Genetic and morphological study of the Black-stripe minnow, *Galaxiella nigrostriata* (Salmoniformes: Galaxiidae), including a disjunct population near Perth, Western Australia

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Abstract – This study was undertaken to measure the relatedness of an outlying population of Black-stripe minnow, *Galaxiella nigrostriata* (Shipway), from Melaleuca Park, 25 km northeast of Perth, to the main populations situated some 350 km to the south between Augusta and Albany. Allozyme electrophoresis was used to assess genetic divergence between populations. Univariate statistical tests were employed to compare the morphology of fish from the different locations. Reproductive isolation of *G. nigrostriata* at EPP 173 has resulted in inbreeding and reduced heterozygosity as well as differences in morphology. *Galaxiella nigrostriata* throughout its range shows unusually low levels of genetic polymorphism. One polymorphic locus was identified from the 27 loci sampled. Polymorphic individuals were restricted to the southern sites; specimens from EPP 173 were entirely monomorphic. Fish from EPP 173 had longer head lengths and shorter caudal peduncles than those in the south.

Keywords: freshwater fish, low genetic variation.

INTRODUCTION

Of the ten species of freshwater fish native to southwestern Australia, six [*Galaxias truttaceus*, *Galaxias maculatus*, *Galaxiella munda* and *Galaxiella nigrostriata* (Galaxiidae); *Lepidogalaxias salamandroides* (Lepidogalaxiidae); and *Nannatherina balstoni* (Nannopercidae)] have very restricted, coastal distributions in peat wetlands between Albany (35°02'S; 117°53'E) and Augusta (34°20'S; 115°09'E). The other four species [*Bostockia porosa* (Percichthyidae), *Edelia vittata* (Nannopercidae), *Galaxias occidentalis* (Galaxiidae), and *Tandanus bostocki* (Plotosidae)] have wider distributions between Two Peoples Bay (34°57'S; 118°11'E) and the Moore River (31°03'S; 115°35'E) (Allen 1982; Morgan *et al.* 1996, 1998). Of these ten species, eight are endemic to southwestern Australia, while *G. maculatus* and *G. truttaceus*, both with marine larval stages in some populations, also occur in Tasmania and Victoria with the geographical range of *G. maculatus* extending to New South Wales, South Australia, Queensland, New Zealand, southern South America, and the Chatham and the Falkland Islands (Allen 1982; Berra *et al.* 1996; McDowall 1996).

With the discovery in recent years of outlier populations of *G. munda* between Gingin (31°21'S; 115°54'E) and Muchea (31°35'S; 115°58'E) (Allen 1982), of *N. balstoni* at Gingin (Morgan *et al.* 1996, 1998), and of *G. nigrostriata* at Bunbury (33°19'S; 115°38'E) (Morgan *et al.* 1996, 1998) and in wetland EPP 173 in Melaleuca Park (31°42'S; 115°57'E), northeast of Perth, Western Australia (Smith *et al.* 2002) (Figure 1), there arises the question of how related are any of these widely disjunct northern populations to those from the centre of distribution of that species. The focus in this study is on the Black-stripe minnow, *Galaxiella nigrostriata* (Shipway 1953) (Teleostei: Salmoniformes: Galaxiidae). *Galaxiella nigrostriata* was originally described as a subspecies of the southeastern Australian species, *Galaxias pusillus*. Subsequently, McDowall (1978) erected the genus *Galaxiella* for the three species of diminutive galaxiids with horizontal body stripes, *G. nigrostriata*, *G. munda* and *G. pusilla*. Supplementary descriptions of *G. nigrostriata* have been provided by McDowall and Frankenberg (1981), Gill and Neira (1994), and Berra and Allen (1989) clarified the morphological differences between *G. nigrostriata* and *G. munda*.

Growth, size composition, diet and aspects of the reproductive biology of *G. nigrostriata* from the main area of distribution have been documented by Pen *et al.* (1993), while dietary studies were undertaken by Pusey and Bradshaw (1996).

The present study determined the relatedness of *G. nigrostriata* from EPP 173 to specimens from within the main area of distribution of the species. Allozyme electrophoresis was used to document genetic polymorphism and provide a basis for comparison of heterozygosity and genetic identity between sites. Morphometric analyses were used to compare the physical similarity of specimens from different geographical locations. We conclude the paper by considering the possible period of time that the northern population of *G. nigrostriata* has been isolated in EPP 173.

MATERIALS AND METHODS

Collection of Fish

Fish were collected from EPP 173 throughout the period May to September, 1996. Specimens were collected from the lower southwest between May and July, 1996; i.e. from roadside pools beside Chesapeake Road (34°42'S; 116°07'E) within the Gardner River watershed in the D'Entrecasteaux National Park and, from Scott Road near Lake Jasper (34°25'S; 115°45'E) in the Donnelly River catchment of the Shannon National Park.

Fish were collected by dragging either a pond net, diameter 85 cm, 100 mm² stretched mesh, or a 5 m wide purse seine net of 9 mm² stretched mesh through open water. Low numbers of fish were sampled in order to cause a minimum impact upon the small resident populations and consequently, where possible, specimens were used in several facets of the study.

Electrophoresis

Specimen numbers were as follows: EPP 173, 24 fish; Chesapeake Road, 16; Lake Jasper, 11. Skeletal muscle tissue was removed under a dissection microscope, homogenised with extractant (2:1, extractant: tissue by volume; extractant of 0.28 M sucrose, 0.1% (v/v) mercaptoethanol, 0.02% (w/v) bromophenol blue in 0.02 M tris, pH 8) in ceramic depression wells, using a glass test tube. The extracts were soaked onto 5 x 6 mm rectangles of chromatography paper and stored overnight at –70°C. Extracts were prepared over an ice pack on the day prior to the run to minimise deterioration of the enzymes.

Gels contained between 18 and 19.5 g of hydrolysed potato starch (Starch Art) and approximately 165 mL of the appropriate buffer solution. Starch gels were prepared 24 hours prior to runs which were made at varying voltages and

currents, for different periods of time according to the requirements of each buffer solution. Staining for enzymes followed the protocols described in Richardson *et al.* (1986). For loci encoding enzymes exhibiting polymorphisms, data were collected running a maximum of 14 individuals from a site on a gel together with a minimum of two individuals of known genotype from an alternative location.

Allelic frequencies at the polymorphic locus were calculated and estimates of heterozygosity obtained according to the method of Nevo (1978). Nei's index of genetic identity was calculated to quantify the similarity between the samples (Nei 1978).

Morphometric Analyses

Sample sizes were as follows: EPP 173, 11; Lake Jasper, 16; Chesapeake Road, 36. Eight measurable characters were assessed for each fish using a dissecting microscope: total length (snout to posterior margin of tail = TL), head length (HL), eye diameter (ED), body depth (BD), eye to snout length (E-S), caudal depth (CD), caudal length (CL), length of base of anal fin (FL) and four countable ray numbers (in the pectoral, anal, dorsal and tail fins).

To eliminate the risk of errors due to preservation artefacts only very recently dead and/or anaesthetised fish were used for analysis. In order to determine the effect of sex on character variation, specimens from the Lake Jasper site were humanely killed by an overdose of anaesthetic, dissected and their sex identified by the presence of ovary or testis within the body cavity. Each character was tested for sexual dimorphism using an unpaired 2-tailed *t*-test. With the exception of body depth (BD), each character was independent of sex (95% confidence interval). Given the limited number of specimens available, and the need to use them in a number of aspects of this study, BD was eliminated from the analyses, with the remaining analyses conducted independent of sex. Fish were not sufficiently abundant to permit the comparison of only similar-sized specimens. Linear regression analyses were performed using the Chesapeake Road sample to test whether the relationship of each measurable character to total length was isometric; all characters increased linearly with body size enabling different sized specimens to be compared by standardising character values. Measurable characters were standardised by dividing them by body length of the specimen. All specimens were longer than 13.2 mm standard length, the length by which all fin rays are formed (Gill and Neira 1994). Levene's homogeneity of variance test was applied to qualify the assumption that means for each character were normally distributed within samples. One-way analyses of variance were calculated between sites for each of the meristic and standardised measurable characters.

Table 1 Proteins showing resolvable banding in *G. nigrostriata* and buffer systems giving best resolution.

Protein	Buffer type	No. Loci	Variability
Adenosine deaminase	TEB	1	M
Adenylate kinase	TC8	2	M
Alcohol dehydrogenase	TM	1	M
Creatine kinase	TC8	2	M
αEsterase	TM	1	M
βEsterase	TM	2	M
Glucose 6-phosphate dehydrogenase	TEB	1	M
α-Glycerophosphate dehydrogenase	TEB	2	M
Icocitrate dehydrogenase	TC8	1	M
Lactate dehydrogenase	TM	2	M
Leucine aminopeptidase	TEB	1	M
Malate dehydrogenase	TM	2	1M, 1P
Peptidases			
L-Leucyl-glycyglycine	TM	1	M
L-Leucyl-Proline	TM	1	M
L-Leucyl-L-Tyrosine	TM	1	M
L-Valyl-Leucine	TEB	1	M
6-Phosphogluconate dehydrogenase	TEB	1	M
Phosphoglucose isomerase	TEB	1	M
Phosphoglucomutase	TEB	1	M
Superoxide dismutase	A	2	M

RESULTS

Electrophoresis

Polymorphism was very low; of the 27 loci encoding 20 enzymes clearly resolvable, just one polymorphism was identified (Table 1) – malate dehydrogenase exhibited 3-banded dimeric variants for two alleles. The level of polymorphism recorded for *G. nigrostriata* from both sites within its main area of distribution was 3%; the EPP 173 specimens were entirely monomorphic (Table 2). An additional 11 enzymes assayed showed unresolvable banding patterns and remained ambiguous despite repeated efforts combining liver and muscle tissue, using alternative buffers and increasing the concentration of the stain twofold.

Heterozygote frequencies of Mdh at the Chesapeake Road and Lake Jasper sites fitted frequencies expected under Hardy-Weinberg equilibrium (p > 0.01). There were significant differences in allele frequencies at the polymorphic locus between all sites (p < 0.05). Average overall heterozygosity at Chesapeake Road and Lake Jasper was 0.018 and 0.008 respectively. Nil heterozygosity was recorded at EPP 173. The values obtained for

Table 2 Allele frequencies at the malate dehydrogenase locus; the only locus found to be polymorphic in Black-stripe minnow.

Collection site	Sample size	"Slow"	"Fast"
Melaleuca Park	24	1	0
Chesapeake Road	16	0.125	0.875
Lake Jasper	11	0.5	0.5

Table 3 Between-site comparisons of Nei's genetic similarity (I).

Comparison	I
Chesapeake Road *versus* Lake Jasper	.996
Chesapeake Road *versus* EPP 173	.999
Lake Jasper *versus* EPP 173	.997

Nei's (1978) similarity indices were very close to the theoretical maximum value of one between all sites (Table 3).

Morphometric analyses

The values of each measurable character increased linearly with increasing total body length; correlation co-efficients were very close to one (Table 4). Standardised head lengths within the EPP 173 population (mean 19.6% B.L.) were found to be significantly larger (p = 0.02) than those from fish collected near Lake Jasper (mean 18.5% B.L.) (Table 5). Similarly, standardised caudal peduncle lengths were shorter at EPP 173

Table 4 Correlation co-efficients of linear regression analyses defining the relationship between each measurable character and total body length.

Character	Correlation co-efficient
Head length	r = 0.952
Eye diameter	r = 0.763
Eye-nose distance	r = 0.870
Caudal depth	r = 0.669
Caudal length	r = 0.860

Table 5   Range and mean (± standard deviation) of morphological characters used to compare *G. nigrostriata* from three locations.

	EPP 173 n = 11		Lake Jasper N = 16		Chesapeake Road N = 35	
TL (mm)	20.9 – 34.0	(28.4 ± 4.7)	25.9 – 35.8	(30.6 ± 2.9)	17.8 – 43.0	(32.4 ± 5.6)
HL (% BL)	17.7 – 21.3	(19.6 ± 0.99)	16.7 – 20.8	(18.5 ± 1.07)	15.9 – 21.2	(18.8 ± 1.04)
ED (% BL)	4.8 – 6.8	(6.0 ±. 0.56)	4.7 – 6.1	(5.5 ± 0.40)	4.7 – 8.1	(5.9 ± 0.70)
E–N (% BL)	3.6 – 5.2	(4.4 ± 0.53)	3.5 – 5.0	(4.2 ± 0.46)	2.9 – 5.9	(4.3 ± 0.51)
CD (% BL)	4.8 – 5.5	(5.1 ± 0.30)	3.8 – 6.1	(4.9 ± 0.52)	2.4 – 9.4	(4.9 ± 0.96)
CL (% BL)	14.1 – 16.8	(16.0 ± 0.80)	15.5 – 18.9	(16.8 ± 0.99)	14.4 – 24.9	(17.4 ± 1.75)
Pectoral fins	11 – 13	12 ± 1.0	11 – 13	11.9 ± 0.5	10 – 13	12 ± 0.6
Anal fins	7 – 10	(9.3 ± 0.9)	9 – 10	(9.5 ± 0.5)	7 – 10	(9.4 ± 1.0)
Dorsal fins	7 – 8	(7.4 ± 0.5)	5 – 7	(6.4 ± 0.7)	5 – 8	(6.8 ± 0.8)
Tail fins	14 – 15	(14.5 ± 0.5)	14 – 15	(14.2 ± 0.4)	14 – 15	(14.1 ± 0.3)

(mean 16% B.L.) compared with the Chesapeake Road (mean 17.4% B.L.) (p = 0.02).

DISCUSSION

Galaxiella nigrostriata recorded approximately 3% polymorphism; of 27 loci assayed, only one was polymorphic. This value, compared to values between 15 and 20% commonly recorded from vertebrates, is very low (Nevo 1978). For example, Watts *et al.* (1995), recorded 20% of loci polymorphic in *G. occidentalis*, a relatively widespread galaxiid endemic to southwestern Australia. While genetic similarity between fish from the three different locations sampled is very high and does not reflect geographic distance, the northern population, in contrast to the main population is entirely monomorphic, indicating that reproductive isolation and small population size at EPP 173 has possibly resulted in inbreeding effects. Furthermore, while differences in two morphological characters were not consistent between EPP 173 specimens and those from the southern locations, the fact that there were no differences between fish collected from within the main southern area of distribution indicates that isolation at EPP 173 is influencing the appearance of the fish.

These small differences which have arisen between the EPP 173 population of *G. nigrostriata* and the main populations indicate that the population at EPP 173 has been reproductively isolated for enough generations for genetic and morphological divergence to occur. Since wide-scale urban and rural development of the Swan Coastal Plain, and the subsequent alteration of intervening wetlands commenced early this century (Bekle and Gentilli 1966; Seddon 1972; Wrigley *et al.* 1988; Halse 1989), *G. nigrostriata* at EPP 173 is likely to have been isolated for at least several decades. We conclude that the population of *G. nigrostriata* at EPP 173 is not the result of recent colonisation, but is a long established population which has persisted there presumably

because this wetland has not been subject yet to significant degradation.

Given the rapid (one year) life cycle of this species (Pen *et al.* 1993), and the likelihood that the population at EPP 173 has been isolated for several decades, it is surprising that genetic differentiation is not more pronounced. For example, the *Galaxias* genotype has been shown capable of quite rapid evolution as a result of landlocking behind hydro-electric impoundments (Fulton 1978) and segregation in different river systems (Watts *et al.* 1995). Indeed, small levels of environmental heterogeneity were regarded by Wallis *et al.*, (2001) to be a sufficient reason explaining genetic divergence in populations of *Galaxias vulgaris* Stokell in the South Island of New Zealand. However, in *G. nigrostriata*, genetic divergence appears to have been limited by low levels of genetic variation within the genome of this species.

There are several possible explanations for the peculiarly low levels of genetic polymorphism detected across the range of *G. nigrostriata*, and subsequently for the absence of pronounced genetic divergence between populations. Although *G. nigrostriata* can occur on two widely separated geomorphic units (namely the Swan and Scott Coastal Plains), it is known only from waters with a narrow range of physical and chemical conditions (Smith *et al.* 2002; Knott *et al.* 2002). Although such ephemeral, acidic (pH 3.25–5.5) black-water (colour >1200 TCU) habitats may embody physiologically extreme conditions in comparison with other Australian freshwater habitats, they probably are reasonably constant in their physical and chemical conditions. The narrow range of environmental conditions (i.e. uniform habitat) occupied by *G. nigrostriata* may have caused directional selection for homozygosity and the fixation of a small range of genotypes. The resulting lack of heterozygosity may have subsequently reduced the ability of this species to adapt to the rapid environmental changes to wetland habitats induced by Europeans this century.

Low levels of genetic variation are likely to reflect the degree to which habitat alteration has reduced the number of *G. nigrostriata* populations on the Swan Coastal Plain, causing inbreeding. Several northern outlier populations of freshwater fishes mainly from the lower south west indicate that stocks remaining at EPP 173 and within the narrow strip of southwest coastal peat flats represent only a minor proportion of the original population size. The absence of heterozygotes at EPP 173 indicates the vulnerability of this species to inbreeding. Heterozygosity within the polymorphic locus of fish from within the main population does not indicate inbreeding; inter-breeding is possibly facilitated by extensive seasonal flooding which causes large wetland areas along the south coast to become connected. Within this main population, however, overall heterozygosity is unusually low and is comparable to that of fishes which have had histories of population depletion.

Low levels of genetic polymorphism (6%) and overall heterozygosity (0.013) have also been detected in the North American paddlefish, *Polydon spathula* (Carlson *et al.* 1982). *P. spathula*, like *G. nigrostriata*, occurs in a very narrow range of environmental conditions and has been significantly adversely affected by habitat alteration and is now absent from major areas of its original range (Carlson *et al.* 1982). The low (4%) level of polymorphism of *Sardinella aurita*, abundant throughout the Mediterranean and off Western Africa, (Chikhi *et al.* 1998), has been attributed to a combination of collapses in fish stocks caused by over fishing, variations in reproductive success and possibly mass extinctions caused by ancient glaciation cycles.

The northern population of *G. nigrostriata* could have been established in comparatively recent times, explaining the lack of genetic divergence from the main population. Colonisation could have been facilitated *via* a spring-fed stream which during winter and early spring connects EPP 173 with Ellen Brook, a tributary of the Swan River. However, the nearest documented population of *G nigrostriata* is over 200 km away at Bunbury, and the wetland harbouring this population is widely separated from the Swan River catchment.

However, the present distribution pattern of the endemic fish fauna, and particularly the disjunct patterns of three species with northern outlier populations [*G. nigrostriata* at EPP 173, *G. munda* (Allen 1982) and *N. balstoni* (Morgan *et al.* 1998) near Gingin] and with specialised breeding requirements (acid, black, temporary water), suggests that previously species had a wider distribution through the southwest corner of Australia, particularly along the Swan Coastal Plain. The presence of Europeans has had a markedly deleterious impact, with extensive urban and rural developments leading to alteration of intervening wetlands. Consequently, the population of *G. nigrostriata* at EPP 173 may be of long-standing but it is impossible as yet to estimate the length of the period of complete isolation – likely to have been several decades, at least. At least one species of invertebrate, *Rak obtusus* (Cladocera), also an inhabitant of EPP 173, is otherwise only recorded from wetlands in the lower southwest; further descriptions of aquatic invertebrates from the wetlands at Melaleuca Park could reveal more relictual faunae. More detailed genetic studies, involving DNA assays and comparisons between the other endemic freshwater fishes, some with outlying populations, are essential to provide additional insight into the effects of wetland alteration upon the freshwater piscine fauna in Western Australia.

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