

Galaxias maculatus: an Explanation of its Biogeography

Tim M. Berra^A, L. E. L. M. Crowley^B, Walter Ivantsoff^B and Paul A. Fuerst^C

^ADepartment of Zoology, The Ohio State University, Mansfield, Ohio, 44906, USA.

^BSchool of Life Sciences, Macquarie University, NSW 2109, Australia.

^CDepartment of Zoology, The Ohio State University, Columbus, Ohio 43210, USA.

Abstract. *Galaxias maculatus* is a small diadromous fish found in Australia, New Zealand, South America and on some oceanic islands. Two hypotheses have been advanced to explain this widespread, disjunct distribution. McDowall promoted dispersal through the sea of salt-tolerant juveniles but Rosen and others claimed that the distribution reflected the break-up of Gondwana and subsequent drift of the southern continents. Allozyme electrophoresis of muscle extracts of specimens of *Galaxias maculatus* from eastern and western Australia, New Zealand and Chile was used to test the hypothesis that populations of *G. maculatus* from the western Pacific and the eastern Pacific do not differ genetically. F_{ST} based on allele frequencies and genotypes was 0.14, suggesting only minor differentiation between eastern and western Pacific populations. Minor differentiation in allele frequency existed at some loci, but no fixation of alternative alleles has occurred. The populations examined appear to be part of the same gene pool, indicating that gene flow via dispersal through the sea occurs today. It is unlikely that South American and Australasian populations would be conspecific if they have exchanged no migrants since the break-up of Gondwana at the end of the Mesozoic.

Introduction

Galaxias maculatus has one of the most widely disjunct distributions of any freshwater fish (Berra 1981). It occurs in eastern and western Australia, Tasmania, Lord Howe Island, New Zealand, Chatham Island, southern Chile, Argentina and the Falkland Islands (Fig. 1; McDowall 1970). The species has been described under at least 17 different specific names (McDowall 1972). In sorting out nomenclatural problems among *G. maculatus*, *G. attenuatus* and *G. alpinus*, Stokell (1966) concluded that only one species was common to Australia, New Zealand and South America, and gave priority to *G. maculatus*. McDowall (1967) concurred, but he argued that populations of *G. maculatus* from widely separated land masses were not distinct subspecifically.

Two hypotheses have been postulated to explain the disjunct distribution of *G. maculatus*: dispersal (movement through the sea) and vicariance (continental drift). McDowall (1970) attributed the wide geographic distribution to trans-oceanic dispersal of the marine whitebait stage (Fig. 2). Juveniles have been taken at sea 700 km from the New Zealand mainland (McDowall *et al.* 1975). The age at migration from the sea of juvenile *Galaxias* is 100–200 days (McDowall *et al.* 1994). McDowall (1970) suggested that *G. maculatus* originated in Australia and dispersed eastward past Tasmania to New Zealand and South America via the East Australian Current and the West Wind Drift (Fig. 1). Because this dispersal would have occurred subsequent to the break-up of Gondwana (McDowall 1971), McDowall (1967, 1978) hypothesized that significant phenotypic evolution should exist among present-day

populations of *G. maculatus* if their distribution dated to the Gondwanan break-up over 65 million years ago (Ma BP), or at least to the separation of Australia from Antarctica that occurred 53 Ma BP (Audley-Charles *et al.* 1981).

Rosen (1974, 1978) presented a vicariant alternative to McDowall's dispersal hypothesis. Rosen suggested that because galaxiids occur on all Gondwanan continents except India, their present distribution reflects an ancient Pangaeon pattern followed by continental drift. Rosen (1974, 1978) and Croizat *et al.* (1974) considered galaxiid fishes to be part of a pan-austral Gondwanan biota that fragmented in the Mesozoic. McDowall (1969, 1978) argued that there was no evidence that *G. maculatus* was part of such a hypothetical biota, and that recent dispersal would easily explain both the widespread distribution of *G. maculatus* and how the speciation process among disjunct, diadromous populations might be retarded. McDowall (1978) also argued that generalized tracks, one component of a vicariant model, assume the existence of an ancestral biota that can be demonstrated only by the fossil record, and that the galaxiid fossil record is very limited (McDowall 1976). Ball (1975) and Craw (1979) entered the debate with comments on the nature of biogeographical hypotheses. Finally, in discussing distributional relationships between African and South American freshwater fish families, Lundberg (1993) wrote that '...drift-related vicariance seemed unlikely...' for the Galaxiidae. Likewise, Nelson (1994) thought it untenable that the distribution of *G. maculatus* dated to the break-up of Gondwana and felt that dispersal via ocean currents was a more parsimonious hypothesis.

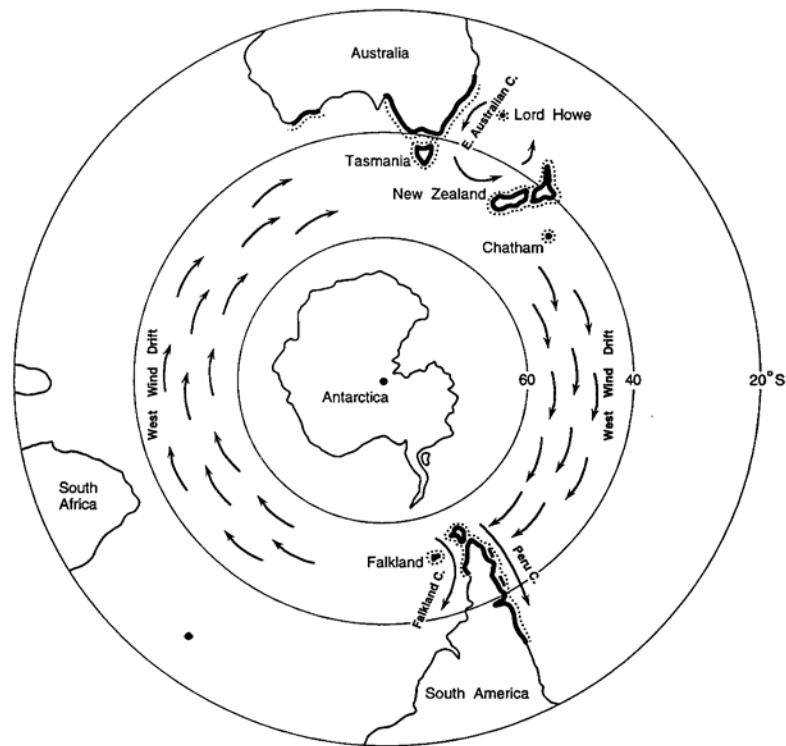


Fig. 1. Geographic distribution of *Galaxias maculatus* (modified from McDowall 1990) and relevant ocean surface currents.

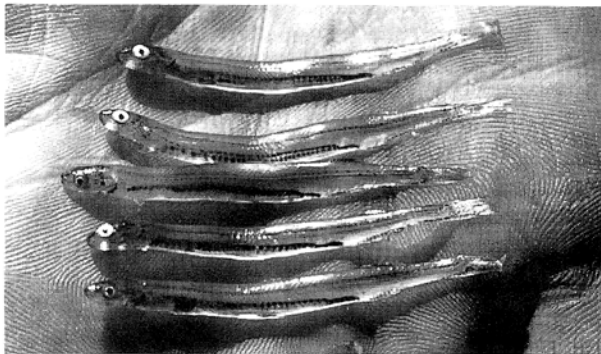


Fig. 2. Salt-tolerant whitebait stage of *Galaxias maculatus* (about 40 mm SL).

This study tested the hypothesis that populations of *G. maculatus* from the western Pacific (Australia and New Zealand) and the eastern Pacific (Chile) do not differ genetically. Significant genetic heterogeneity between populations would be consistent with a vicariant model, whereas its absence would support a dispersal hypothesis.

Materials and Methods

Galaxiids were collected from: Pallinup River, Western Australia (34°04'S, 118°06'E); Gore Creek, Sydney, New South Wales (34°36'S, 149°50'E); Christchurch, New Zealand (43°33'S, 172°40'E); and the Biobio River, Chile (36°50'S, 73°03'W). Specimens from Western Australia, New Zealand and Chile were air-freighted to Macquarie University, Sydney, on dry ice. Specimens from Gore Creek were placed immediately in liquid nitrogen. All material was stored in a freezer at -76°C. Identifications were confirmed by referring to McDowall (1971, 1990) and McDowall and Frankenberg (1981). Meristic and morphometric examination showed that all specimens conformed to the phenotype of *G. maculatus*.

Allozyme electrophoresis was carried out on cellogel following the methods of Richardson *et al.* (1986). Specimens from each population were assayed for 14 enzymes representing 15 genetic loci. Epaxial muscle of at least five specimens from each population was assayed for each enzyme. The following enzymes were examined: adenylate kinase (Ak-A; EC 2.7.4.3), aspartate transaminase (mAta-A; EC 2.6.1.1), creatine kinase (CK-A; EC 2.7.3.2), fructose-bisphosphatase (Fbp-A; EC 3.1.3.11), fructose-bisphosphate aldolase (Fba-A; EC 4.1.2.13), fumarate hydratase (Fumh-A; EC 4.2.1.2), glucose dehydrogenase (Gcdh-A; EC 1.1.1.118), glucose-6-phosphate isomerase (Gpi-A and Gpi-B; EC 5.3.1.9), isocitrate dehydrogenase (NADP+) (mIcdh-A; EC 1.1.1.42), L-lactate dehydrogenase (Ldh-A; EC 1.1.1.27), malate dehydrogenase (mMdh-A; EC 1.1.1.37), malate dehydrogenase (NADP+) (mMdhp-A; EC 1.1.1.40), mannose-6-phosphate isomerase (Mpi-A; EC 5.3.1.8) and phosphoglucomutase (Pgm-A; EC 5.4.2.2). Locus nomenclature follows Buth (1983), with minor modifications.

The BIOSYS-1 program of Swofford and Selander (1981) was used to calculate measures of genetic variation, agreement with Hardy-Weinberg expectations within geographic samples (exact test, using Levene's [1949] correction for small sample size), F -statistics and a contingency χ^2 analysis of allele frequency and genotype homogeneity among populations.

Results

There was very little genetic divergence among populations. Sixteen of 21 alleles observed were common and at the same or similar frequency in all four populations. Nine loci (*mAta-A*, *Fba-A*, *Fbp-A*, *Fumh-A*, *Gcdh-A*, *mIcdh-A*, *mMdhp-A*, *Mpi-A* and *Pgm-A*) were monoallelic for the same allele in each population. Six of the monoallelic loci were scored from five specimens in each sample; *sAta-A*, *Fumh-A* and *Gcdh-A* were scored from ten specimens in each sample. Genotype arrays for the six polyallelic loci are shown in Table 1.

Estimates of average heterozygosity in the four populations (Table 1) revealed a deficit of (expected) heterozygotes. In all four populations exact probabilities revealed a significant ($P < 0.05$) deficit of heterozygotes at *Gpi-B*. Similarly, a significant deficit of heterozygotes at *mMdh-A* was found in the sample from New Zealand.

Table 1. Genotype arrays and mean heterozygosity estimates for four populations of *Galaxias maculatus*

Locus	Genotype	Number of Specimens			
		Western Australia	New South Wales	New Zealand	Chile
<i>Ak-A</i>	aa	0	0	0	1
	ab	0	1	1	0
	bb	5	4	4	4
<i>Ck-A</i>	ab	2	1	3	0
	bb	3	4	2	5
<i>Gpi-A</i>	aa	3	2	0	1
	ab	5	2	0	1
	bb	7	11	15	13
<i>Gpi-B</i>	aa	5	10	9	10
	ab	0	1	2	1
	bb	10	4	4	4
<i>Ldh-A</i>	aa	5	4	2	3
	ab	0	1	2	1
	bb	0	0	1	1
<i>mMdh-A</i>	aa	1	0	3	1
	ab	1	0	0	0
	bb	3	5	2	4
Direct-count heterozygosity (\pm s.e.)		0.062 (0.035)	0.053 (0.022)	0.089 (0.047)	0.022 (0.014)
Expected heterozygosity ^A (\pm s.e.)		0.117 (0.053)	0.091 (0.037)	0.146 (0.058)	0.120 (0.048)

^AUnbiased estimate of Nei (1978).

Unbiased genetic distance values (Table 2), estimated by using Nei's (1978) correction for small sample size, showed that all populations were very similar. There were no unique Chilean alleles and no 'private alleles' (*sensu* Slatkin 1985).

An analysis of homogeneity of allele frequency among populations showed that significant differences exist at the two *Gpi* loci and the *mMdh* locus. Analysis of gene diversity indicated that the F_{ST} (calculated by either allele frequencies or genotype frequencies) was 0.14. This is consistent with a historical migration level of between one and two individuals per generation ($N_e m = 1.6$, where N_e is effective population size and m is migration rate). A homogeneity test of allele frequency differences combined over all six variable loci was highly significant ($\chi^2 = 49.6$, 18 d.f., $P = 0.00009$), indicating that the estimated F_{ST} value also differs significantly from zero.

Table 2. Matrix of Nei's (1978) genetic similarity/distance coefficients. Below diagonal, unbiased genetic distance; above diagonal, unbiased genetic identity

Population	WA	NSW	NZ	Chile
Western Australia (WA)	—	0.987	0.972	0.980
New South Wales (NSW)	0.014	—	0.971	1.000
New Zealand (NZ)	0.028	0.030	—	0.991

Discussion

$F_{ST} = 0.14$ is consistent with a set of historically connected interbreeding populations. Similar values are given by Ward *et al.* (1994) for many anadromous and freshwater fishes. Minor differentiation was present, which indicates that the population is not panmictic, but significant differentiation between the western Pacific and eastern Pacific populations was absent and speciation has not occurred. The heterogeneity that is present is not isolated in Chile but is scattered among the four populations. We suggest that cross-oceanic migration occurs regularly and that migrants may come from any of the western Pacific populations. These populations may even exchange fewer genes with each other than they do with the eastern Pacific population (Table 2).

Although there were slight variations in proportion of alleles present in different populations, the overall allozymic similarity strongly suggests that the populations are conspecific. The low genetic divergence among populations despite their wide geographic distribution suggests that great oceanic distances are not necessarily a long-term barrier to gene flow; this supports the dispersal model of McDowall (1970, 1978). Our results also imply that gene flow continues today, as suggested by McDowall and Whitaker (1975).

There was minor variation within the Western Australian populations at *Gpi-A* and *Gpi-B* (Table 1). Similar differences were found by Barker and Lambert (1988) and Allibone and Wallis (1993) among populations of *G. maculatus* from New Zealand. Campos (1989) found that protein variation among populations of *G. maculatus* from Chile, Australia and New Zealand was small.

The reason for the observed deficit in heterozygotes shown in Table 1 is unknown. Some of the assumptions necessary for equilibrium may be violated, or there could be localized inbreeding in each population. Regardless, our interpretation of low but persistent exchange of genes between localities is not compromised, because the significant F_{ST} value is not due to heterozygote deficiency. The F_{ST} value calculated from genotype frequencies was the same as that calculated from allele frequencies. In fact, the F_{ST} calculated from the *Gpi-B* locus was only 0.10, even though this locus had significant heterozygote deficits in all populations.

The meristic and morphological data presented in McDowall's papers, combined with the allozyme data presented here, support the concept of a single species that has dispersed around the Southern Hemisphere. Gene flow via dispersal would tend to maintain a gene pool with low interpopulation differentiation, as encountered in this study. Moreover, it is extremely unlikely that populations of *G. maculatus*, if isolated in South America for the past 65 million years or so, would appear to be conspecific with populations from Australia and New Zealand today, given normal rates of mutation and drift between long-isolated populations, even assuming that similar types of selection may have been operating in these populations.

Under a neutral model, only a small amount of gene flow is necessary to prevent accumulation of genetic differences by random drift. Allendorf and Phelps (1981), citing the work of Wright (1943), suggested that one migrant individual per local population per generation generally was sufficient to obscure any disruptive effects of drift. The marine larval stage of *G. maculatus* has a six-month period to traverse the distance between the southern continents (McDowall *et al.* 1994) and could provide enough gene flow to deter fixation for alternative alleles.

Ovenden *et al.* (1993) found little allozymic variation between landlocked and diadromous species of *Galaxias*, but they did find considerable differentiation in mitochondrial DNA. They attributed the differences to reproductive isolation of landlocked populations. MtDNA analysis in progress may shed more light on genetic divergence among geographic populations of *G. maculatus*.

Acknowledgments

We thank V. Ruiz (Chile), G. and M. Allen (Western Australia), and R. McDowall and J. Sykes (New Zealand) for shipment of frozen specimens; and D. Buth for conducting

BIOSYS-1 analyses and for advice on the interpretation of the analyses. We also thank R. Cashner, J. Gold, R. McDowall, A. Meyer, G. Wallis and J. Williams for comments on earlier drafts of the manuscript. Figure 2 was printed by J. Sprang from a colour slide. The first author was supported by travel funds provided by The Ohio State University.

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Manuscript received 8 January 1996; revised and accepted 26 February 1996