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Mitochondrial and allozyme genetics of two Tasmanian galaxiids (*Galaxias auratus* and *G. tanycephalus*, Pisces: Galaxiidae) with restricted lacustrine distributions

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Galaxias auratus and *G. tanycephalus* have a restricted distribution in Tasmanian highland lakes and were probably derived from a landlocked population of the diadromous *G. truttaceus* during the last 100,000 years. We have analysed the mitochondrial DNA and the allozyme products of 22 nuclear loci for each lake species. Four mitochondrial DNA haplotypes, defined by 40–42 six-base restriction sites, were identified amongst 26 *G. auratus* and three haplotypes, defined by 38–39 restriction sites, were found in six *G. tanycephalus*. The evolutionary genetics of reproductive isolation appears to have inflated mitochondrial but not nuclear DNA diversity between the lake species and their hypothesized progenitor, *G. truttaceus*. The amount of intraspecific mitochondrial and nuclear DNA diversity for *G. auratus* and *G. tanycephalus* is consistent with their having an extended and independent evolutionary history in reproductive isolation.

Keywords: allozymes, evolution, *Galaxias*, landlocking, mitochondrial DNA.

Introduction

Nucleotide sequence diversity of mitochondrial DNA (mtDNA) between and within closely related animal species provides an excellent record of evolutionary history (Brown, 1985; Wilson *et al.*, 1985; Avise *et al.*, 1987). The mitochondrial genome is particularly useful because mutations are generally passed from mother to offspring (Lansman *et al.*, 1983; Gyllensten *et al.*, 1985) and are never transferred out of lineages by recombination (Avise *et al.*, 1987). In an interbreeding, reproductively isolated population, the rate of lineage loss depends on the variance of family size and individual survivorship to reproductive age (Avise *et al.*, 1984), while the rate of lineage gain depends on the rate of faulty mtDNA replication and repair and stochastic forces associated with oocyte production (Birky *et al.*, 1983).

Mitochondrial DNA analysis has been particularly appropriate for the exploration of evolutionary history within and between closely related freshwater fish species. A relative lack of mtDNA diversity is often the only clue that a bottleneck event (Carson, 1968) may have occurred, especially if the event was transitory. For example, 61 *Galaxias truttaceus* sampled from central Tasmanian lakes were found to represent only two mtDNA lineages compared to the 58 lineages which were found amongst 150 stream fish of the same species (Ovenden & White, 1990). In the same study, the relatively high amount of nuclear DNA diversity in the landlocked, compared to the stream populations of *G. truttaceus* was deduced to be a natural consequence of their youth. Bermingham & Avise (1986) and Ovenden *et al.* (1988) found that the branching order of phylogenies describing mtDNA evolution in populations of *Amia*, *Lepomis* and *Gadopsis* species could be independently verified by the known geological history of their habitats. This was attributed to the pronounced degree of geographical isolation experienced by populations in different catchments.

The Galaxiidae is a family of small scaleless freshwater fish found in Australia, New Zealand, South America and South Africa as well as some small Pacific islands (Berra, 1981). The greatest species diversity occurs in Australia which has 20 species, including 18 endemics, and New Zealand, which has 13 species of which 11 are endemic. In Australia, 15 species are found in Tasmania, 10 of which are restricted to the

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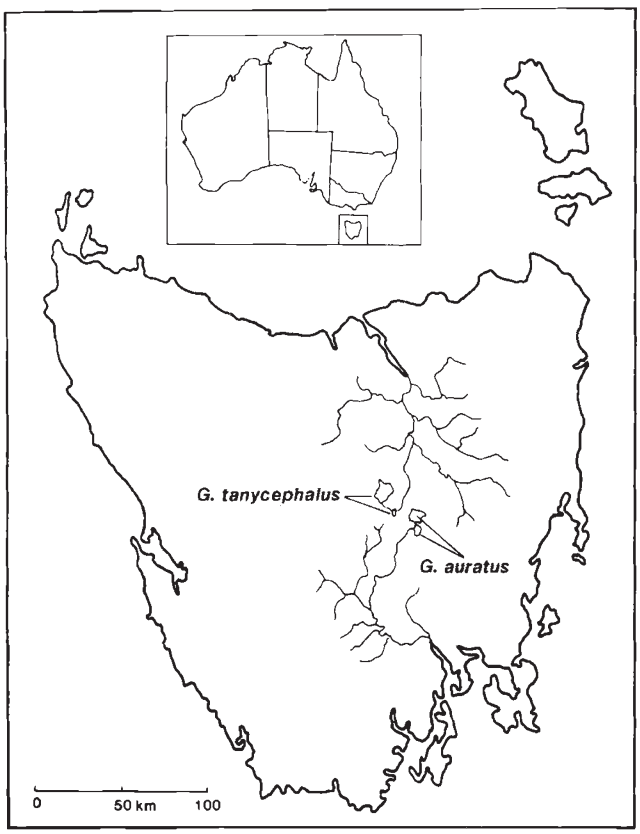

Fig. 1 Distribution of *G. auratus* (Lakes Sorell and Crescent) and *G. tanycephalus* (Arthurs and Woods Lakes).

island state (McDowall & Frankenberg, 1981). The family is represented by diadromous and landlocked freshwater species in riverine and lacustrine habitats.

Three species (*G. truttaceus, G. auratus* and *G. tanycephalus*) form a distinct clade within the genus. This has been confirmed by studies of their morphology (Fulton, 1978; McDowall & Frankenberg, 1981; Johnson *et al.*, 1983) and karyology (Johnson *et al.*, 1981). *G. tanycephalus* is found only in Arthurs and Woods Lakes on the Central Plateau of Tasmania, while *G. auratus* is found only in Lakes Crescent and Sorell. The third species, *G. truttaceus*, is widespread in coastal drainages in south eastern Australia, as well as in a few landlocked lakes on the Central Plateau. Adult riverine *G. truttaceus* spawn in autumn and the larvae spend the winter at sea before returning to freshwater in spring. Lacustrine *G. tanycephalus, G. auratus* and *G. truttaceus* spawn in spring and have an entirely freshwater life cycle (Humphries, 1989).

The derivation of landlocked, entirely freshwater species from inland invading diadromous species is thought to be a major speciating force in the genus (McDowall, 1972; Andrews, 1976). Accordingly, *G. auratus* and *G. tanycephalus* are assumed to be landlocked derivatives of the ubiquitous *G. truttaceus* (Fulton, 1978; Humphries, 1989). In a previous study (Ovenden & White, 1990), the mitochondrial and allozyme genetics of a landlocked population of *G. truttaceus* were characterized in comparison to those of representative diadromous populations. The genetic character of the landlocked population: an almost complete lack of mtDNA diversity but without a concommitant decrease in nuclear gene heterozygosity, was hypothesized to be associated with incipient speciation. In this study, we present mtDNA and allozyme data from populations of *G. auratus* and *G. tanycephalus* and speculate that they have experienced similar events in their evolutionary history.

Materials and methods

Galaxias auratus (Lake Crescent 42°10′S 147°10′E) and *G. tanycephalus* (Arthurs Lake 42°00′S 146°55′E, Fig. 1) were collected by electrofishing. The mitochondrial genomes of 26 *G. auratus* and six *G. tanycephalus* were analysed. Nuclear allele frequencies were assayed from 40 *G. auratus* and six *G. tanycephalus*. The rarity and secretive habits of *G. tanycephalus* made it impractical to obtain a larger sample. The techniques used for the analysis of mtDNA and for the assay of alleles at 22 enzymic loci have been reported (Ovenden & White, 1990).

The location of restriction sites recognized by 13 endonucleases (*Apa*LI, *Bam*HI, *Bcl*I, *Bgl*II, *Bst*EII, *Hin*dIII, *Nco*I, *Pst*I, *Pvu*II, *Sac*II, *Sal*I, *Xba*I, *Xho*I) were mapped within the mtDNA of *G. auratus* and *G. tanycephalus*. This was done by comparing the size and number of digestion products with those produced from *G. truttaceus* mtDNA (Ovenden & White, 1990). Novel sites were mapped using the double digestion technique (Ovenden *et al.*, 1992).

Haplotypes were a composition of the alphabetic restriction site morphs for each of the 13 restriction enzymes used. Restriction site presence and absence data were used to calculate sequence diversity (number of base substitutions per base pair) between each pair of haplotypes (Nei & Li, 1979; Nei & Tajima, 1983). In addition, mean intraspecific and interspecific diversity, with their appropriate standard errors (Nei & Jin, 1989), were calculated for all genomes assayed. Nei's genetic distances and their standard errors were calculated from nuclear allele frequencies (Nei, 1972; Tomiuk & Graur, 1988). The expected nuclear gene heterozygosity and its variance (Nei & Roychoudhury, 1974), corrected for small sample size for *G. tanycephalus* (Nei, 1978), were calculated for each species.

Results

Mitochondrial DNA

Three haplotypes were found amongst the six *G. tanycephalus*. Four fish possessed haplotype no. 2 (ABBADABAAAABB), while haplotypes no. 1 (ABBADABAAAABA) and no. 3 (ABBAEABAAAABB) were found in one fish each. There were four *G. auratus* haplotypes. Haplotype no. 4 (ABBADADABAGBB) was the most common, being found in 22 fish. Two fish possessed haplotype no. 5 (ABCADADABAGBB), and haplotype no. 6 (ABFADADABAGBB) and no. 7 (ABBADCDABAGBB) were found in one fish each. The restriction site composition of each haplotype is shown in Table 1 and the diversity between them in Table 2.

The sequences of the mitochondrial genomes of the 26 *G. auratus* samples were more similar to each other (mean intraspecific mitochondrial genome diversity across all genomes, 0.075 ± 0.054 per cent) than were those of the six *G. tanycephalus* samples (0.146 ± 0.119 per cent; Table 3). In terms of interspecific mtDNA sequence diversity, the *G. auratus* samples were more similar to those of *G. tanycephalus* (0.864 ± 0.448 per cent) than either was to the *G. truttaceus* sampled from stream habitats by Ovenden & White (1990, Table 4).

The degree of restriction site similarity of *G. tanycephalus* and *G. auratus* to *G. truttaceus* (Ovenden & White, 1990) suggests that their mtDNA is the same size. None of the *G. tanycephalus* and *G. auratus* restriction sites which were present in some, but not all, of the haplotypes fell within the 4260 bp region, which was previously identified as being highly conserved in *G. truttaceus* (Ovenden & White, 1990). No cases of intra-individual or inter-individual genome length heteroplasmy were recorded.

Allozymes

Of the 22 loci assayed in *G. auratus* and *G. tanycephalus*, 18 showed the same or similar allele frequencies in both species (Table 5). Genetic differences were detected between the remaining five loci. There was a fixed difference at the peptidase-D locus. At the guanine deaminase locus, the alleles Gda^c and Gda^e were the most common in *G. auratus* while Gda^a and Gda^d were the most common in *G. tanycephalus*. At the malate dehydrogenase-2 locus, allele *b* was fixed in *G. auratus*, but present at low frequency in *G. tanycephalus* and accompanied by allele *a*. Three alleles were present at the phosphoglucomutase-1 locus (*a*, *c* and *e*) while alleles *d* and *e* were present in *G. tanycephalus*.

Nuclear gene heterozygosity was 0.065 ± 0.014 (mean across all loci $\pm$ variance) for *G. auratus* and 0.083 ± 0.017 for *G. tanycephalus*. Twelve or 55 per cent of the 22 loci were polymorphic in the sample of *G. auratus*, while 7 or 32 per cent of the loci were polymorphic in *G. tanycephalus* (Table 5). There was an average of 1.68 alleles per locus in the *G. auratus* sample and an average of 1.32 alleles per locus in the *G. tanycepalus* sample.

Nei's modified genetic distance between *G. auratus* and *G. tanycephalus* was 0.253 ± 0.112 ($\pm$ standard error). Unlike the mitochondrial genome, nuclear genome similarities indicated that *G. tanycephalus* was more closely related to stream populations of *G. truttaceus* (0.086 ± 0.049 units) analysed by Ovenden & White (1990), than to *G. auratus*. *Galaxias auratus* was separated by 0.149 ± 0.079 units of Nei's modified genetic distance from the stream populations of *G. truttaceus* analysed by Ovenden and White (Table 4).

Discussion

Extended evolution in an isolated habitat of finite size may be responsible for the lack of nuclear gene heterozygosity within *G. auratus* (0.065) and *G. tanycephalus* (0.083) relative to that of the recently formed lacustrine population of *G. truttaceus*. In the previous analysis by Ovenden & White (1990), the lacustrine population of *G. truttaceus* had a similar amount of nuclear gene heterozygosity (0.104) to their diadromous relatives (0.116) which occur in coastal streams. The limited resources available to these lake populations places an upper limit on population size, which does not affect genetic variability in the short term. This is shown by the large amount of allozyme heterozygosity retained from their diadromous ancestors by the recently formed lacustrine population of *G. truttaceus*. However, we suggest that genetic drift has led to a decrease in heterozygosity in *G. auratus* and *G. tanycephalus* which has not been augmented by immigration, as each population is reproductively isolated, or by mutation, as each population is too small.

Estimates of the amount of mtDNA variation within *G. auratus* (0.075 per cent) and *G. tanycephalus* (0.146 per cent) populations are significantly higher than that of the lacustrine *G. truttaceus* (effectively 0 per cent) previously analysed by Ovenden & White (1990). mtDNA variation was probably reduced in lake populations of *G. truttaceus* by a severe, but brief, bottleneck event, possibly associated with the establishment of the population. Populations of *G. auratus* and *G. tanycephalus* may have experienced bottleneck events in the past, but their greater age and possible abundance have masked the possible influence of the

Table 1 The presence (1) or absence (0) of restriction sites in mitochondrial haplotypes in two species of galaxiids. The composite restriction morph of each haplotype is ABBADABAAAABA (1), ABBADABAAAABB (2), ABBAEABAAAABB (3), ABBADADABAGBB (4), ABCADADABAGBB (5), ABFADADABAGBB (6) and ABBADCDABAGBB (7). The sites are described as the number of base pairs clockwise from the single *Bgl*II site. Sites not previously identified amongst 216 *G. truttaceus* genomes (Ovenden & White, 1990) are *Bcl*I sites 3, 4 and 5, *Xho*I site 6 and *Xba*I site 5

Site position		*G. tanycephalus*			*G. auratus*			
		1	2	3	4	5	6	7
*Bgl*II site 1	0	1	1	1	1	1	1	1
*Pst*I site 1	0	1	1	1	1	1	1	1
*Hin*dIII site 1	100	1	1	1	1	1	1	1
*Bcl*I site 2	613	0	0	0	0	1	0	0
*Hin*dIII site 2	984	1	1	1	1	1	1	1
*Bst*EII site 1	1341	1	1	1	1	1	1	1
*Xba*I site 2	1700	1	1	1	1	1	1	1
*Pst*I site 3	1707	1	1	1	1	1	1	1
*Pvu*II site 1	2800	1	1	1	1	1	1	1
*Bam*HI site 1	2883	1	1	1	1	1	1	1
*Sal*I site 3	3088	1	1	1	1	1	1	1
*Nco*I site 1	3100	0	0	0	1	1	1	1
*Nco*I site 2	3300	1	1	1	0	0	0	0
*Xho*I site 2	3700	1	1	1	1	1	1	1
*Nco*I site 3	4183	1	1	1	1	1	1	1
*Sal*I site 4	4452	1	1	1	1	1	1	1
*Xho*I site 3	4825	0	1	1	1	1	1	1
*Xho*I site 4	4888	1	1	1	1	1	1	1
*Pvu*II site 3	5117	1	1	1	1	1	1	1
*Bst*EII site 3	5187	1	1	1	1	1	1	1
*Nco*I site 4	5733	1	1	1	1	1	1	1
*Hin*dIII site 4	5934	1	1	1	1	1	1	1
*Pvu*II site 5	7018	0	0	0	1	1	1	1
*Sal*I site 6	7475	1	1	1	1	1	1	1
*Sal*I site 5	7540	0	0	0	1	1	1	1
*Bst*EII site 5	7687	1	1	1	1	1	1	1
*Sac*II site 3	9094	1	1	1	1	1	1	1
*Hin*dIII site 5	9180	1	1	1	1	1	1	1
*Apa*LI site 1	9616	1	1	1	1	1	1	1
*Xba*I site 3	9626	1	1	1	1	1	1	1
*Xho*I site 6	9812	1	1	1	1	1	1	1
*Xba*I site 4	9853	1	1	1	1	1	1	1
*Apa*LI site 2	10709	1	1	1	1	1	1	1
*Sac*II site 4	10950	1	1	1	1	1	1	1
*Pst*I site 6	11412	1	1	1	1	1	1	1
*Pvu*II site 6	11876	1	1	1	1	1	1	1
*Hin*dIII site 6	11954	1	1	1	1	1	1	0
*Hin*dIII site 7	13075	1	1	1	1	1	1	1
*Bcl*I site 4	13143	0	0	0	0	0	1	0
*Bst*EII site 6	13347	1	1	0	1	1	1	1
*Hin*dIII site 8	14018	1	1	1	1	1	1	1
*Xba*I site 5	14234	1	1	1	1	1	1	1
*Bcl*I site 5	15260	1	1	1	1	1	0	1
*Nco*I site 7	16231	1	1	1	1	1	1	1

Table 2 The sequence diversity (number of base substitutions per base pair, above diagonal) and standard errors (below diagonal) between three *G. tanycephalus* and four *G. auratus* haplotypes. Haplotypes are numbered in Table 1

G. tanycephalus, 1	—	0.00218	0.00445	0.01092	0.01302	0.01550	0.01337
G. tanycephalus, 2	0.00219	—	0.00218	0.00856	0.01064	0.01302	0.01092
G. tanycephalus, 3	0.00319	0.00219	—	0.01092	0.01302	0.01550	0.01337
G. auratus, 4	0.00504	0.00439	0.00504	—	0.00202	0.00412	0.00207
G. auratus, 5	0.00553	0.00491	0.00553	0.00203	—	0.00614	0.00412
G. auratus, 6	0.00613	0.00553	0.00613	0.00295	0.00361	—	0.00630
G. auratus, 7	0.00568	0.00504	0.00568	0.00208	0.00295	0.00371	—

Table 3 Mitochondrial and nuclear DNA variability within three species of galaxiid fish

	Intraspecific mtDNA diversity (%)	*n*	Nuclear gene heterozygosity	*n*
G. auratus	0.075	26	0.065	40
G. tanycephalus	0.146	9	0.083	6
*G. truttaceus**	0.624	150	0.116	82
G. truttaceus‡	0	66	0.104	40

*Sampled from 12 (mtDNA) or two (nuclear DNA) coastal drainages in south-eastern Australia (Ovenden & White, 1990).
‡Sampled from four (mtDNA) or one (nuclear DNA) central Tasmanian lake (Ovenden & White, 1990).

Table 4 The number of mtDNA base substitutions per base pair (corrected for intraspecific diversity, above diagonal) and Nei's modified genetic distance (below diagonal) between three species of galaxiid fish

	G. auratus	*G. tanycephalus*	*G. truttaceus*
G. auratus	—	0.864%	1.915%
G. tanycephalus	0.253	—	1.025%
*G. truttaceus**	0.149	0.086	—

*Sampled from 12 (mtDNA) or two (nuclear DNA) coastal drainages in south-eastern Australia (Ovenden & White, 1990).

event upon mtDNA diversity. Interestingly, the most abundant present day population, *G. auratus*, does not have the greatest mtDNA diversity.

Speciation following landlocking of diadromous populations of *G. truttaceus* is the most tenable explanation of the evolution of *G. auratus* and *G. tanycephalus*, given their biogeography and phenotypic relationship to *G. truttaceus* and the tendency of *G. truttaceus* to intrude gradually into inland waters (McDowall, 1972; Andrews, 1976; Fulton, 1978). The formation of lacustrine populations of *G. truttaceus via* landlocking, and its genetic implications, have previously been studied by Ovenden & White (1990).

The habitats of *G. auratus* and *G. tanycephalus* (Lakes Sorell and Crescent and Arthurs and Woods Lakes) are more ancient than the shallow, post-glacial lakes currently occupied by *G. truttaceus*. The exact ages of the older lakes are unknown but it is likely that they were formed by geomorphological activity less than 100,000 years ago and occur outside the areas affected by the Pleistocene glaciation (Davies, 1974). While the ancestors of the lacustrine *G. truttaceus* may have become landlocked 3000–7000 years ago (Ovenden & White, 1990), it is possible that the ancestors of *G. auratus* and *G. tanycephalus* may have invaded the precursors of their current habitats as long as 100,000 years ago.

Arthurs and Woods Lakes drain to the north while Lakes Sorell and Crescent flow to the south. It is possible, however, that Arthurs and Woods Lakes previously drained southwards as they share an endemic galaxiid genus (*Paragalaxias*; McDowall & Fulton, 1978) and a syncarid crustacean species (*Paranaspides lacustris*; Fulton, 1982) with the nearby Great Lake which currently drains southward. The direction of drainage of Arthurs and Woods Lakes may have been affected by tilting of strata in response to the melting of

Table 5 Allele frequencies for 22 loci from 40 *G. auratus*, and six *G. tanycephalus*. Allele designations correspond to Ovenden & White (1990)

	Locus																					
	Ada	*Est*	*Fum*	*Gda*	*Glo*	*Got-1*	*Got-2*	*Gpd*	*Gpi-1*	*Gpi-2*	*Gsr*	*Ldh-1*	*Ldh-2*	*Mdh-1*	*Mdh-2*	*Mpi*	*Pep-A*	*Pep-D*	*Pgm-1*	*Pgm-2*	*Tpi-2*	*Ugpp*
G. auratus																						
a	0.00	0.03	0.00	0.09	0.00	1.00	0.00	0.00	0.00	0.00	0.01	0.86	0.01	0.00	0.00	0.99	0.00	0.00	0.14	0.00	0.00	0.00
b	0.08	0.97	0.97	0.00	1.00		0.00	1.00	1.00	0.00	0.98	0.14	0.99	1.00	1.00	0.01	1.00	0.00	0.00	0.00	0.00	0.99
c	0.91	0.00	0.03	0.68			1.00			0.00	0.01	0.00						1.00	0.85	0.99	0.03	0.01
d	0.01			0.00						1.00									0.01	0.01	0.00	
e				0.23						0.00									0.00		0.97	
G. tanycephalus																						
a	0.00	0.00	0.00	0.83	0.17	1.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.92	1.00	0.00	0.00	0.00	0.00	0.00	0.00
b	0.08	1.00	1.00	0.00	0.83		0.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	0.08	0.00	1.00	0.00	0.00	0.00	0.00	0.92
c	0.92	0.00	0.00	0.00			1.00			0.00	0.00	0.00					0.00	0.00	1.00	0.00	0.00	0.08
d	0.00			0.17						0.67								0.08	0.00	0.00		
e				0.00						0.33								0.92			1.00	

the ice-sheets at the end of the glaciation (Banks, 1973) or to tectonic activity (Davies, 1965).

The ancestors of *G. auratus* and *G. tanycephalus* may have invaded inland freshwater drainages from the south, when the primordial Lakes Sorell and Crescent and Arthurs and Woods Lakes were the endpoints of southern drainages. A permanent drop in the water table, followed by the tilting of Arthurs and Woods Lakes to the north may have initiated the reproductive isolation of the ancestral *G. auratus* and *G. tanycephalus*. Alternatively, the primordial lakes may have been invaded independently from both the south and the north after they attained the direction of their present day drainage.

The evolutionary relationship of *G. auratus* and *G. tanycephalus* to *G. truttaceus*, and hence the most likely landlocking scheme, cannot be resolved in this instance with phylogenetic techniques. Pamilo & Nei (1988) warn that phylogenetic trees constructed from DNA sequences at a genetic locus (mtDNA) do not necessarily agree with the evolutionary pathway of the species. The most common cause of this discrepancy is the genetic polymorphism of the ancestral species and drift associated with bottlenecks across a short span of evolutionary time. All three conditions exist within the *G. auratus, G. tanycephalus* and *G. truttaceus* assemblage. Another problem for the testing of alternative topologies between *G. auratus, G. tanycephalus* and *G. truttaceus* is the lack of suitable outgroups. While allozyme data do exist for most other galaxiid species (R. W. G. White & J. R. Ovenden, in preparation), the next most closely related galaxiid to this triad is *G. olidus*, which shares virtually no mtDNA restriction sites with them (J. R. Ovenden, unpublished observations).

A considerable amount of mtDNA sequence divergence has accumulated between diadromous populations of *G. truttaceus* analysed by Ovenden & White (1990) and either *G. auratus* (1.915 per cent) or *G. tanycephalus* (1.025 per cent, Table 3). This magnitude of mtDNA divergence is similar to that cited by Avise *et al.* (1987) for most pairs of closely related vertebrate species. By way of contrast, Nei's genetic distances separating the species pairs (*G. auratus* and *G. truttaceus*, 0.149; *G. tanycephalus* and *G. truttaceus*, 0.086) are among the smallest reported between congeneric species of fish (Avise & Aquadro, 1982). The abrupt and permanent effect of bottlenecks upon the intraspecific mtDNA diversity of *G. auratus* and *G. tanycephalus* in their confined lake habitat may have emphasized mitochondrial, but not nuclear, DNA divergence between them and *G. truttaceus*.

If the ancestral populations of *G. auratus* and *G. tanycephalus* were derived from an inland invasion of diadromous *G. truttaceus* from the south, it is possible

that gene flow between the primitive landlocked lake populations continued after their connection with the coastal drainages was severed. Gene exchange amongst primitive lake populations also may have occurred following independent inland invasions from the north and south, especially if drainage patterns were in a state of flux. However, the magnitude of net mtDNA divergence (0.864 per cent), and notably Nei's genetic distance (0.253), between *G. auratus* and *G. tanycephalus* compared to that between them and the populations of *G. truttaceus* analysed by Ovenden and White (1990, Table 3), is large enough to suggest that the lake species have been reproductively isolated during most of their evolutionary history.

The mitochondrial and allozyme genetics of *G. auratus* and *G. tanycephalus* are consistent with their having experienced evolution in reproductive isolation with a finite population size. Their close genetic relationship to *G. truttaceus*, in both the mitochondrial and nuclear genomes, does not conflict with the hypothesis of their possible speciation from landlocked *G. truttaceus* ancestors. Furthermore, it is conceivable that the pattern of mitochondrial and nuclear DNA found in *G. auratus* and *G. tanycephalus* is the logical extension of evolution in lacustrine isolation of which the lake population of *G. truttaceus* is an example.

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