Phosphoglucomutase	PGM	5.4.2.2	1	TM	P
Superoxide dismutase	SOD	1.15.1.1	1	TM	M

Results

Significant departures from Hardy–Weinberg expectations were observed in the samples from Sites ND3 and ND6. The ND3 sample showed significant deficits of heterozygotes at the *PEPB** and *PEPC** loci and a non-significant deficit of heterozygotes at the *GPI** locus (Table 2). There was a complete absence of heterozygotes at the *PEPC** locus, despite the nearly equal frequency of the *b* and *c* alleles. Similarly, the ND6 sample showed a significant deficit of heterozygotes at the *PEPC** and *GPI** loci and a non-significant deficit of heterozygotes at the *PEPB** locus (Table 2).

Table 2. Allele frequencies at four polymorphic loci for samples of *G. occidentalis* from Sites ND3 and ND6

The proportional departure of heterozygosity from Hardy–Weinberg equilibrium expectation at each locus is given in parentheses. Sample sizes (*n*) are also given. **P* < 0·05; ***P* < 0·01; ****P* < 0·001; n.s., *P* > 0·05; —, no test possible

Site	Allele	*PEPB**	*PEPC**	*PGM**	*GPI**
ND3	(*n*)	21	21	21	21
	a	0·357	0·000	0	0
	b	0·643	0·476	0	0·309
	c	0	0·524	1·000	0·691
	d	0	0	0	0
		(–0·494*)	(–1·000***)	(—)	(–0·456)
ND6	(*n*)	31	19	32	32
	a	0·129	0·054	0	0
	b	0·871	0·474	0	0·422
	c	0	0·474	0·953	0·578
	d	0	0	0·047	0
		(–0·153 n.s.)	(–0·626**)	(0·033 n.s.)	(–0·811***)

Further examination of the genotypic structure of the ND3 and ND6 samples revealed there was non-random association of genotypes among loci in these samples (Table 3). The individuals in the ND3 sample were separated into two distinct genetic groups: Population ND3(i) comprising 11 individuals homozygous for *PEPC*c*, *PEPB*b* and *GPI*c*, and Population ND3(ii) comprising 10 individuals homozygous for *PEPC*b* (Table 3). The same two genetic groups were also identifiable within the ND6 sample, which was divided into two groups for further analysis: Population ND6(i) comprising seven individuals homozygous for *PEPC*c*, *PEPB*b* and *GPI*c*, and Population ND6(ii) comprising eight individuals either homozygous for *PEPC*b* or heterozygous for *PEPC*ab* (Table 3). Two individuals (Nos 8 and 9) from the ND6 sample that had the *PEPC*bc* genotype could not be clearly divided into either group so were not included in further analyses.

Genetic distances between pairs of samples are summarized by the phenogram (Fig. 2). This shows that the

Table 3. Distribution of individuals from Samples ND3 and ND6 among genotypes at the *PEPB, *PEPC** and *GPI** loci**

Values are numbers of individuals of a given genotype at each locus

Site	Locus	Genotype	*PEPC** locus			
			ab	*bb*	*bc*	*cc*
ND3	*PEPB**	*aa*	–	5	–	–
		ab	–	5	–	–
		bb	–	–	–	11
	*GPI**	*bb*	–	4	–	–
		bc	–	5	–	–
		cc	–	1	–	11
ND6	*PEPB**	*aa*	–	1	–	–
		ab	–	2	–	–
		bb	1	4	2[A]	7
	*GPI**	*bb*	1	6	–	–
		bc	–	1	–	–
		cc	–	–	2[A]	7

[A]Individuals 8 and 9.

samples divide into two distinct genetic groups. With one exception, all samples collected from sites in the Darling Range cluster into one group and all samples collected from the Swan Coastal Plain cluster into a second group. These two groups are hereinafter referred to as the Darling Range genetic group and the Swan Coastal Plain genetic group respectively. There were distinct differences in allelic frequencies between the two genetic groups, especially at the *PEPC** and *GPI** loci where they are almost fixed for different alleles (Table 4). The exception, Population ND3(ii) from a headwater stream in the North Dandalup River, clustered with samples from the Swan Coastal Plain. At Site ND6 in the transition zone, Population ND6(i) clustered with samples from the Darling Range, whereas Population ND6(ii) clustered with samples from the Swan Coastal Plain (Fig. 2).

There were highly significant differences in allelic frequencies among samples within each of the rivers: at three loci in the North Dandalup River and at four loci in the Canning River (Table 5). This is to be expected because the two genetic groups of *G. occidentalis* occur in each river system.

There were no significant differences in allelic frequencies among samples of the Darling Range genetic group in either the North Dandalup or the Canning River (Table 5). In contrast, in this genetic group there were significant genetic differences between samples from different rivers at the *PEPB**, *PEPC** and *GPI** loci. Samples from the North Dandalup River were less variable than those from the Canning River at three loci (Table 4).

There was significant genetic heterogeneity among populations of the Swan Coastal Plain genetic group at the *PEPB** and *GPI** loci in the North Dandalup River (Table

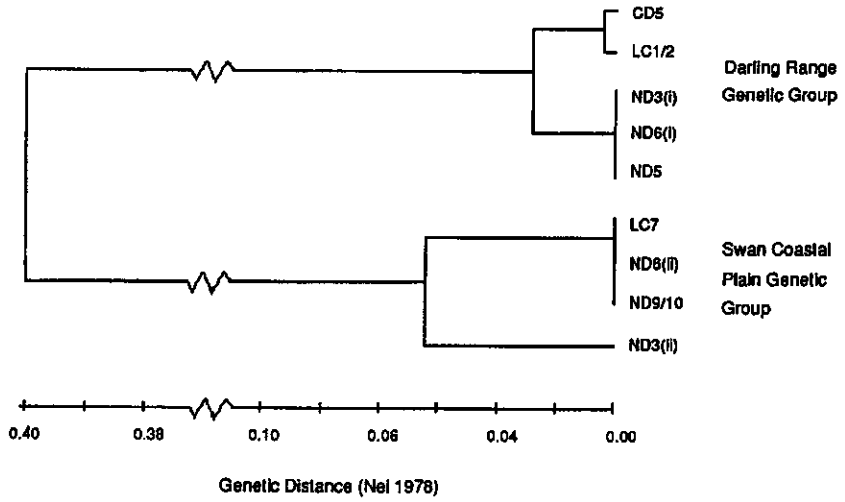

Fig. 2. UPGMA phenogram of Nei's (1978) genetic distances for samples of *Galaxias occidentalis* from Western Australia.

Table 4. Allele frequencies at four polymorphic loci in samples of *G. occidentalis* from the Canning River and North Dandalup River in Western Australia

Samples are presented in the same order as in the phenogram (Fig. 2). Sample sizes (*n*) are also shown

Locus	Allele	Darling Range group					Swan Coastal Plain group			
		CD5	LC1/2	ND3(i)	ND6(i)	ND5	LC7	ND6(ii)	ND9/10	ND3(ii)
*PEPB**	(*n*)	50	24	11	7	48	34	8	48	10
	a	0·07	0·08				0·41	0·25	0·29	0·75
	b	0·93	0·92	1·00	1·00	1·00	0·59	0·75	0·71	0·25
PEPC*	(*n*)	49	24	11	7	48	34	8	47	10
	a							0·06	0·01	
	b	0·24	0·38			0·01	0·93	0·94	0·95	1·00
	c	0·76	0·62	1·00	1·00	0·99	0·07		0·04	
PGM*	(*n*)	50	24	11	7	48	34	8	48	10
	a								0·01	
	b						0·02			
	c	1·00	1·00	1·00	1·00	1·00	0·88	0·81	1·00	
	d						0·10	0·19	0·09	
	e								0·02	
GPI*	(*n*)	50	24	11	7	48	34	8	48	10
	a						0·03			
	b	0·12	0·08			0·01	0·85	0·94	0·92	0·65
	c	0·88	0·92	1·00	1·00	0·99	0·09	0·06	0·07	0·35
	d						0·03			
	e								0·01	

5). However, when Population ND3(ii) was excluded from the analysis, there were no significant differences in allelic frequencies in this group. The differences between Population ND3(ii) and the other populations in the Swan Coastal Plain genetic group reflect the anomaly of its grouping, since it was collected from the Darling Range. No comparison could be made among samples of the Swan Coastal Plain group from the Canning River, as there was only one sample representing this group. When Sample ND3(ii) was excluded from the analysis, there were no significant differences in allelic frequencies for the comparison between samples from the two rivers (Table 5).

Morphometric and meristic data on which the discriminant analyses were based are presented in Table 6.

Table 5. Summary of heterogeneity χ^2 tests of allelic frequencies among samples of *G. occidentalis*
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., $P > 0.05$; —, no test possible

Comparison	*PEPB**	*PEPC**	*PGM**	*GPI**
Within rivers				
North Dandalup River				
1. All samples [ND9/10, ND6(i), ND6(ii), ND5, ND3(i), ND3(ii)]	***	***	n.s.	***
2. Darling Range group [ND3(i), ND5, ND6(i)]	—	n.s.	—	n.s.
3. Coastal plain group [ND6(ii), ND9/10, ND3(ii)]	***	n.s.	n.s.	**
4. Coastal plain group excluding ND3(ii)	n.s.	n.s.	n.s.	n.s.
Canning River				
1. All samples [CD5, LC1/2, LC7]	***	***	**	***
2. Darling Range group [CD5, LC1/2]	n.s.	n.s.	—	n.s.
3. Coastal plain group	—	—	—	—
Between rivers				
1. Darling Range group [CD5, LC1/2, ND3(i), ND5, ND6(i)]	*	***	—	*
2. Coastal plain group [LC7, ND6(ii), ND9/10, ND3(ii)]	***	n.s.	n.s.	*
3. Coastal plain group excluding ND3(ii)	n.s.	n.s.	n.s.	n.s.

The discriminant analysis of all samples correctly classified 85·7% of the Darling Range genetic group and 79·4% of the Swan Coastal Plain genetic group. The variables that contributed most to the discrimination between the two genetic groups were upper jaw length, lower jaw length, and caudal length. In contrast, all four other discriminant analyses using individuals from the North Dandalup River only, the Canning River only, Site ND3 only, and Site ND6 only, resulted in all individuals being correctly classified according to their genetic grouping.

Discussion

This study shows that two distinct genetic forms of *G. occidentalis* occur in the North Dandalup and Canning Rivers in Western Australia. The distribution of the Darling Range genetic group extended from the headwater streams to the transition zone where the Darling Range meets the Swan Coastal Plain. The distribution of the Swan Coastal Plain genetic group was mainly restricted to the transition zone and the Swan Coastal Plain, but one population of this group was collected from a site in the ranges. The two forms were found to occur sympatrically at two sites: Site ND6, in the transition zone of the North Dandalup River, and Site ND3, in the headwaters of the North Dandalup River. The distinctiveness of these two genetic groups in both river systems was shown by clear discontinuities in gene frequencies between populations from the Darling Range and the Swan Coastal Plain. This discontinuity parallels the differences in macroinvertebrate community structure and environmental variables that exist between the headwater and coastal plain sites in the two river systems (Storey and Edward 1989; Storey *et al.* 1990).

The genetic differences between the Darling Range and the Swan Coastal Plain genetic groups cannot be interpreted as a response to environmental differences between the two areas, despite the sample from Site ND6, from the transition zone, having allelic frequencies that were intermediate between those of populations from the Darling Range and the Swan Coastal Plain. The significant deficits of heterozygotes from Hardy–Weinberg expectations and the non-random association of genotypes among loci in the ND6 sample strongly suggest that this sample comprises individuals belonging to two different gene pools. Deficits of heterozygotes and the non-random association of genotypes are characteristic when a sample comprises individuals from different gene pools (Chakraborty and Leimar 1987) and have been observed in several fish species where discrete gene pools occur sympatrically (Allendorf *et al.* 1976; Kirkpatrick and Selander 1979; Ryman *et al.* 1979; Ferguson and Mason 1981; Ferguson 1989; Verspoor and Cole 1989). Most importantly, the sample from Site ND3 in the headwaters of the North Dandalup River also comprised the same two genetic groups. This observation clearly invalidates any possibility that the different genetic groupings simply reflect environmental differences between the ranges and the plain.

The demonstration of fixed allelic differences between sympatric groups has been suggested to provide evidence that the groups have evolved an effective means of reproductive isolation (Shaklee *et al.* 1982). In *G. occidentalis* there were almost fixed allelic differences at two of the four loci examined and there were also clear morphological differences between the two genetic forms when they occurred in the same catchment or at the same site. Despite the lack of fixed differences between the two genetic forms of *G. occidentalis*, this study has shown that the two forms can occur sympatrically and maintain their genetic differences. This suggests that they may have evolved a mechanism to keep their gene pools separate. For example, it is possible that the two different forms may have different reproductive styles, as there are distinct environmental differences between the habitats in the headwaters and on the coastal plain. One possibility is that the coastal plain form may have a marine larval stage similar to that of *G. maculatus* from eastern Australia (Allen 1982). Although the conclusions of Pen and Potter (1991) do not provide any support for the possibility of a marine larval stage in this species, their study was restricted to sites in the Darling

Table 6. Mean values for each of the morphometric and meristic variables measured on *G. occidentalis*
Values for pectoral rays and anal rays are given as counts. Values for all other variables are shown in millimetres. Standard errors are given in parentheses

Variable	Darling Range group					Swan Coastal Plain group			
	CD5	LC1/2	ND3(i)	ND6(i)	ND5	LC7	ND6(ii)	ND9/10	ND3(ii)
Pectoral rays	13·50	13·71	13·22	13·67	13·43	12·43	13·20	13·00	13·33
	(0·76)	(1·11)	(0·67)	(0·57)	(0·53)	(1·71)	(0·45)	(0·58)	(0·87)
Anal rays	14·75	14·57	14·33	15·00	15·29	13·43	15·40	14·86	14·44
	(0·46)	(0·53)	(0·71)	(1·00)	(0·49)	(2·44)	(0·89)	(0·38)	(0·73)
Standard length	60·71	59·09	60·60	49·70	63·59	61·54	61·39	54·61	62·52
	(13·75)	(8·59)	(7·50)	(0·26)	(19·72)	(11·10)	(2·43)	(4·97)	(5·27)
Body depth	7·16	8·13	7·72	6·23	7·84	7·63	7·62	6·48	7·98
	(1·61)	(1·60)	(1·22)	(0·81)	(2·25)	(0·89)	(0·42)	(0·64)	(1·96)
Caudal length	8·52	8·04	9·41	7·97	9·34	8·54	8·62	7·60	8·38
	(2·01)	(1·21)	(0·95)	(0·83)	(2·78)	(1·66)	(0·25)	(0·82)	(0·94)
Caudal depth	4·10	4·60	4·53	3·97	4·56	4·18	4·51	3·84	4·60
	(0·97)	(0·79)	(0·76)	(0·21)	(1·27)	(0·69)	(0·30)	(0·53)	(0·64)
Pelvic to anal fin	14·39	15·24	15·43	12·38	16·38	16·34	15·97	13·95	15·47
	(3·37)	(1·85)	(1·93)	(0·66)	(5·49)	(2·96)	(1·65)	(1·64)	(1·69)
Head length	13·28	13·19	14·01	11·53	13·71	13·72	13·47	12·19	14·35
	(2·54)	(1·46)	(1·95)	(0·06)	(3·90)	(2·41)	(1·12)	(1·29)	(1·08)
Eye diameter	3·49	3·49	3·75	3·23	3·76	3·59	3·70	3·41	3·92
	(0·45)	(0·37)	(0·39)	(0·15)	(0·63)	(0·33)	(0·12)	(0·17)	(0·27)
Upper jaw length	5·16	5·04	5·43	4·37	5·36	5·42	5·56	5·04	5·97
	(1·20)	(0·77)	(0·90)	(0·06)	(1·79)	(0·69)	(0·29)	(0·63)	(0·48)
Lower jaw length	5·74	5·59	5·93	4·67	5·68	5·86	5·79	5·40	6·36
	(1·39)	(0·83)	(0·96)	(0·15)	(1·82)	(1·03)	(0·21)	(0·72)	(0·50)
Snout to eye	3·71	3·82	3·76	3·22	3·61	3·76	3·76	3·44	3·88
	(0·95)	(0·51)	(0·56)	(0·33)	(1·15)	(0·82)	(0·19)	(0·62)	(0·32)
Base of anal fin	8·99	9·00	8·49	7·57	8·89	9·12	9·61	8·23	9·41
	(1·82)	(1·19)	(0·88)	(0·15)	(2·09)	(2·03)	(0·80)	(0·89)	(0·86)

Range. Thus, if the Darling Range and Swan Coastal Plain genetic forms of this species were to have different reproductive styles, the differences would not have been detected by Pen and Potter's (1991) study. If future research were to demonstrate that the two groups have different reproductive styles, then they should clearly be recognized as different species.

It is possible that hybridization may occur between the two genetic groups of *G. occidentalis*. For example, Individuals 8 and 9 from the ND6 sample have the *PEPC*bc* genotype, whereas the Darling Range group are all homozygous for *PEPC*c* and the Swan Coastal Plain group has only the *PEPC*a* or *PEPC*b* alleles. However, since the Darling Range group are fixed for *GPI*c* and the Swan Coastal Plain group are almost fixed for *GPI*b*, it is unlikely that these two individuals are F_1 hybrids because they do not have the *GPI*bc* genotype. The possibility that there has been hybridization between the two genetic forms of *G. occidentalis* would not weaken the possibility that they may be two species because interspecific hybridization has been well documented in fishes (Schwartz 1972).

With the exception of Population ND3(ii) from the headwaters of the North Dandalup River, there appears to be a very clear pattern of distribution of the two genetic forms of *G. occidentalis*. One of the genetic groups identified in this study occurs mainly on the coastal plain and in the transition zone and the other genetic group occurs only in the ranges and the transition zone. However, the pattern of distribution of the two forms is not as straightforward as it first appears because both forms occur at Site ND3 in the headwaters of the North Dandalup River. Thus, individuals belonging to the Swan Coastal Plain genetic group were observed to occur at a site in the ranges. There are several different hypotheses that can explain this pattern of

distribution. One explanation is that the Swan Coastal Plain genetic group may be distributed throughout the plain and the ranges. If this were the case, it would be expected that other individuals of the Swan Coastal Plain group would occur at other sites in the ranges. Further study of the distribution of the two forms would confirm or refute this hypothesis. Alternatively, the distribution of the Swan Coastal Plain genetic group may have historically included sites both on the plain and in the ranges but may now be largely restricted to the coastal plain and transition zone. Under this hypothesis a formerly continuously distributed coastal plain genetic group could have become subdivided as a result of the tectonic uplift of the Darling Plateau, which is generally assumed to have begun during the mid Tertiary (Lambeck 1987). The Darling Scarp, which is coincident with the Darling Fault, marks the transition between the ranges and the coastal plain. It is quite possible that small waterfalls that occur in this transition zone may have provided, and may still provide, a physical barrier to migration of *G. occidentalis*. In addition, the differences in water velocity and substratum type between the Darling Range and Swan Coastal Plain sections of the North Dandalup and Canning Rivers (Storey *et al.* 1990) may provide environmental barriers that limit exchange between populations from these two areas. Under this hypothesis Population ND3(ii) could be a relic of this formerly continuously distributed group. The observation that there were significant genetic differences between Population ND3(ii) and the other three populations in the Swan Coastal Plain genetic group provides support for this hypothesis. Another explanation for the pattern of distribution of the two forms of *G. occidentalis* is that some individuals of the Swan Coastal Plain genetic group may have been translocated nearby to Site ND3(ii). This explanation seems unlikely, however, as Site ND3 is in a very isolated area and access to this stream is very difficult. If translocation to Site ND3 has occurred, then the distribution of the Swan Coastal Plain genetic group may extend only from the transition zone to the coastal plain. Future studies of the distribution of *G. occidentalis* will clarify the ranges of the two genetic forms.

In addition to the large genetic differences between the Darling Range and Swan Coastal Plain genetic groups, there was also significant genetic differentiation observed among populations within each genetic group. For example, there was significant differentiation within the Darling Range genetic group between populations from the North Dandalup and Canning Rivers at three of the four loci examined. This suggests that gene exchange within this group between catchments is either absent or very limited. In contrast, there was no significant variation in allelic frequencies among populations of the Darling Range genetic group within either the North Dandalup or the Canning River, despite the occurrence of large impoundments between Sites ND3 and ND5 in the North Dandalup River and between Sites CD5 and LC1/2 in the Canning River. Since these impoundments have been present for only a relatively short time, it is not surprising that the populations that are divided by them have not diverged. However, since impoundments are known to be effective barriers to the upstream movement of several fish species (Ruhr 1957; Raymond 1979), and even small V-notch gauging stations have been observed to limit the upstream migration of this species (Pusey *et al.* 1989), it is possible that these divided populations may show genetic differences in the future.

There was also significant heterogeneity within the Swan Coastal Plain genetic group between populations from the North Dandalup and Canning Rivers. These differences were due to Population ND3(ii), which was collected from a site in the Darling Ranges but clustered with populations from the Swan Coastal Plain. The divergence of Population ND3(ii) from other populations in the Swan Coastal Plain genetic group is probably due to its geographic isolation from the other populations in this group. When Population ND3(ii) was excluded from the analysis, there were no significant differences between populations of this genetic group from different rivers. This suggests that gene exchange may be occurring, or may have occurred in the recent past, between populations from different coastal plain rivers. One possible avenue for gene flow between populations from different rivers is via the ocean. If the Swan Coastal Plain group were to have a marine larval stage, similar to that of *G. maculatus* from eastern Australia (Allen 1982), then gene flow between populations of the Swan Coastal Plain genetic group from different rivers could be maintained. Alternatively, gene flow could occur, or may have recently occurred, between these populations via the wetlands of the coastal plain. Prior to the recent draining of 70% of the lakes and swamps of the Swan Coastal Plain (Riggert 1966), the rivers of the Swan Coastal Plain were potentially linked during wet periods by a series of interconnecting wetlands (Seddon 1972). Thus, in the recent past there may have been the opportunity for gene flow to occur between populations from different rivers, which would limit the opportunities for populations to diverge.

In summary, this study has shown that there are two different genetic forms of *G. occidentalis* occurring in the North Dandalup and Canning Rivers in Western Australia. The fact that these two forms can occur sympatrically suggests that *G. occidentalis* may consist of two distinct species, but there is insufficient evidence to resolve the taxonomic status of these forms at this stage. As this study was restricted to only two river systems and four polymorphic loci, further research on this species should be undertaken to determine the distribution, population

structure and taxonomic status of the two genetic forms. In particular, a full survey of protein loci should be undertaken. Most importantly, the reproductive biology of the two forms should be determined by using samples from several rivers. These types of studies would provide essential information so that effective and relevant conservation and management programmes for this species complex can be undertaken.

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