Full Paper

A PRELIMINARY STUDY ON ALLOZYME GENETIC CHARACTERIZATION OF *Galaxias truttaceus* VALENCIENNES (PISCES : GALAXIIDAE) IN WESTERN VICTORIA, AUSTRALIA

STUDI AWAL KARAKTERISASI GENETIK ALLOZIM *Galaxias truttaceus* VALENCIENNES (PISCES : GALAXIIDAE) DI BAGIAN BARAT VICTORIA, AUSTRALIA

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Abstract

The purpose of this study was to determine allozyme genetic characters of the spotted galaxid in western Victoria, Australia. Two populations, Barongarook Creek (landlocked population) and Dutton Creek (diadromous population), of the spotted galaxias (*Galaxias truttaceus*) was investigated on their genetic characters by allozyme electrophoresis methods. From 23 system enzymes and general protein, 39 putative enzyme-coding loci were resolved, 3 of which were polymorphic at Esterase-1 (*Est-1*), Glycerol-3-phosphate dehydrogenase-2 (*Gpdh-2*), and Phosphogluconate hydrogenase (*6pgd*). Fixed allozyme differences were found at *Est-1* locus indicating that incipient change in genetic character have been started in the landlocked population.

Key words : allozyme electrophoresis, *Galaxias truttaceus*, genetic characterization

Introduction

The spotted galaxias, *Galaxias truttaceus*, is mainly found in coastal drainages of southern Victoria, Tasmania, the islands of Bass Strait, and Western Australia. The fish is mostly abundant in low elevation streams which are relatively close to the sea and the fish can also be found in lakes and lagoons, often at higher elevation (Merrick and Schmida, 1984; McDowall, 1996). The fish species is normally 120-140 mm in total length, but will attain over 200 mm. The fish appears to be carnivorous, feeding on small invertebrates and surface prey such as beetles, spiders, bugs, and ants (Merrick and Schmida, 1984).

Life cycle of these spotted galaxiids are diverse. These fish species spend their entire lives in freshwater but display considerable variation in reproductive patterns and characteristics. Most of the spotted galaxias is known as riverine or diadromous fish which ussually spawn in autumn and the larvae spend 3 months of winter at sea before returning to fresh water in spring (Ovenden and White, 1990). The fish then require a further 18 months or more in freshwater habitats before reproductive maturity is reached (Mc Dowall, 1996). Generally riverine fish species have the potential for long-distance dispersal of larvae, resulting in genetic connection between populations over large distances (Ward *et al*., 1994). In some cases, however, lacustrine or landlocked spotted galaxiids have no marine larval stage and have limited migrating capabilities of the adults because their life history is confined to freshwater. This lacustrine spotted galaxias ussually spawn in spring and spend the entire life cycle in lakes

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(Humpries, 1989). Some characteristics of fish species which have the typical life cycle may produce genetic divergence among populations due to the fish species restricted gene flow (Carvalho, 1993). A consequence of life history divergences of the spotted galaxias fish is thought to be important factor to account for high diversity of galaxiid fishes in Australia (Humpries, 1990; Ovenden and White, 1990; Ovenden *et al*. 1993).

The main objective of this study was to determine allozyme genetic characters of the spotted galaxias in western Victoria, Australia at 23 enzyme systems and general proteins representing 39 presumptive gene loci. This investigation is needed to examine whether different reproductive patterns of the galaxiid species reveal change in the fish species allozyme genetic characters. The findings of this research are expected to yield valuable information concerning incipient speciation due to landlocking of this galaxiid species together with further studies.

Materials and Methods

Specimen collection and storage
Specimens of *G. truttaceus* from Barongarook Creek ($143^0 39'$E, $38^0 22'$S; 5 samples) and Dutton Creek ($141^0 30'$E; $38^0 20'$S; 5 samples) were caught using either baited fish traps or a seine net. Based on morphological characters identified from McDowall and Frankenberg (1981) and McDowall (1996), all individuals collected in this study were *G. truttaceus*. The collection of specimens was made under Permit No. FSP/CW/225 (2-3) from the Department of Natural Resources and Environment, Victoria, Australia. Procedures for handling of live fish and their euthanasia used in this study were approved by Deakin University's Annual Ethics Committee.

The fish were transferred alive to the laboratory and killed in iced water. Subsequently, muscle, liver, and gill tissue were dissected from each partially thawed fish and placed immediately into 1.5 ml screw cryogenic vials for storage at -80^o C until required for allozyme analyses.

Sample preparation
Approximately 0.5 grams of each sample of epaxial muscle, eye and liver was ground with grinding solution containing 10% (w/v) sucrose, 0.1% (w/v) bromophenol blue and 0.1% (v/v) mercaptoethanol using a Microson Ultrasonic Cell Disruptor (Model XL 2005) operated at approximately 20% power for 3-5 second. Homogenised extracts were then centrifuged at 13,000 rpm to pellet tissue debris. Next, the supernatant was absorbed onto Whatman filter paper rectangles (5x3 mm in size) for starch gel electrophoresis. The supernatant was also applied for cellulose acetate gel electrophoresis (CAGE) using the Helena applicator system described by Hebert and Beaton (1993).

Allozyme electrophoresis procedures
Allozyme electrophoresis procedures followed those described in Shaklee and Keenan (1986) and Murphy *et al*. (1996) for starch gel electrophoresis. The method of Richardson *et al.* (1986) and Hebert and Beaton (1993) was used for cellulose acetate electrophoresis. Enzymes were referred to the Enzyme Commision (EC) Number and nomenclature for enzyme coding loci followed the recommendations of Richardson *et al*. (1986). Buffer systems and volumes, running conditions, and staining procedures were essentially those of Richardson *et al.* (1986), Shaklee and Keenan (1986) and Murphy *et al.* (1996) for starch gel electrophoresis and Hebert and Beaton (1993) for cellulose acetate gel electrophoresis (Table 1). Staining procedures utilised agar overlays for all systems except esterase (EST), glutamate oxaloacetate (GOT), and general protein (GP). For EST and GOT enzyme gels were presoaked in

substrate solution before adding fast blue BB. General protein resolved by staining gels with a solution of amido black followed by a series of washes in a methanol : distilated water : acetic acid (5:5:1) solution. In order to determine the identical zymograms, the other galaxiid fishes (*G. maculatus*, *G. olidus*, and *G. occidentalis*) were included as an output group and used as comparative purposes.

Data Analysis

The most common allele was assigned arbitrarily the value 100. Slower and faster bands on the zymograms representing other alleles were given lower or higher numbers corresponding to their relative mobilities respectively. Calibration of alleles was accomplished by running individuals from different populations on the same electrophoretic membrane.

Electrophoretic banding patterns were interpreted using standard procedures (Richardson *et al.*, 1986) and converted to genotypes. Genotype data were then analyzed using the BYOSIS-1 program (Swofford & Selander, 1981) which demontrated levels of genetic variability between populations of this species. From this program, the frequencies of each allele from each locus for each population were also determined. In addition, the genetic identity (I) explained by Nei (1978) were calculated to evaluate genetic relationship between populations investigated in this study.

Result and Discussion

The allozyme electrophoretic patterns revealed that the enzymes examined were controlled by genes at 39 presumptive loci. For each population investigated, no more than two alleles per locus were observed. In 36 loci, all individuals exhibited single bands of identical mobility. This is assumed that these enzymes are encoded by single, monomorphic loci at which every individual is homozygous for the same allele. Analysis of allozyme data also revealed that *Est-1, Gpdh-2, 6pgd* were polymorphic (Table 2) with two banded phenotypes found at *Est-1* and three banded phenotypes found at *Gpdh-2* and *6pgd*. Such phenotypes indicate that the active enzyme is a monomer for *Est-1* and a dimer for *Gpdh-2* and *6pgd*, and the typical two and three banded phenotypes represent heterozygotes.

It can be seen as in Table 2 that variation at the *Est-1* locus is principally responsible for genetic differences between the normal diadromous population (Dutton Creek) and the landlocked population (Barongarook Creek). Two alleles were observed at the *Est-1* locus with the *Est-1**100 occuring at high frequency (0.70) in Dutton Creek, but is absent in Barongarook Creek. A possible explanation of this genetic differences is that the change in life history of the diadromous *G. truttaceus* population. This was also commonly found in the diadromous *G. truttaceus* populations in Tasmania (Humpries, 1989; Humpries, 1990; Ovenden and White, 1990) and *G. maculatus* populations in western Victoria, Australia (Arisuryanti, 1999). A consequence of change in life history is therefore change in genetic characters and over sufficiently long periods of time this can lead to speciation. Speciation due to landlocking is thought to be a frequent occurrence within Galaxias (Ovenden and White, 1990) and is thought to be important factor to account for high diversity of galaxiid fishes in Australia (Watts, 1995; Arisuryanti, 2000). In addition, the landlocked population has probably experienced at least one, severe, but transitory bottleneck possibly induced by natural selection for life-history characters essential for survival in the lacustrine habitat.

Table 1. Stains and buffer used in the electrophoretic analysis of *Galaxias truttaceus* samples

Enzyme name	Locus	E.C.[1] number	No. of loci	Buffer[2]	Tissue[3]	Electro-phoresis[4]
Alcohol dehydrogenase	*Adh*	1.1.1.1	1	TG	L	CA
Creatine kinase	*Ck*	2.7.3.2	1	TC8	L	S
Esterase	*Est*	Non-specific	2	TC8	L	S
Fructose biphosphate	*Fbp*	3.1.3.11	2	TC8	L	S
Fructose biphosphate aldolase	*Fba*	4.1.1.13	2	TC8	M	S
General proteins	*Gp*	Non-specific	6	Poulik	M	S
Glucose-6-phosphate dehydrogenase	*G6pdh*	1.1.1.49	1	TC6	G	S
Glutamate oxaloacetate	*Got*	2.6.1.1	2	TC8	L	S
Glutamate dehydrogenase	*Gdh*	1.4.1.2	1	TC8	L	S
Glycerol-3-phosphate dehydrogenase	*Gpdh*	1.1.1.8	2	TG	L	CA
Isocitrate dehydrogenase	*Idh*	1.1.1.42	1	TC8	L	S
Lactate dehydrogenase	*Ldh*	1.1.1.27	1	TC8	L	S
Malate dehydrogenase	*Mdh*	1.1.1.37	1	TC8	L	S
Mannose-6-phosphate isomerase	*Mpi*	5.3.1.8	1	TG	L	CA
Peptidase L-leucyl-glycyl-glycine	*Pep-LGG*	3.4.11.4	2	Poulik	L	S
Peptidase L-leucyne-proline	*Pep-LP*	3.4.13.9	1	Poulik	L	S
Peptidase L-leucyl-L-tyrosine	*Pep-LT*	3.4.13.11	2	Poulik	M	S
Peptidase glycyl-L-leucine	*Pep-GL*	3.4.x.x	2	Poulik	M	S
Phosphoglucomutase	*Pgm*	5.4.2.2	2	TC8	L	S
Phosphogluconate hydrogenase	*6pgd*	1.1.1.44	1	TC6	G	S
Glucose-6-phosphate isomerase	*Pgi*	5.3.1.9	2	TC8	M	S
L-iditol-dehydrogenase	*Sdh*	1.1.1.14	2	TC8	L	S
Superoxide dismutase	*Sod*	1.15.1.1	1	TG	L	CA

[1] EC = Enzyme Commision Number
[2] See Murphy *et al.* (1996) and Hebert and Beaton (1993) for buffer recipes
[3] Tissue : L = Liver; M = Muscle; G = Gill
[4] Electrophoresis : S = Starch gel; and CA = Cellulase acetate gel

Table 2. Allele frequencies of polymorphic loci. Sample size in parentheses

Locus	Allele	Sample	
		Barongarook Creek (5)	Dutton Creek (5)
Est-1	108	1.00	0.30
	100	-	0.70
Gpdh-2	109	0.90	1.00
	91	0.10	-
6pgd	95	-	0.40
	90	1.00	0.60

On the basis of of genetic identity values, the two population of *G.truttaceus* are still to be conspecific (I=0.980). According to Richardson *et al.* (1986), genetic identity levels can be used as a guide to taxonomic status. In general, authors taking this approach consider populations separated by genetic identity values of less than 0.85 to belong to separate species and those that have genetic identity values of more than 0.85 to be conspecific. Nevertheless, the finding of the diagnostic locus revealing genetic difference found in the two *G. truttaceus* populations may indicate incipient speciation by the recent establishment of their reproductive pattern. Moreover, on the basis of this finding, it is demonstrated that morphological similarity does not necessarily equate with genetic similarity. Morphological plasticity, convergent evolution, and cryptic speciation appear to be relatively common phenomenon for freshwater organisms (Austin & Knott, 1996).

Further investigations from other genetic characters such as mitochondrial gene regions will be needed to clarify more precisely the change in genetic characters due to change in life history of *G. truttaceus* in western Victoria.

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References

Arisuryanti, T. 1999. Molecular Genetic Variation in *Galaxias maculatus* (Osteichthyes : Galaxiidae). M.Sc. Thesis Deakin University, Victoria, Australia. 31-47.

Arisuryanti, T. 2000. A preliminary study of genetic variation of *Galaxias olidus* (Slamoniformes : Galaxiidae) in western Victoria, Australia. Biologi 9 (2) : 487-498.

Austin, C.M. and B. Knott. 1996. Systematic of the freshwater crayfish genus *Cherax* (Decapoda : Parastacidae) in south western Australia : Electrophoretic, morphological and habitat variation. Australian Journal of Zoology 44 : 223-258.

Carvalho, G. R. 1993. Evolutionary aspects of fish distribution: genetic variability and adaptation. Journal of Fish Biology 43 : 53–73.

Hebert, P.D.N. and Beaton, M.J. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Department of Zoology, University of Guelph. 32 p.

Humpries, P. 1989. Variation in the life history of diadromous and landlocked populations of the

spotted galaxias, *Galaxias truttaceus* Valenciennes, in Tasmania. Australian Journal of Marine and Freshwater Research 40 : 510-518.

Humpries, P. 1990. Morphological variation in diadromous and landlocked populations of the spotted galaxias, *Galaxias truttaceus*, in Tasmania, south-eastern Australia. Environmental Biology of Fishes 27 : 97-105.

McDowall, R.M.1996. Freshwater fishes of South-eastern Australia. Reed Books. Australia. 77 p.

McDowall, R.M. and R.S. Frankenberg. 1981. The galaxiid fishes of Australia (Salmoniformes : Galaxiidae). Records of the Australian Museum 33 : 443-605.

Merrick, J.R. and G.E. Schmida. 1984. Australian freshwater fishes : Biology and Management. Griffin Press Limited. Australia. 105 p.

Murphy, R.W., J.W.J. Sites, D.G. Buth, C.H. Haufler. 1996. Proteins : Isozyme Electrophoresis. In : Molecular Systematics. Hillis, D.M., C. Moritz and B.K. Mable. Sinauer Associates, Inc., Sunderland. USA. 51-120.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89 : 583-590.

Ovenden, J.R. and R.W.G. White. 1990. Mitochondrial and Allozyme Genetics of Incipient Speciation in a Landlocked Population of *Galaxias truttaceus* (Pisces: Galaxiidae). Genetics 124(3): 701–716.

Ovenden, J.R., R.W.G. White, and M. Adams. 1993. Mitochondrial and allozyme genetics of two Tasmanian galaxiids (*Galaxias auratus* and *Galaxias tanycephalus* (Pisces : Galaxiidae) with restricted lacustrine distributions. Heredity 70 : 223-230.

Richardson, B.J., P.R. Baverstock, and M. Adams. 1986. Allozyme electrophoresis. Academic Press. Australia. 377 p.

Shaklee, J.B. and C.P. Keenan. 1986. A practical laboratory guide to the techniques and methodology of electrophoresis and its application to fish fillet identification. Australian CSIRO Marine Laboratory Rep. : 177.

Swofford, D.L. and M.B. Selander. 1981. BIOSYS-1. A fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72 : 281-283.

Ward, R. D., M. Woodwark and D. O. F. Skibinski. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. Journal of Fish Biology 44 : 213–232.

Watts, R.J., A.W. Storey, D.R. Hebbert and D.H.D. Edward. 1995. Genetic and morphological differences between populations of the Western Minnow, *Galaxias occidentalis*, from two river systems in south-western Australia. Marine and Freshwater Research 46 : 769-777.