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# Mitochondrial and Allozyme Genetics of Incipient Speciation in a Landlocked Population of *Galaxias truttaceus* (Pisces: Galaxiidae)

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ABSTRACT

*Galaxias truttaceus* is found in coastal rivers and streams in south-eastern Australia. It spawns at the head of estuaries in autumn and the larvae spend 3 months of winter at sea before returning to fresh water. In Tasmania there are landlocked populations of *G. truttaceus* in a cluster of geologically young lakes on the recently glaciated Central Plateau. These populations have no marine larval stage and spawn in the lakes in spring. Speciation due to land locking is thought to be a frequent occurrence within *Galaxias.* To investigate the nature of the speciation event which may be occurring within lake populations of *G. truttaceus* we studied the mitochondrial DNA (mtDNA) and allozyme diversity of both lake and stream populations. Using the presence or absence of restriction sites recognized by 13 six-base restriction endonucleases, we found 58 mtDNA haplotypes among 150 fish collected from 13 Tasmanian and one south-east Australian mainland stream populations. The most parsimonious network relating the haplotypes by site loss or gain was starlike in shape. We argue that this arrangement is best explained by selection upon slightly beneficial mutations within the mitochondrial genome. Gene diversity analysis under Wright's island model showed that the populations in each drainage were not genetically subdivided. Only two of these stream haplotypes were found among the 66 fish analyzed from four lake populations. Despite the extreme lack of mtDNA diversity in lake populations, the observed nuclear DNA heterozygosity of 40 lake fish (0.10355) was only slightly less than that of 82 stream fish (0.11635). In the short time (3000–7000 years) that the lake fish have been landlocked, random genetic drift in a finite, stable-sized population was probably not responsible for the lack of mtDNA diversity in the lake populations. We infer the lake populations have 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. If speciation is occurring in the landlocked populations of *G. truttaceus*, then it may be driven by genetic transilience.

SPECIATION is the acquisition by populations of unique and mutually incompatible genetic profiles. From a population genetic perspective, TEMPLETON (1981) divides theories of speciation into two categories: divergence and transilience. Divergent speciation occurs when barriers to interbreeding are acquired by allopatric populations due to the action of some form of natural selection. Transilient speciation occurs when two or more populations experiencing similar selective forces become reproductively isolated due to the disruption of at least one major coadapted gene complex. Gene complexes can be destabilized by chromosomal rearrangements and hybrid incompatibilities, but the most influential theory of destabilization is that of a founder effect which can cause rapid adaptive shifts in a previously stable genetic system (TEMPLETON 1981). MAYR (1954, 1963, 1982) and later CARSON (1975) discussed founder effects in terms of the genetic changes that occur in small populations founded by a few colonizing individuals. A bottleneck event may induce a founder effect in a geographically isolated population if the population experiences a catastrophic decrease, and subsequent recovery of, population size.

Landlocking is thought to be a major mechanism of speciation in galaxiid fish. This family is found in Australia, New Zealand, South America and South Africa as well as in some small Pacific islands (BERRA 1981). Sixteen of the 21 Australian species are found in Tasmania, ten of which are endemic to the island state. New Zealand has the next richest galaxiid fauna with 13 species, 11 being endemic (MCDOWALL and FRANKENBERG 1981). The existence of most of the freshwater species has been explained by the chance inland isolation, and subsequent speciation, of populations of diadromous species (MCDOWALL 1972; ANDREWS 1976; FULTON 1978). *Galaxias auratus* Richardson and *Galaxias tanycephalus* Fulton, for example, which are confined to inland lakes, are thought to be derived from the widespread, diadromous *Galaxias truttaceus* (Valenciennes). Speciation in response to land locking may occur by divergence or transilience. A landlocked, lacustrine population may experience different selective forces to the riverine, diadromous

population in which case speciation may occur by divergence. However, being landlocked in an inland lake may make the population susceptible to bottlenecks, and hence, to speciation via the founder effect.

In this study we investigate the nature of speciation which may generally be involved with landlocking among the galaxiids. Diadromous populations of *G. truttaceus* are common in coastal streams in Tasmania and southern mainland Australia. Adults cannot tolerate full strength seawater. In late summer adults congregate in fresh water at the head of the stream estuary to spawn. On hatching the larvae are immediately washed to sea where they grow and develop. In spring the juvenile fish reenter the freshwater habitat. Landlocked populations of *G. truttaceus* also occur in isolated, shallow lakes that were formed by the most recent retreat of glaciers from central Tasmania. Lacustrine *G. truttaceus* are morphologically identical to riverine *G. truttaceus*, but their entire life cycle takes place in fresh water. Lacustrine fish tend to produce fewer but larger eggs than do the stream fish (HUMPHRIES 1989). These lake populations of *G. truttaceus* may be on the brink of speciation. To study the type of speciation that may be occurring within lacustrine populations of *G. truttaceus* we have compared mitochondrial DNA (mtDNA) sequence variation and allozyme heterozygosity in diadromous and landlocked populations.

Mitochondrial genomes are maternally inherited and are composed of genes in total linkage disequilibrium (WILSON *et al.* 1985; AVISE *et al.* 1987). Within an interbreeding population, the genomes form bifurcating, non-anastomosing trees which "grow" from an ancestral genome through time and are "pruned" by lineage extinction (AVISE *et al.* 1987). The amount of sequence divergence in mtDNA between and within reproductively isolated populations, produced by the rapid rate of sequence evolution (BROWN, GEORGE and WILSON 1979) and the maternal inheritance of the genome, often bears close resemblance to the biogeographical history of the assemblage of the populations (LANSMAN *et al.* 1983; BERMINGHAM and AVISE 1986; OVENDEN, WHITE and SANGER 1988). Mitochondrial DNA is also useful for the detection of bottleneck events within species. Most animals possess only one mitochondrial haplotype (but see BIRKY, FUERST and MARUYAMA 1989; RAND and HARRISON 1989). The expected proportion of heterozygous nuclear loci per individual can be high, equal to the average proportion of heterozygotes per locus in the population (NEI 1987). Each pair of animals that successfully survive a bottleneck event will pass to their offspring about 75% of the nuclear DNA variation of the pre-bottleneck population (NEI, MARUYAMA and CHAKRABORTY 1975). If the post-bottleneck population rapidly recovers its pre-bottleneck size, little or no trace of the event will be evident in the nuclear genome of the descendants of the bottleneck survivors (NEI, MARUYAMA and CHAKRABORTY 1975). In contrast, the post-bottleneck population will contain only those mtDNA haplotypes possessed by the few females which survived the bottleneck event and successfully reproduced. Our finding that lacustrine populations have reduced mtDNA diversity suggests that the opportunity has or did exist for the disruption of major adaptive gene complexes which may be instrumental in speciation by the founder effect.

During this study we were alert to any potential affects of selection upon the molecular biology and evolutionary relationships of the mitochondrial genome within a species. The use of mtDNA sequence divergence for the study of intraspecific evolutionary history may be compromised if the genome is visible to selective forces. Every nucleotide in the mitochondrial genome, except a few thousand in a region devoted to the regulation and initiation of replication, is part of 37 functional genes which produce RNA (messenger, ribosomal and transfer) and a special suite of respiratory protein subunits. In vertebrates, mtDNA base sequence pliability coexists with functional constraints because substitutions occur either at the third position in codons (MORITZ, DOWLING and BROWN 1987) or are structurally similar nucleotide transitions and are not transversions (BROWN *et al.* 1982). The effect of base sequence variation on the relative efficiency of cellular respiration and ultimately on the fitness of individuals carrying the mutated mtDNA has never been measured. Several studies (ADAMS and ROTHMAN 1982; AVISE, BALL and ARNOLD 1988; MACRAE and ANDERSON 1988) have suggested that selection does operate on mtDNA, but no consensus has been reached about its characteristics. We find that selective forces affecting mitochondrial genomes are likely to be weak.

## MATERIALS AND METHODS

*Galaxias truttaceus* individuals were collected by electrofishing from coastal drainages and from lakes on the Central Plateau of Tasmania. Eleven Tasmanian stream populations were sampled: Allens Creek (42 fish, 43°04'S 147°52'E), Doctors Creek (4, 41°15'E 148°17'S), Don River (5, 41°22'S 146°18'E), Fortescue Lagoon Creek (57, 43°08'S 147°57'E), Griffiths Creek (9, 42°39'S 147°57'E), Hughes Creek (8, 41°08'S 148°18'E), Leven River (3, 41°11'S, 146°04'E), Manuka Creek (5, 42°09'S 145°18'E), Meredith River (5, 42°07'S 148°05'E), Mesa Creek (2, 43°26'S 146°54'E) and Snug Creek (3, 43°04'S 147°16'E). Four lake populations were sampled: Carters Lake (2, 41°52'S 146°32'E), Lake Augusta (1, 41°53'S 146°31'E), Isabella Lagoon (52, 41°52'S 146°29'E) and Little Blue Lagoon (6, 41°52'S 146°28'E). Seven fish were sampled from the mainland of south-east Australia (Apollo Bay, Victoria, 38°46'S 143°40'E) (Figure 1).

mtDNA was extracted from fresh or liquid nitrogen frozen ovary or liver tissue using a method similar to that

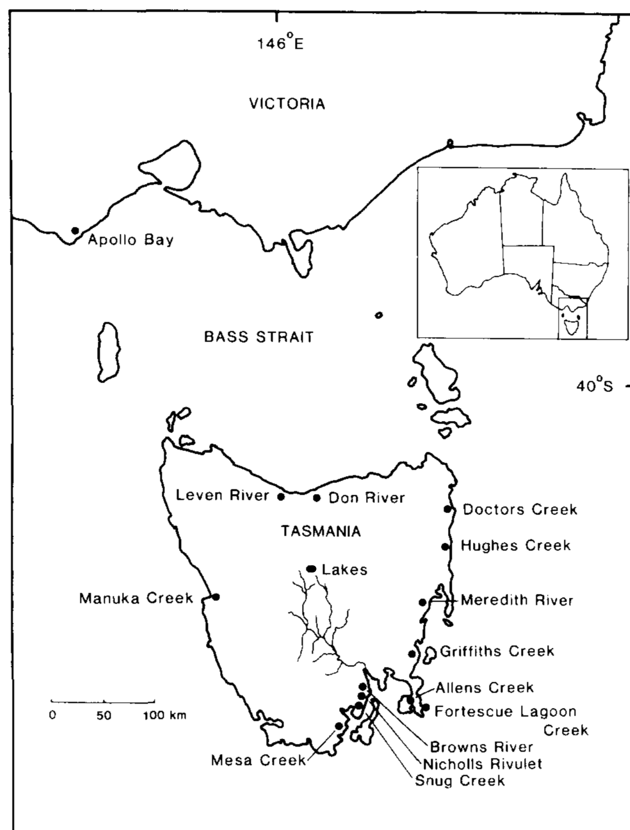


FIGURE 1.—Sampling locales: the location of the 14 stream sites and four inland lake sites from which *G. truttaceus* were sampled.

described by CHAPMAN and POWERS (1984). DNA was digested to completion with each restriction endonuclease according to the supplier's (New England Biolabs, Beverly, Massachusetts) directions. A second endonuclease was often added, with the appropriate amount of 2.5 M NaCl, to produce double-digested fragments. Fragments were end-labeled with [$\alpha$-$^{32}$P]deoxycytosine triphosphate in the presence of deoxyadenosine, deoxythymidine and deoxyguanosine triphosphate using the exonuclease and polymerase activity of the Klenow fragment of DNA polymerase I (OVENDEN, SMOLENSKI and WHITE 1989). Fragments were separated according to size by electrophoresis in 1.0 to 1.4% agarose gels (OVENDEN, WHITE and SANGER 1988). Fragment position was observed by autoradiography and fragment size was estimated by comparison with the position of fragments of lambda DNA produced by *Hin*dIII.

The positions of 39 restriction sites recognized by 14 endonucleases [*Apa*LI (recognition sequence GTGCAC), *Bam*HI (GGATCC), *Bcl*I (TGATCA), *Bgl*II (AGATCT), *Bst*EII (GGTNACC), *Hin*dIII (AAGCTT), *Nco*I (CCATGG), *Nde*I (CATATG), *Pst*I (CTGCAG), *Pvu*II (CAGCTG), *Sac*II (CCGCGG), *Sal*I (GTCGAC), *Xba*I (TCTAGA) and *Xho*I (CTCGAG)] were mapped onto the circular genome of a single stream fish using partial and double digestion techniques (OVENDEN and WHITE 1988). The remaining 210 fish DNAs were mapped for the presence or absence of these restriction sites for all enzymes except *Nde*I which was too expensive for this large scale survey. In a single genome two types of recognition sequences were often assayed at the same time by digesting the genome first with one enzyme, then another. Pairs of enzymes were chosen which produced fragments which did not have the same gel mobility and were larger than about 1000 bp. This double digestion survey technique facilitated the mapping of the position of variant sites and allowed numerous sites per genome to be surveyed during each experiment. The type and position of each variant site was confirmed by single digests with each of the identifying enzymes and by double digests with further enzymes if the site position was uncertain. *Hin*dIII sites were not assayed with the double digestion technique as two pairs of *Hin*dIII sites were close together, separated by only 800 and 900 bp. For each restriction enzyme, every variant genome, or morph, was given an uppercase letter to describe its set of restriction sites. The haplotype of each genome was described by a 13 letter summary of these morph designations.

The mean and variance of the position of the majority of restriction sites was calculated from the length of restriction fragments produced. To begin, the mean genome size was calculated by summing the genome size from successful digests and dividing by the total number of digests. The sizes of the fragments in each digest were then standardized by multiplying each by the mean genome size and dividing by the fragment sum for that digest. After standardizing in this way, the fragments in each digest summed to the mean genome size. Beginning with the first appropriate restriction site clockwise of the single *Bgl*II site at map position zero, the position of the next site for that enzyme was calculated for each digested genome by adding the standardized size of the appropriate fragment to the chosen map position. The mean map position obtained was used as a reference point for the calculation of the position of the next clockwise site using the same procedure. These cycles were repeated until all remaining sites were positioned. Means and variances of site position were obtained for every site except the first appropriate site clockwise of map position zero which was used to initiate the cycle of fragment summations. The accuracy of the site positions were compromised by the number of times a site was identified among the genomes assayed and the distance, in base pairs, between each site. Galaxiid mtDNA sequence data from our laboratory will test the efficiency of this method of determining restriction site position.

To measure the amount of genetic subdivision between stream populations of *G. truttaceus*, we calculated the intrademe (I) and interdeme (J) identity probability of TAKAHATA and PALUMBI (1985) from the presence or absence of restriction sites in each population. As little is known of the dispersal ability of larvae between stream mouths, our null hypothesis was that the population conformed to the finite island model of WRIGHT (1943). This model assumes that a subpopulation is a reproductive unit; that it is not composed of genetically distinct subgroups but it is panmictic and that all subpopulations are of equal size. It also assumes that $N_e m$, the number of reproductively successful migrants, are equally likely to have come from any subpopulation. The identity probability is the probability that two randomly sampled homologous DNA molecules will be identical. The magnitude of these identity probabilities are primarily determined by the effective migration rate between populations and can be used to calculate $G_{ST}$ which represents the fraction of genetic variation within an entire population that is due to interdeme differences. We used Equations 17 and 19 of TAKAHATA and PALUMBI (1985) to estimate $I$ and $J$ respectively: $I = 1/[l \cdot n\,(n-1)]\ \Sigma\ C_i\,(C_i - 1)$ and $J = 1/l \cdot n \cdot n'\ \Sigma\ C_i.C_i'$; where $n$ and $n'$ are the number of mitochondrial genomes sampled from each deme, $l$ is the number of restriction sites identified within the $n$ genomes from each deme, $C_i$ and $C_i'$ are the numbers of genomes cut at restriction site $i$. Bootstrapping (PALUMBI and WILSON 1990) was used to evaluate the significance of the $G_{ST}$ values obtained.

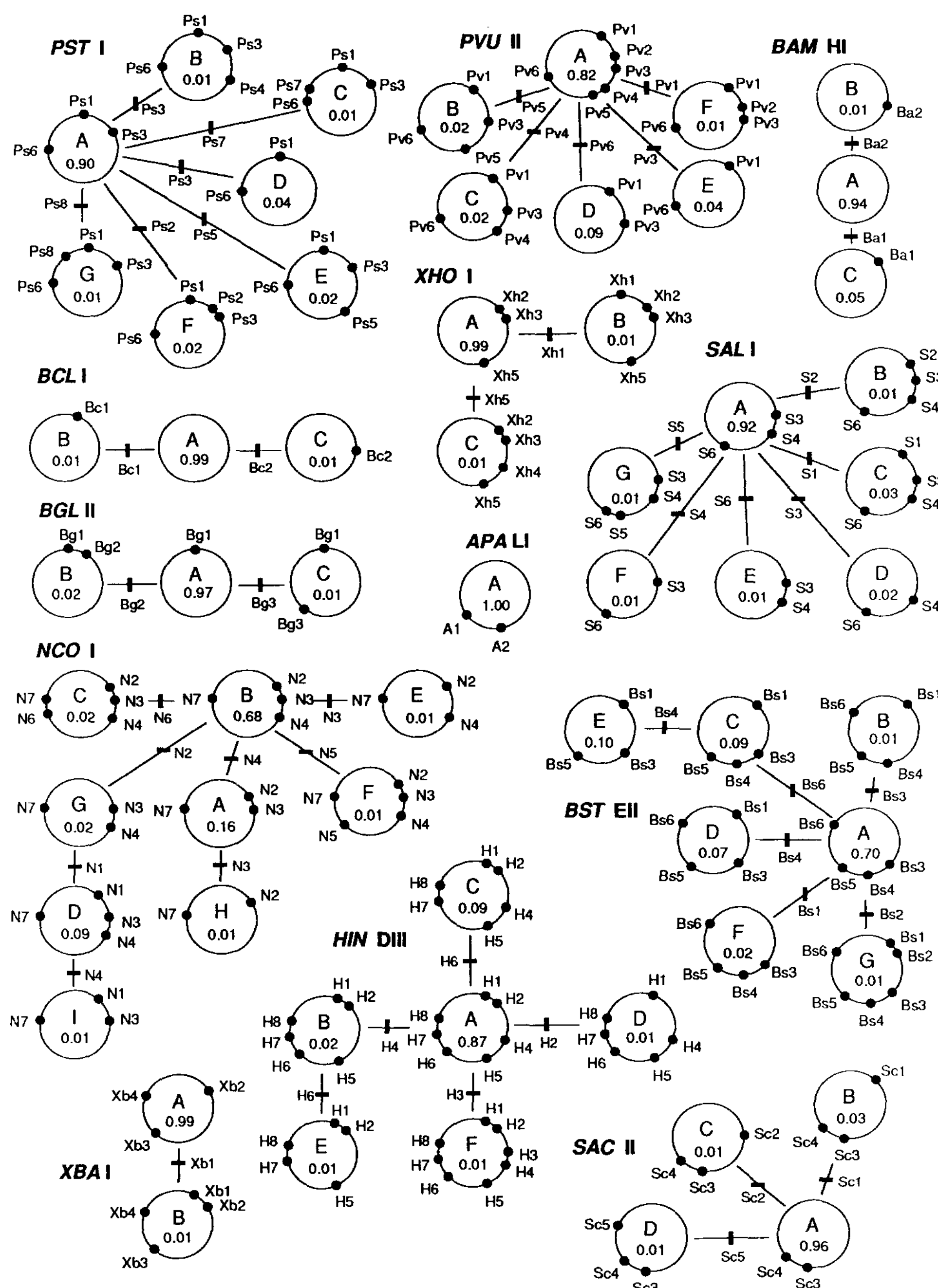


FIGURE 2.—Parsimonious, unrooted networks showing evolutionary relationships between restriction site morphs. The position of restriction sites shown on these maps is approximate only. The mapped position, as the number of base pairs clockwise of the single *Bgl*II site, of each site is shown in Table 2. The site which is either gained or lost between morphs is shown adjacent to the appropriate branch. Networks connecting *Hin*dIII, *Bst*EII and *Nco*I maps have at least one alternative topology. Characters which have been either convergently lost or gained are sites 4 and 6 for the *Hin*dIII network, sites 4 and 6 for the *Bst*EII network and sites 3 and 4 for the *Nco*I network. The frequency of each morph among the stream fish mitochondrial genomes is shown.

Subdivision between stream populations was also measured by $G_{ST}$ calculated using haplotypes as "alleles." Subpopulation heterozygosity ($H_0$) was calculated according to $1 - \Sigma x_i^2$, where $x_i$ was the frequency of the $i$th haplotype. If the total number of haplotypes sampled per population ($n$) was less than 50, $H_0$ was corrected for small sample size by $2n \cdot (H_0)/(2n - 1)$. The mean of $H_0$ across subpopulations was used to calculate $G_{ST}$. Overall heterozygosity ($H_T$) was $1 - \Sigma y_i^2$ where $y_i$ was the mean frequency of the $i$th haplotype across all subpopulations. This approach is similar to one used by RAND and HARRISON (1989) for size classes of cricket mtDNA. The significance of the $G_{ST}$ obtained was evaluated against a series of jackknifed $G_{ST}$ values obtained by omitting one subpopulation in turn.

The maximum likelihood estimate of number of substitutions per base pair ($\pi$, NEI and TAJIMA 1983) between individual genomes was also calculated. Diversity between and within populations ($\partial$), was calculated by averaging the value of $\pi$ across pairwise comparisons of individuals. The magnitude of the standard deviation of $\partial$ increased as the number of pairwise $\pi$ values increased. Thus, a measure of variation for $\partial$ is not presented although methods to do so have recently become available (NEI and JIN 1989).

A further 122 fish were collected to determine nuclear gene diversity. Two stream populations (Allens Creek, 40 fish, Fortescue Lagoon Creek, 42) and one lake population (Isabella Lagoon, 40) were sampled. Aliquots of the supernatant of crushed and centrifuged heads were electrophoresed at 200 V on cellulose acetate for between 1.25 and 2.5 hr, depending on the enzyme to be resolved (RICHARDSON, BAVERSTOCK and ADAMS 1986). Allozyme variation at 22 loci identified by the following enzymes was scored:

**TABLE 1**

**Locality and sample size of 58 *G. truttaceus* mtDNA haplotypes**

| No. | Haplotype | Locality | *N* | No. | Haplotype | Locality | *N* |
|---|---|---|---|---|---|---|---|
| 1 | AAAAAAAAAAAAA | Allens Ck | 3 | 23 | AAAAAAGAACAAA | Fortescue Lagoon Ck | 1 |
| | | Doctors Ck | 2 | | | Manuka Ck | 1 |
| | | Don R. | 2 | 24 | AAAAAAHAAAAAA | Fortescue Lagoon Ck | 1 |
| | | Fortescue Lagoon Ck | 5 | 25 | AAAAABBAAAAAA | Allens Ck | 1 |
| | | Hughes Ck | 1 | | | Fortescue Lagoon Ck | 1 |
| | | Leven R. | 1 | 26 | AAAAABBADAAAA | Fortescue Lagoon Ck | 1 |
| | | Manuka Ck | 1 | 27 | AAAAACAAAAAAA | Allens Ck | 1 |
| | | Snug Ck | 1 | 28 | AAAAACBADAAAA | Allens Ck | 3 |
| | | Carters Lake | 2 | | | Fortescue Lagoon Ck | 4 |
| | | Lake Augusta | 1 | | | Manuka Ck | 1 |
| | | Isabella Lagoon | 51 | | | Meredith R. | 1 |
| | | Little Blue Lagoon | 6 | | | Snug Ck | 1 |
| 2 | AAAAAAAACAAAA | Allens Ck | 1 | 29 | AAAAACDAAAAAA | Griffiths Ck | 1 |
| 3 | AAAAAAAAFAAAA | Fortescue Lagoon Ck | 1 | 30 | AAAAACFADAAAA | Fortescue Lagoon Ck | 1 |
| 4 | AAAAAABAAAAAA | Allens Ck | 6 | 31 | AAAAADBAAADAA | Fortescue Lagoon Ck | 1 |
| | | Doctors Ck | 2 | 32 | AAAAAEBADAAAA | Fortescue Lagoon Ck | 1 |
| | | Don R. | 1 | 33 | AAAABABAEBAAA | Allens Ck | 1 |
| | | Fortescue Lagoon Ck | 12 | 34 | AAAACAAAAAAAA | Allens Ck | 1 |
| | | Griffiths Ck | 1 | | | Isabella Lagoon | 1 |
| | | Hughes Ck | 1 | | | Manuka Ck | 1 |
| | | Mesa Ck | 1 | 35 | AAAACABAAAAAA | Fortescue Lagoon Ck | 5 |
| 5 | AAAAAABAAAAAB | Allens Ck | 1 | | | Meredith R. | 1 |
| 6 | AAAAAABAAAAAC | Fortescue Lagoon Ck | 1 | 36 | AAAACABAAACAA | Fortescue Lagoon Ck | 2 |
| 7 | AAAAAABAAAABA | Allens Ck | 1 | 37 | AAAACABAAAFAA | Allens Ck | 1 |
| 8 | AAAAAABAAABAA | Allens Ck | 1 | 38 | AAAACABAADAAA | Fortescue Lagoon Ck | 1 |
| 9 | AAAAAABAAACAA | Allens Ck | 1 | 39 | AAAACAEAAAAAA | Snug Ck | 1 |
| | | Fortescue Lagoon Ck | 2 | 40 | AAAADAAAAAAAA | Fortescue Lagoon Ck | 2 |
| 10 | AAAAAABAAADAA | Allens Ck | 1 | | | Hughes Ck | 1 |
| | | Don R. | 1 | 41 | AAAADABAAAAAA | Allens Ck | 2 |
| 11 | AAAAAABABAAAA | Allens Ck | 1 | | | Fortescue Lagoon Ck | 4 |
| 12 | AAAAAABAEAAAA | Allens Ck | 1 | | | Leven R. | 1 |
| 13 | AAAAAABAEBEAA | Hughes Ck | 1 | 42 | AAAADADAAAAAA | Hughes Ck | 1 |
| 14 | AAAAAABDAAAAA | Allens Ck | 3 | 43 | AAAAEABAAAAAA | Allens Ck | 1 |
| | | Fortescue Lagoon Ck | 2 | | | Fortescue Lagoon Ck | 3 |
| | | Griffiths Ck | 1 | | | Hughes Ck | 2 |
| 15 | AAAAAABEAAAAA | Leven R. | 1 | 44 | AAAAEABCAAAAA | Fortescue Lagoon Ck | 1 |
| 16 | AAAAAABFAAAAA | Allens Ck | 2 | 45 | AAAAGABAEAAAA | Allens Ck | 1 |
| | | Don R. | 1 | 46 | AAABAABAAAAAA | Fortescue Lagoon Ck | 1 |
| 17 | AAAAAACAAAAAA | Fortescue Lagoon Ck | 1 | 47 | AAABAABAEBEAA | Allens Ck | 1 |
| | | Griffiths Ck | 2 | 48 | AAACAADAAAAAA | Allens Ck | 1 |
| 18 | AAAAAACACAAAA | Griffiths Ck | 1 | | | Meredith R. | 1 |
| 19 | AAAAAADAAAAAA | Allens Ck | 2 | 49 | AACAAAAAAAAAA | Meredith R. | 1 |
| | | Fortescue Lagoon Ck | 2 | 50 | AABAAABBBAAAA | Griffiths Ck | 1 |
| | | Griffiths Ck | 2 | 51 | ABAAAABAAAAAA | Apollo Bay, Victoria | 2 |
| | | Manuka Ck | 1 | 52 | ABAAAABDAAAAA | Hughes Ck | 1 |
| | | Meredith R. | 1 | 53 | ABAAACBADAAAA | Apollo Bay, Victoria | 1 |
| | | Mesa Ck | 1 | 54 | ABAABFBAEBCAA | Apollo Bay, Victoria | 1 |
| 20 | AAAAAADEAAAAA | Allens Ck | 1 | 55 | ABAACABAAAAAA | Apollo Bay, Victoria | 1 |
| 21 | AAAAAAEAAAAAA | Allens Ck | 1 | 56 | ABAACABFAAAAA | Apollo Bay, Victoria | 1 |
| 22 | AAAAAAGAAAAAA | Allens Ck | 1 | 57 | ABAADABAAAAAA | Apollo Bay, Victoria | 1 |
| | | Fortescue Lagoon Ck | 1 | 58 | ACABACBADAAAA | Allens Ck | 1 |

CR = creek; R = river.

adenosine deaminase (Enzyme Commission Number 3.5.4.4, running buffer 0.02 M phosphate pH 7.0), esterase (3.1.1.1, 0.05 M tris-maleate pH 7.8), fumarate hydratase (4.2.1.2, 0.02 M phosphate pH 7.0), guanine deaminase (3.5.4.3, 0.02 M phosphate pH 7.0), glyoxalase 1 (4.4.1.5, 0.02 M phosphate pH 7.0), glutamate-oxaloacetate transaminase (2.6.1.1, 0.02 M phosphate pH 7.0), glycerol-3-phosphate dehydrogenase (1.1.1.8, 0.025 M tris-glycine pH 8.5), glucose-phosphate isomerase (5.3.1.9, 0.02 M phosphate pH 7.0), glutathione reductase (1.6.4.2, 0.05 M tris-maleate pH 7.8), lactate dehydrogenase (1.1.1.27, 0.02 M phosphate pH 7.0), malate dehydrogenase (1.1.1.37, 0.01 M citrate/phosphate pH 6.4), mannose-phosphate isomerase (5.3.1.8, 0.02 M phosphate pH 7.0), dipeptidase (3.4.13.11, 0.05 M tris-maleate pH 7.8), peptidase (3.4.13.9, 0.05 M tris-maleate pH 7.8), phosphoglucomutase (5.2.4.2, 0.05 M tris-maleate pH 7.8), triose-phosphate isomerase (5.3.1.1, 0.02 M phosphate pH 7.0 and 0.025 M tris-glycine pH 8.5), and

UDP glucose pyrophosphorylase (2.7.7.9, 0.05 M tris-maleate pH 7.8). Loci for this study were selected from the 43 resolved by R. W. G. WHITE and J. R. OVENDEN (unpublished results) with the addition of the esterase and peptidase loci. The average heterozygosity and expected variance (NEI and ROYCHOUDHURY 1974) were calculated for each population. The magnitude of the heterozygosities was not absolute because enzyme loci were not chosen at random. The amount of subdivision between stream populations was estimated using the $G$ statistic (NEI 1973).

The computer programs MIX (PHYLIP version 3.2; FELSENSTEIN 1989) and MacClade (version 2.1, written by WAYNE MADDISON and DAVID MADDISON, Harvard University) were used to construct parsimonious networks between haplotypes based on the presence or absence of restriction sites. Compatibility analyses on the same data set were performed with CLIQUE (FELSENSTEIN 1989).

## RESULTS

**Stream populations:** There was no evidence of size variation among the mitochondrial genomes surveyed. Based on the presence or absence of restriction sites, one (*Apa*LI), two (*Xba*I), three (*Bam*HI, *Bcl*I, *Bgl*II, *Xho*I), four (*Sac*II), six (*Hin*dIII, *Pvu*II), seven (*Bst*EII, *Sal*I, *Pst*I) and nine (*Nco*I) morphs were identified per enzyme (Figure 2). *Bam*HI morph A and *Bcl*I morph A did not possess any sites. The frequency of the most common morph for each restriction enzyme varied from 0.99 to 0.68. In most cases less common morphs were related to the common morph by the gain or loss of a single restriction site. However, the rare *Bst*EII morph E was related to another rare morph, C, by a single site gain or loss. The rare *Hin*dIII morph E was most closely related to rare morph B. *Nco*I rare morph I was a single site gain or loss from rare morph D which was similarly related to rare morph G. *Nco*I rare morph H was also more closely related to another rare morph than to the common morph (Figure 2).

Fifty-eight mtDNA haplotypes, identified by a 13-letter summary of restriction enzyme morphs, were found in the 150 *G. truttaceus* sampled from stream populations (Table 1). The average number of substitutions per base pair between each pair of haplotypes was 0.0088 ± 0.0037 ($n$ = 1653 comparisons) or a minimum of 3.63 ± 1.48 mutations. The frequency of classes of these measures of diversity between haplotypes was significantly skewed to the left and platykurtic ($\pi$, $t_{g1,\infty} = 3.21$, $P < 0.001$; $t_{g2,\infty} = 12.45$, $P < 0.001$; minimum number of mutations, $t_{g1,\infty} = 9.84$, $P < 0.001$; $t_{g2,\infty} = 10.15$, $P < 0.001$; Figure 3). Thus, the haplotypes were more likely to differ by three to four mutations than a lesser or greater amount and relatively more genomes were closely related than were distantly related. Similar pairwise diversity trends were found by CANN, STONEKING and WILSON (1987) between human mitochondrial genomes. The most common haplotype (AAAAAABAAAAAA, #4) was present in 24 of the 150 stream fish. The remaining 57 haplotypes were represented by one to ten fish each. Six haplotypes were identified among the seven genomes sampled from Apollo Bay, Victoria, and none of these was found in Tasmanian streams. However, 32 of the 52 Tasmanian haplotypes were also found in one locality only (Table 1).

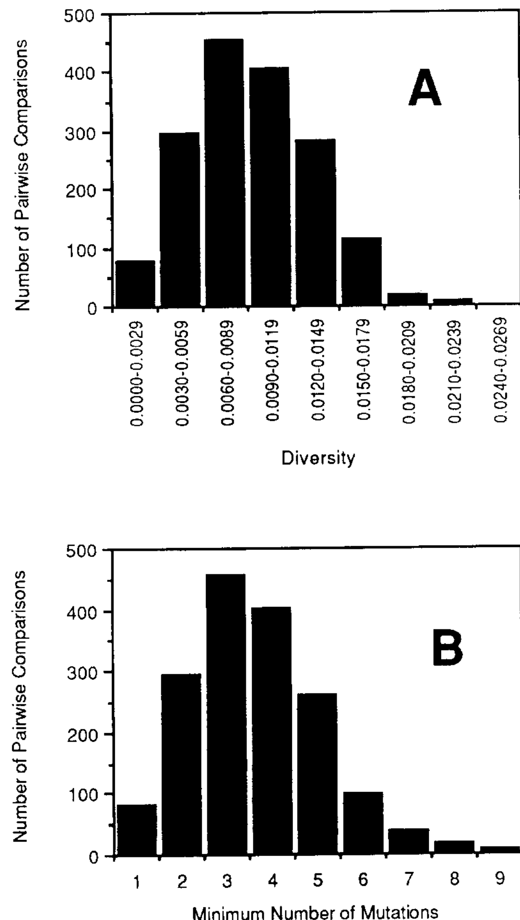


FIGURE 3.—The frequency distribution of the number of base substitutions per base pair (A) and the minimum numbers of mutations (B) between 58 *G. truttaceus* haplotypes.

The 13 six-base restriction enzymes identified a total of 62 different restriction sites across all genomes analyzed. Of these, we surveyed between 33 and 39 six-base restriction sites in each of the 58 stream haplotypes. This was equivalent to directly comparing the sequence of *ca.* 204 bp at the same location in all the mitochondrial genomes analyzed. The site positions are presented in Table 2. The standard deviation of the mapped position of the 32 sites whose position was estimated on between 50 and 310 separate occasions varied from 18 to 218 bp. This degree of statistical variance of site position is similar to the "guestimates" of site position variance which are routinely presented with mitochondrial DNA restriction site maps (ARAYA *et al.* 1984; OVENDEN and WHITE 1988).

No variant sites were found between *Sal*I site 5 (7540 bp clockwise of *Bgl*II site 1) and *Pst*I site 7 (11,800 bp clockwise of *Bgl*II site 1; Figure 4). Nine

TABLE 2

**Mapped position, in nucleotides clockwise of the single *Bgl* II site, of 64 six-base restriction sites among the 150 stream genomes surveyed**

| Site Name | Mean | Standard deviation | *N* |
|---|---|---|---|
| *Bgl* II site 1 (ref) | 0 | 0 | 0 |
| *Pst* I site 1 (ref) | 0 | 0 | 0 |
| *Hin*dIII site 1 (ref) | 100 | 0 | 0 |
| *Bcl* I site 1* | 363 | 0 | 2 |
| *Hin*dIII site 2 | 984 | 48 | 80 |
| *Pst* I site 2 | 1226 | 0 | 1 |
| *Bst*EII site 1 | 1341 | 25 | 268 |
| *Xba* I site 1 | 1488 | 0 | 1 |
| *Xba* I site 2 (ref) | 1700 | 0 | 0 |
| *Pst* I site 3 | 1707 | 18 | 195 |
| *Xho* I site 1 | 1712 | 51 | 4 |
| *Sal* I site 2 | 1793 | 62 | 2 |
| *Sal* I site 1 | 1798 | 29 | 4 |
| *Bst*EII site 2 | 2069 | 0 | 1 |
| *Sac*II site 1 | 2254 | 42 | 2 |
| *Pvu*II site 1 (ref) | 2800 | 0 | 0 |
| *Bam*HI site 1 | 2883 | 20 | 3 |
| *Sal* I site 3 | 3088 | 104 | 196 |
| *Nco* I site 1 (ref) | 3100 | 0 | 0 |
| *Pst* I site 4 | 3164 | 0 | 1 |
| *Nco* I site 2 (ref) | 3300 | 0 | 0 |
| *Bgl* II site 2 | 3481 | 27 | 2 |
| *Xho* I site 2 (ref) | 3700 | 0 | 0 |
| *Nco* I site 3 | 4183 | 52 | 257 |
| *Sal* I site 4 | 4452 | 99 | 199 |
| *Pst* I site 5 | 4476 | 0 | 1 |
| *Bcl* I site 2 | 4553 | 13 | 2 |
| *Pvu*II site 2* | 4726 | 0 | 1 |
| *Xho* I site 3 | 4888 | 34 | 288 |
| *Pvu*II site 3 | 5117 | 18 | 303 |
| *Bst*EII site 3 | 5187 | 86 | 270 |
| *Hin*dIII site 3 | 5636 | 0 | 1 |
| *Nco* I site 4 | 5733 | 100 | 177 |
| *Hin*dIII site 4 | 5934 | 80 | 79 |
| *Bgl* II site 3 | 6164 | 0 | 1 |
| *Pvu*II site 4 | 6275 | 23 | 3 |
| *Bam*HI site 2 | 6307 | 23 | 56 |
| *Bst*EII site 4 | 6426 | 47 | 194 |
| *Xho* I site 4 | 6528 | 14 | 3 |
| *Sac*II site 2 | 6713 | 0 | 1 |
| *Pvu*II site 5 | 7018 | 27 | 53 |
| *Sal* I site 6 | 7475 | 61 | 191 |
| *Bst*EII site 5 | 7687 | 161 | 272 |
| *Sac*II site 3 | 9094 | 93 | 265 |
| *Hin*dIII site 5 | 9180 | 96 | 82 |
| *Apa*LI site 1 | 9616 | 47 | 310 |
| *Xba* I site 3 | 9626 | 88 | 250 |
| *Xho* I site 5 | 9812 | 218 | 342 |
| *Apa*LI site 2 | 10709 | 53 | 310 |
| *Sac*II site 4 | 10950 | 106 | 265 |
| *Pst* I site 6 | 11412 | 55 | 199 |
| *Pst* I site 7 | 11800 | 0 | 1 |
| *Pvu*II site 6 | 11876 | 88 | 296 |
| *Hin*dIII site 6 | 11954 | 108 | 75 |
| *Hin*dIII site 7 | 13075 | 55 | 82 |
| *Bst*EII site 6 | 13347 | 73 | 246 |
| *Nco* I site 5 | 13513 | 0 | 1 |
| *Sac*II site 5 | 13610 | 0 | 1 |
| *Hin*dIII site 8 | 14018 | 49 | 82 |
| *Xba* I site 4 | 14234 | 52 | 230 |
| *Nco* I site 6 | 15195 | 0 | 3 |
| *Nco* I site 7 | 16231 | 96 | 265 |

Some sites were used as references (ref) to map the remaining sites and do not have map position statistics. The position of two sites (*) was mapped to one of two alternate positions.

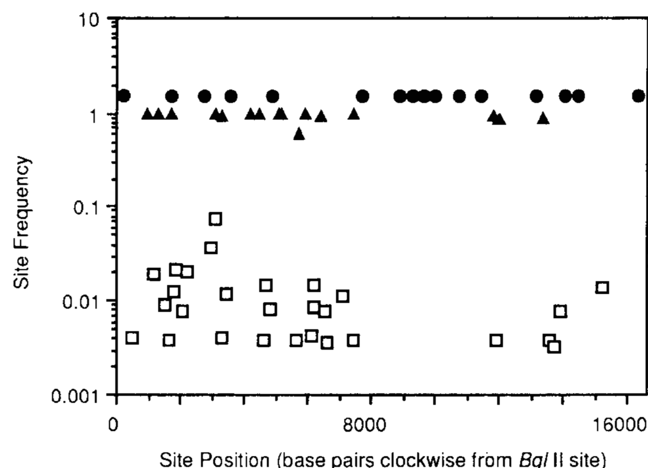


FIGURE 4.—The position and frequency of invariant (circle), high frequency variant (triangle) and low frequency variant (square) restriction sites in 263 *G. truttaceus* mitochondrial genomes. The frequency of the invariant sites (circle) has been artificially set at 1.5, instead of 1.0, to distinguish them from the high frequency variant sites (triangle).

invariant sites were identified in this 4260-bp region including two *Sac*II sites (#3, 9,094 bp; #4, 10,950 bp) separated by about 1,800 bp. The distance between these invariant *Sac*II sites suggests that they are homologous to those reported in completely sequenced genomes and are found in the 12S and 16S rRNA genes (CARR, BROTHER and WILSON 1987; WALLIS 1987). We infer that the 12S and 16S rRNA genes of the *G. truttaceus* mitochondrial genome are located in this 4260-bp segment.

Of the 62 restriction sites available for phylogenetic analysis, 21 of them were present in all haplotypes (*Apa*LI sites 1 and 2, *Bgl*II site 1, *Bst*EII sites 1 and 5, *Hin*dIII sites 1, 5, 7 and 8, *Nco*I site 7, *Pvu*II site 1, *Pst*I sites 1 and 6, *Sac*II sites 3 and 4, *Xba*I sites 2, 3 and 4, *Xho*I sites 2, 3 and 5), 18 sites were present or absent in one haplotype only (*Bam*HI site 2, *Bcl*I sites 1 and 2, *Bgl*II site 3, *Bst*EII site 2, *Hin*dIII sites 2 and 3, *Nco*I site 5, *Pst*I sites 4 and 7, *Pvu*II site 2, *Sac*II sites 2 and 5, *Sal*I sites 2 and 4, *Xba*I site 1, *Xho*I sites 1 and 4) and 23 sites were present or absent in more than one haplotype (*Bam*HI site 1, *Bgl*II site 2, *Bst*EII sites 3, 4 and 6, *Hin*dIII sites 4 and 6, *Nco*I sites 1, 2, 3, 4 and 6, *Pst*I sites 2, 3 and 5, *Pvu*II sites 3, 4, 5 and 6, *Sal*I sites 1, 3 and 6, *Sac*II site 1; Table 2). The degree of homoplasy among the 23 synapomorphic characters was great. The most parsimonious trees constructed using the 23 synapomorphic characters were 44 character state changes in length. The largest number of compatible synapomorphic characters was 13. This high degree of convergence in the character set did not strongly support the arrangement of the 58 haplotypes into any particular cladistic network. The majority of the haplotypes were one to five character states changes from the most frequent haplotype (AAAAAABAAAAA, #4) (Figure 5). The

FIGURE 5.—A parsimonious, unrooted network describing the evolutionary relationship between 58 *G. truttaceus* mitochondrial DNA haplotypes in terms of the presence or absence of restriction sites. The area of each circle is proportional to the numbers of fish with that haplotype. Haplotypes are numbered as in Table 1. The site which is either gained or lost along each branch is named. A full description of these sites can be found in Table 2. Sites which are present or absent only in one haplotype are represented by an open bar. Sites which are present or absent in more than one, but not all haplotypes are represented by a hatched bar if they appear in the network more than once and by a solid bar if they appear only once. Refer to the text for a description of sites which occur in all haplotypes.

average number of mutations which separated haplotype #4 from the remaining 57 haplotypes was 1.98. The most distantly related haplotype was #54 (ABAABFBAEBCAA) from Apollo Bay, Victoria (Table 1), which was an average of 7.28 mutations from the remaining 57 haplotypes. The most robust clade consisted of haplotypes #12, 13, 33, 45, 47 and 54 which all had lost *Pvu*II site 3 with respect to the remainder of the haplotypes. Four of the members of this clade (#13, 33, 47 and 54) possessed the *Sac*II site 1. Two of them (#13 and 47) had lost *Sal*I site 6 and the other two (#33 and 54) had lost the *Bst*EII site 3. The fidelity of this clade was compromised by the possession of *Bgl*II site 2 by haplotype #47 and nonclade members #46 and 58 and the possession of *Bam*HI site 1 by clade member #54 and nonclade members #51, 52, 53, 55, 56 and 57.

For the 12 stream populations from which more than one fish was sampled, intrademe identity probability ($I$) varied from a maximum of 0.97685 (Doctors Creek) to a minimum of 0.68436 (Allens Creek; Table 3). The mean value of $I$ was 0.86899. Pairwise comparisons of the stream populations produced interdeme identity probabilities ($J$) ranging from 0.59968 (Allens Creek-Fortescue Lagoon Creek) to 0.94864 (Doctors Creek-Don River). The mean value of $J$ was 0.80439 ($n$ = 66). The means of $I$ and $J$ were used to calculate the average gene diversity of subpopulations [$H_0 = (1 - I)$] and the average gene diversity of the total population, [$H_T = 1 - \{(1/L) \cdot I + (1 - 1/L) \cdot J\}$] where $L$ is the number of subpopulations (TAKAHATA and PALUMBI 1985). The average gene diversity of each stream population was 0.13101 and the average gene diversity of the total population was 0.19022. Thus the amount of mtDNA restriction site variation that was due to subdivision between streams ($G_{ST}$) was 31%. However, as 500 estimates of $G_{ST}$ from randomized data ranged from 26% to 35% in an approximately normal distribution we regard the $G_{ST}$ value of 31% not to be an indication of population subdivision. A similar result was obtained from the $G_{ST}$ calculated from haplotype frequencies in each subpopulation. The value of $G_{ST}$ (10.2%) lay within the range of 11 jackknifed estimates (7.6–11.4%). A lack of stream population subdivision detected with mtDNA was confirmed by allelic variation at 22 nuclear loci. $G_{ST}$ for nuclear DNA calculated for two southern Tasmanian streams, Allens Creek and Fortescue Lagoon Creek whose mouths are 99 km apart (Table 3), was effectively zero. Average nuclear gene diversity within Allens Creek was 0.11756. Within Fortescue Lagoon Creek it was 0.11390. Nuclear gene diversity between streams was 0.11637.

There was no difference in the magnitude of $\partial$, the number of substitutions per base pair between pairs of mitochondrial genomes, within and between streams. The five genomes collected from the Meredith River were the most diverse (mean $\partial$ = 0.00867) while the six genomes collected from Doctors Creek were the most similar (mean $\partial$ = 0.00160). Interstream divergence ranged from a high of 0.00847 between the Hughes Creek sample ($n$ = 8) and the Meredith River sample ($n$ = 5), to a low of 0.00214 between the Doctors Creek sample ($n$ = 4) and the Don River sample ($n$ = 5). The range of diversities ($\partial$) between Apollo Bay, Victoria and Tasmanian stream samples was 0.00731 to 0.01039.

**Lake populations:** Of the 61 lacustrine genomes assayed, one fish from Lake Augusta, two fish from Carters Lake, six fish from Little Blue Lagoon and 51

TABLE 3

**Interdeme identity probability (above diagonal), intrademe identity probability (underlined), and the minimum distance by sea (km, below diagonal) separating 12 stream populations of *G. truttaceus***

| Locality | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Allens Creek (1) | 0.68436 | 0.65705 | 0.69285 | 0.69085 | 0.59968 | 0.65308 | 0.66112 | 0.68968 | 0.66377 | 0.66760 | 0.69357 | 0.67111 |
| Apollo Bay (2) | 841 | 0.85481 | 0.84146 | 0.83972 | 0.68290 | 0.73319 | 0.80484 | 0.81859 | 0.78405 | 0.77077 | 0.82142 | 0.81765 |
| Doctors Creek (3) | 335 | 506 | 0.97685 | 0.94864 | 0.75209 | 0.83201 | 0.87500 | 0.94594 | 0.90263 | 0.88205 | 0.94594 | 0.94444 |
| Don River (4) | 550 | 360 | 215 | 0.94324 | 0.73370 | 0.81033 | 0.85000 | 0.91754 | 0.87589 | 0.85700 | 0.91842 | 0.91531 |
| Fortescue Lagoon Creek (5) | 99 | 742 | 236 | 451 | 0.74261 | 0.69037 | 0.70239 | 0.73323 | 0.73493 | 0.70789 | 0.75152 | 0.72946 |
| Griffiths Creek (6) | 159 | 682 | 176 | 391 | 60 | 0.83664 | 0.77367 | 0.80878 | 0.79689 | 0.78484 | 0.83994 | 0.80511 |
| Hughes Creek (7) | 284 | 557 | 51 | 266 | 185 | 125 | 0.85347 | 0.85208 | 0.83312 | 0.81707 | 0.87500 | 0.84615 |
| Leven River (8) | 570 | 355 | 235 | 20 | 471 | 411 | 286 | 0.93693 | 0.87350 | 0.85500 | 0.91666 | 0.91291 |
| Manuka Creek (9) | 441 | 403 | 629 | 414 | 417 | 477 | 680 | 394 | 0.87894 | 0.84600 | 0.90526 | 0.87543 |
| Meredith River (10) | 221 | 669 | 163 | 378 | 122 | 62 | 112 | 398 | 539 | 0.86666 | 0.88974 | 0.85641 |
| Mesa Creek (11) | 131 | 713 | 343 | 558 | 107 | 167 | 292 | 704 | 310 | 229 | 0.94594 | 0.91441 |
| Snug Creek (12) | 65 | 779 | 306 | 521 | 70 | 130 | 255 | 770 | 376 | 192 | 66 | 0.90740 |

fish from Isabella Lagoon had identical mtDNA haplotypes (AAAAAAAAAAAAA, #1). This haplotype was also found in stream populations on the east (Doctors Creek, $n = 2$; Hughes Creek, 1; Allens Creek, 3; Fortescue Lagoon Creek, 3; Snug Creek, 1), west (Manuka Creek, 1) and north coasts (Don River, 2; Leven River, 1) of Tasmania. One fish from Isabella Lagoon had the same haplotype (AAAACAAAAAAAA, #34) as did one fish from Allens Creek and one fish from Manuka Creek (Table 1).

Of the 17 loci which were polymorphic in either the stream and lake populations, seven loci (*Est, Fum, Gsr, Mdh-1, Ugpp, Pep A* and *Pep D*) were reduced from two alleles in the stream to one in the lake, three loci (*Ada, Got-2*, and *Ldh-1*) were reduced from three to one allele, two loci (*Gda, Gpi-2*) were reduced from five to one alleles, one locus (*Tpi-2*) was reduced from five alleles to two, one locus was reduced from three to two alleles (*Pgm-1*) and one locus from four to three alleles (*Pgm-2*; Table 4). The commonest stream allele was generally retained in the lake population. The second most common *Gda* stream allele ($Gda^c$) was present in the lake population. The most common *Pgm* stream allele ($Pgm\text{-}2^c$) was lost and two rarer alleles ($Pg\text{-}2^a$, $Pgm\text{-}2^b$) were found in the lake. Three of the four rarest stream *Tpi-2* alleles were not present in the lake sample ($Tpi\text{-}2^a$, $Tpi\text{-}2^b$, $Tpi\text{-}2^e$) but the other rare allele ($Tpi\text{-}2^c$) was present in the lake at a higher frequency than in the stream sample. The polymorphic loci which did not have reduced numbers of alleles in the lake population were *Gpd* and *Gpi-1*. In the lake sample, $Gpd^a$ and $Gpd^b$ had approximately equal frequencies while in the stream only one chromosome out of 164 was assayed as possessing $Gpd^a$. The lake fish sampled possessed an allele at *Gpi-1* ($Gpi\text{-}1^a$) which was not observed in the stream sample. The average nuclear heterozygosity of the Isabella Lagoon population was $0.10355 \pm 0.05318$ (mean across all loci ± variance, $n = 40$). In comparison, the average nuclear heterozygosity of Allens Creek and Fortescue Lagoon Creek combined was $0.11635 \pm 0.03880$ (mean across all loci ± variance, $n = 82$).

## DISCUSSION

**Stream populations are not genetically subdivided:** Using the island model of WRIGHT (1943), analysis of the mitochondrial and nuclear gene diversity of stream populations of *G. truttaceus* shows that the amount of inbreeding within the total population that is due to population subdivision is minimal. The *G. truttaceus* populations in Tasmania, and arguably Victoria also, may form a single interbreeding population. The similarity of $G_{ST}$ values calculated from nuclear and mitochondrial gene diversity suggests that there is no gross systematic bias in the sex of breeding individuals exchanged between populations. In the

**TABLE 4**

**Allele frequencies for 22 nuclear DNA Loci in 40 *G. truttaceus* from Allens Creek, 42 from Fortescue Lagoon Creek and 40 from Isbella Lagoon**

| | *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* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Allens Creek | | | | | | | | | | | | | | | | | | | | | | |
| a | 0.01 | 0.99 | 0.01 | 0.44 | 1.00 | 1.00 | 0.06 | 0.01 | 0.00 | 0.01 | 1.00 | 0.93 | 1.00 | 0.00 | 1.00 | 1.00 | 0.02 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 |
| b | 0.29 | 0.01 | 0.99 | 0.00 | | | 0.01 | 0.99 | 1.00 | 0.00 | 0.00 | 0.06 | | 1.00 | | | 0.98 | 1.00 | 0.58 | 0.14 | 0.05 | 1.00 |
| c | 0.70 | | | 0.30 | | | 0.93 | | | 0.01 | | 0.01 | | | | | | | 0.43 | 0.84 | 0.13 | |
| d | | | | 0.03 | | | | | | 0.98 | | | | | | | | | | 0.00 | 0.83 | |
| e | | | | 0.24 | | | | | | 0.00 | | | | | | | | | | | | |
| Fortescue Lagoon Creek | | | | | | | | | | | | | | | | | | | | | | |
| a | 0.00 | 1.00 | 0.00 | 0.43 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.99 | 0.98 | 1.00 | 0.02 | 1.00 | 1.00 | 0.00 | 0.01 | 0.01 | 0.05 | 0.01 | 0.02 |
| b | 0.38 | | 1.00 | 0.01 | | | 0.00 | 1.00 | 1.00 | 0.01 | 0.01 | 0.02 | | 0.98 | | | 1.00 | 0.99 | 0.58 | 0.05 | 0.05 | 0.98 |
| c | 0.62 | | | 0.26 | | | 1.00 | | | 0.00 | | 0.00 | | | | | | | 0.40 | 0.81 | 0.11 | |
| d | | | | 0.06 | | | | | | 0.98 | | | | | | | | | | 0.10 | 0.82 | |
| e | | | | 0.24 | | | | | | 0.01 | | | | | | | | | | | 0.01 | |
| Observed number of alleles for two streams combined | | | | | | | | | | | | | | | | | | | | | | |
| | 3.00 | 2.00 | 2.00 | 5.00 | 1.00 | 1.00 | 3.00 | 2.00 | 1.00 | 5.00 | 2.00 | 3.00 | 1.00 | 2.00 | 1.00 | 1.00 | 2.00 | 2.00 | 3.00 | 4.00 | 5.00 | 2.00 |
| Isabella Lagoon | | | | | | | | | | | | | | | | | | | | | | |
| a | 0.00 | 1.00 | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | 0.46 | 0.23 | 0.00 | 1.00 | 1.00 | 1.00 | 0.00 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.48 | 0.00 | 0.00 |
| b | 0.00 | | 1.00 | 0.00 | | | 0.00 | 0.54 | 0.78 | 0.00 | | | | 1.00 | | | 1.00 | 1.00 | 0.21 | 0.40 | 0.48 | 1.00 |
| c | 1.00 | | | 1.00 | | | 1.00 | | | 0.00 | | | | | | | | | 0.79 | 0.00 | 0.53 | |
| d | | | | | | | | | | 1.00 | | | | | | | | | | 0.13 | | |
| e | | | | | | | | | | | | | | | | | | | | | | |
| Observed number of alleles | | | | | | | | | | | | | | | | | | | | | | |
| | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 2.00 | 2.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 2.00 | 3.00 | 2.00 | 1.00 |
| Expected number of alleles | | | | | | | | | | | | | | | | | | | | | | |
| | 2.04 | 1.06 | 1.06 | 3.30 | 1.00 | 1.00 | 1.32 | 1.06 | 1.00 | 1.23 | 1.06 | 1.41 | 1.00 | 1.06 | 1.00 | 1.00 | 1.06 | 1.06 | 2.05 | 3.32 | 2.22 | 1.11 |

The expected number of alleles in Isabella Lagoon was calculated assuming that the population was derived from population having the same gene frequencies as Allens and Fortescue Lagoon Creeks combined and that the effective size of the population during the bottleneck ($N_0$) was five.

absence of sex-specific migration between populations, the $G$ statistic calculated from mitochondrial and nuclear gene diversity between populations should be identical when the populations are in equilibrium. This is because the value of $G_{ST}$ is approximately independent of mutation rate if allowance is made for the effective population size of mitochondrial genomes being less than that for nuclear genomes (TAKAHATA and PALUMBI 1985; CHAKRABORTY and LEIMAR 1987). The rate of approach of $G_{ST}$ to equilibrium depends on the relative strength of genetic drift within subpopulations and the amount of gene flow between them. Genetic drift can be measured by the inverse of effective population size and gene flow is measured as the number of reproductively active individuals in a subpopulation which originated from outside that population (CHAKRABORTY and LEIMAR 1987). At a given rate of gene flow between subpopulations following an alteration in gene flow or drift, $G_{ST}$ estimated from mitochondrial gene diversity will approach equilibrium more rapidly than $G_{ST}$ estimated from nuclear gene diversity because the effective population size applicable to mitochondrial genomes is one quarter that of nuclear genomes. Thus, if the pattern of gene flow between populations has been recently perturbed, population subdivision will be measurable from the mitochondrial genome diversity before the nuclear genome.

The larval stage of riverine *G. truttaceus* spends 3 months at sea before reentering fresh water. No information is available on the extent of movement of larvae, either passive or active, during this marine phase. As the larval phase is the only stage during the life history of *G. truttaceus* when genetic interchange could occur between streams it is likely that some larvae are widely dispersed from their parental spawning streams during winter. Unlike some of their anadromous salmoniform relatives (HASLER 1971), it is unlikely that *G. truttaceus* seek out their spawning stream during their return to fresh water. No nuclear gene subdivision was reported in three populations of *G. maculatus* in adjoining streams in New Zealand (BARKER and LAMBERT 1988); this galaxiid has a life history essentially similar to that of *G. truttaceus*.

Isolation-by-distance may occur between stream populations if gene flow is proportional to geographic distance. Seven Victorian fish had different mtDNA haplotypes to the 204 Tasmanian fish surveyed. Nucleotide sequence diversity between Victorian and Tasmanian samples was larger than between Tasmanian streams, although the interdeme identity probability values between localities did not follow this trend. During the last glacial period, 10,000–20,000 yr ago, a land bridge connected Tasmania to south-east mainland Australia (DAVIES 1974). At this time southern Victorian coastal drainages merged with northern Tasmanian drainages before flowing westward into the sea, allowing reproductive contact between Tasmanian and Victorian galaxiids. If divergence has occurred between extant Tasmanian and Victorian populations of *G. truttaceus* it probably began when rising sea levels severed the connection between the coastal drainages inhabited by *G. truttaceus*. Correcting for intraspecific divergence (NEI and LI 1979, equation 25), the mean number of base substitutions per base pair between them is 0.0021. In a similar study of southern Victorian and northern Tasmanian populations of *Gadopsis marmoratus*, a fish strictly confined to fresh water, no net divergence was reported between populations (OVENDEN, WHITE and SANGER 1988).

**Lack of genetic diversity in lake populations:** Lacustrine *G. truttaceus* are geographically isolated from and have a different life history strategy to riverine *G. truttaceus*. Their mitochondrial and, to a lesser extent, their nuclear genomes were less diverse than their stream cousins. Reduced mitochondrial genome diversity is not a general feature of lacustrine freshwater fish populations. For example, BILLINGTON and HEBERT (1988) reported nine mitochondrial haplotypes from 141 assayed walleyes (*Stizostedion vitreum*) in the recently formed Laurentian Great Lakes system. Assuming the founders of the lake *G. truttaceus* population were as diverse, and possibly indistinguishable from, extant stream populations, random genetic drift or a pronounced bottleneck event may have been responsible for the observed genetic homogeneity in lacustrine populations.

The north-west Central Plateau of Tasmania was heavily glaciated 15,000–25,000 yr ago when glaciers were at their maximum extent in both the southern and northern hemispheres (DAVIES 1974). Between 9,000 and 20,000 yr ago melting glaciers created a multitude of lakes which were dammed by glacial moraines or formed by glacial erosion (JENNINGS and AHMAD 1957; DERBYSHIRE 1972). Precipitation rates and the water table were high, ensuring that the lakes were large and drained into the headwaters of the south-easterly flowing Nive and Ouse Rivers of the Derwent River system. About 3,000–7,000 yr ago the connection between the lakes and the south-easterly drainage was severed by decreasing rainfall (DAVIES 1974). A simultaneous increase in evaporation rates began the fragmentation of the larger lakes into the extant assemblage of small lakes.

*G. truttaceus* may have been continuously distributed throughout lakes and streams in the Derwent Valley when the two habitats were contiguous 9,000–20,000 yr ago. At this time, the lakes were presumably large and productive and may have supported large numbers of *G. truttaceus*. The genetic diversity of *G. truttaceus* in the lakes at this time was probably equal

to that of stream populations which we assume to be similar to the diversity measured in this study. Breeding adults from the lake and upper parts of the river drainage probably migrated to the river mouth each year to reproduce. When the connection between the lake and the river was severed 3000–7000 yr ago, large numbers of fish may have been isolated in the prototype lake. Mitochondrial haplotypes in the four lakes sampled were identical, implying that the process which produced the observed patterns of mtDNA diversity began after lake and stream contact was severed and was completed before the lakes were fragmented, a period of only a few thousand years. Alternatively, the four lake populations may have identical mitochondrial haplotypes due their colonization by genetically uniform fish from a nearby lake during a period of high precipitation and subsequent flooding.

The newly isolated lake population would have rapidly become extinct unless it was able to reproduce in the lacustrine environment. If the stream-derived adults persisted in their autumn spawning habits, eggs or larvae may not have survived in the cold, unproductive lake during winter. Modern populations of lake fish have acquired the ability to spawn in spring when larvae take advantage of increased water temperatures and the resulting increase in lake productivity. To account for an alteration in life history, spawning stimuli may have been absent in the lake during autumn but were present in the lake environment in spring. For example, spawning may be induced by a temporary stabilization of temperature that was a feature of the spring but not the autumn climate on the Central Plateau. Little is known about environmental stimuli controlling the synchronization of spawning except that HUMPHRIES (1989) observed spawning in streams during periods of decreasing temperature and photoperiod. There is no evidence that stream populations spawn in both autumn and spring.

If the newly formed lake population of *G. truttaceus* was preadapted to the lacustrine environment by being able to delay spawning until spring, the size of the lake population would have been dependent on the carrying capacity of the lake and its mtDNA diversity would have been equal to that of its founding stream population. At this time we assume lake fish were numerous as the lake was presumably large and productive. It is difficult to account for the observed lack of mtDNA diversity in present day lake populations by random genetic drift, given the large size and high diversity of the original population and the short time since its formation. AVISE, NEIGEL and ARNOLD (1984) have modeled genetic drift of mitochondrial genomes in the form of stochastic lineage extinction associated with reproduction. In simulations performed under conditions where the carrying capacity of the environment is equal to the number of founders, as it may have been in the lacustrine population, they predicted that four times the population size in generations must elapse to account for the survival of the two observed haplotypes from a heterogeneous population. Given a generous estimate of elapsed time since the severing of the contact between the lake and the river and the fragmentation of the prototype lake as 10,000 yr, and assuming generation length is 2 yr, the founding lake population could have consisted of no more than 1250 breeding females. We consider this to be an underestimate of female effective population size and suggest that random genetic drift alone cannot account for the lack of genetic diversity in the lake population.

When *G. truttaceus* became isolated from its riverine habitat and if it was not preadapted to the lacustrine environment, natural selection may have been responsible for the species' life history switch from autumn to spring spawning to suit its new habitat. Natural selection would have rapidly eliminated from the population the offspring of the majority of adults which could not delay spawning until after winter. As life history traits are highly heritable in domesticated salmonids, we assume that a proportion of the offspring of adults which did spawn after winter inherited the life history strategy of their parents. A selective force of this magnitude may have created a pronounced population size bottleneck which could have been responsible for the observed lack of mtDNA diversity in extant lake fish. Natural selection on life history traits is not the only force which could have been responsible for a bottleneck in the lake population. Sudden and substantial mortality of lake fish could have been caused by any number of adverse environmental conditions from prolonged freezing of the lake surface to a decrease in lake productivity leading to lack of food.

The nuclear genetics of Isabella Lagoon fish are consistent with the population having experienced a pronounced, but transitory, bottleneck. The minimum effective number of individuals ($N_0$) which may have given rise to a post-bottleneck population can be calculated from $H_s = \{1 - [1/(2N_0)]\} \cdot H_0$ (NEI, MARUYAMA and CHAKRABORTY 1975) where $H_0$ and $H_s$ are the heterozygosities of the pre- and post-bottleneck populations, respectively. Using the observed average nuclear heterozygosities of two stream populations ($H_0$; 0.11635) and the Isabella Lagoon population ($H_s$; 0.10355) the proposed bottleneck may have involved only five fish. This is a crude estimate only as the variances of the average heterozygosity measurements are large and overlapping. The estimate of bottleneck size can be used to calculate the expected number of alleles retained at each locus after a transitory bottle-

neck according to $E(n) = m - \Sigma(1 - P_j)N_0$ (DENNISTON 1978) where $m$ is the original number of alleles, $P_j$ is the frequency of the $j$th allele and $N_0$ is population size during the bottleneck. There is an approximate concordance between the observed and expected number of alleles assuming the Isabella Lagoon population was derived from a random sample of five fish from Allens and Fortescue Lagoon Creeks (Table 4).

The ability of stream galaxiids to switch life history strategies upon encountering a lacustrine environment is important to the evaluation of the relative importance of bottlenecking and drift. Translocation experiments, where stream fish are introduced into lakes and vice versa, could be done to test the flexibility of the life history strategy of this galaxiid. In mixed populations of lake and stream fish the presence or absence of *Nco*I restriction site 4 in mtDNA haplotypes could be used to distinguish between descendants of translocated stream or lake fish. The apparent rapidity (3000–7000 yr) with which major population genetic and adaptive changes have occurred in lacustrine populations of *G. truttaceus* are not without precedent. Speciation among the cichlid fish of Lake Victoria in Africa has occurred swiftly. One hundred and seventy species of cichlids (Haplochromis) are found in the lake, which is only 500,000–750,000 yr old. Lake Nabugabo, which was part of Lake Victoria as recently as 4000 yr ago, contains several endemic species of cichlids (FRYER and ILES 1972).

If the lacustrine populations of *G. truttaceus* have experienced a severe, but transitory, bottleneck, it is possible that speciation may be occurring due to a genetic revolution aided by the major genetic disruptions that are thought to be associated with a founder event (TEMPLETON 1980). However, the demonstration that lacustrine populations of *G. truttaceus* have experienced a bottleneck does not exclude the hypothesis that speciation of the landlocked isolate may occur by divergence. The biogeographic observations made by MAYR (1954, 1963) that small population size on islands leads to the proliferation of new species is evidence for the occurrence of speciation due to founder effects. The intense adaptive radiation of the 700 Hawaiian drosophilid species upon islands some of which are less than 400,000 yr old (CARSON 1976) also suggests that speciation can occur during rapid genetic shifts. The relative importance of speciation by founder events (CARSON and TEMPLETON 1984) compared to speciation by gradual, sequential allele substitutions (BARTON and CHARLESWORTH 1984) can only be accurately evaluated by the detailed analysis of a major gene complex in the process of disruption during speciation. Lacustrine populations of *G. truttaceus* which are possibly on the brink of speciation by the founder effect may be suitable species in which to search for a gene complex involved in a shift from one adaptive peak to another.

**Natural selection upon galaxiid mtDNA:** Natural selection on mtDNA haplotypes has been implied from studies of the molecular biology of the genome, population genetic analyses and breeding experiments. ADAMS and ROTHMAN (1982) implicated selection in the nonrandom distribution of restriction sites throughout the human mitochondrial genome. LANSMAN *et al.* (1983) compared site variability for eight 6-base restriction enzymes in the mitochondrial genome of several subspecies of *Peromyscus maniculatus* and reported no variable sites in the 12S and part of the 16S rRNA genes. We also found a complete lack of variable restriction sites in the 12S and 16S rRNA genes in *G. truttaceus* mtDNA. Ribosomes have considerable secondary and tertiary structure, stabilized by complementary base pairing, which is vital for translation of polypeptides (HIXSON and BROWN 1986). Individuals carrying substitutions in their mitochondrial genomes which destabilize the essential structure of mitochondrial ribosomes may never join the population, reflecting strong selection for all, or parts of the base sequence of the 12S and 16S rRNA genes. The observation of interspecies transfer of mtDNA across zones of hybridization (POWELL 1983; TEGELSTRÖM 1987) may be explained by mtDNA haplotypes from one species conferring a selective advantage upon hybrid and backcrossed offspring. MACRAE and ANDERSON (1988) tested the assumption of neutrality for *Drosophila pseudoobscura* mtDNA haplotypes by monitoring their frequencies for at least 10, and in one case 32, generations. They concluded that the haplotypes were not always neutral and may be subject to sporadic bouts of selection.

If mtDNA evolution conforms to the neutral (KIMURA 1983) or nearly neutral model (OHTA 1974, 1976), the expected amount of mtDNA sequence divergence within an interbreeding population can be predicted from $N_e$, the effective population size, and the rate of base substitutions. However, OVENDEN, MACKINLAY and CROZIER (1987) and AVISE, BALL and ARNOLD (1988) have shown that the amount of intraspecific mtDNA divergence in large, panmictic populations cannot be accurately predicted from the censused population size and the accepted rate of mtDNA evolution. Assuming the population is in equilibrium, OVENDEN, MACKINLAY and CROZIER (1987) compared the observed amount of mtDNA sequence divergence in three species of rosella parrots to that expected from twice the product of the censused population size, the rate of mtDNA evolution (BROWN, GEORGE and WILSON 1979) and the generation length (NEI and LI 1979). AVISE, BALL and ARNOLD (1988) compared the observed number of generations to shared ancestry of pairs of mitochon-

drial genomes drawn at random from American eel, hardhead catfish and red-winged blackbird populations to that expected from inbreeding theory (TAJIMA 1983) using reasonable estimates of population size and the calculated rate of mtDNA evolution (BROWN, GEORGE and WILSON 1979). Both studies reached the same conclusion that the rate of genome evolution is much slower than calibrated or the effective population size is two to three orders of magnitude below the estimated total population size. OVENDEN, MACKINLAY and CROZIER (1987) rejected the hypothesis of a much slower rate of mtDNA evolution because mtDNA haplotypes were species specific and accepted the alternative hypothesis of reduced $N_e$ because the $N$ calculated for the three species of rosellas from nuclear genetic diversity was about twice that calculated from mitochondrial genome diversities. AVISE, BALL and ARNOLD (1988) also concluded that $N_e$ was much smaller than censused population sizes due to the large amount of mtDNA sequence heterogeneity observed in the selected species and their close relatives. Thus the measured amount of mtDNA divergence and the accepted rate of mtDNA evolution suggests that, at least for eels, catfish, blackbird and parrot populations, the effective population size is two to three orders of magnitude below the estimated population sizes. Selection may be responsible for this reduction in effective population size.

If selection is operating in mtDNA haplotypes it may be a strong or weak force. A mitochondrial genome possessing a slightly beneficial mutation may cause a slow revolution among the mitochondrial lineages of the interbreeding population to which it belongs. The female carrying the genome containing the slightly beneficial sequence would be more likely to survive and reproduce, as would be her daughters, causing the genome to increase in frequency among the population. With time, mutations will occur in the germ line of some of the females in this lineage, creating a cluster of closely related lineages. In the meantime, lineages not containing the slightly beneficial sequence would become less numerous as they were replaced by lineages that did contain the sequence.

Several features of mtDNA lineage relationships within stream populations of *G. truttaceus* suggests that a slow revolution has occurred, or may be occurring within the species. Haplotype #4 may be the descendant of a genome in which a slightly beneficial mutation arose. This would account for its central position in the haplotype phylogeny (Figure 5) and the platykurtic nature of the frequency distribution of diversity values between haplotypes (Figure 3). The notion that haplotype #4 may be visible to selection is supported by its being the most common haplotype among the 150 fish sampled from stream populations. This haplotype may represent a successful lineage which became more frequent in the population because it conferred upon its carriers a higher fitness. The other lineages in the population may differ from this central lineage by having one or numerous selectively neutral mutations.

An alternative explanation for the central position of a single haplotype in the evolutionary tree of *G. truttaceus* mitochondrial genomes is that the population has recently experienced a severe population size bottleneck, through which only a few haplotypes survived. Bottleneck events have been tendered to explain the topology of many mtDNA phylogenies (WILSON *et al.* 1985 and references therein) and may be a general feature of the recent history of some animal populations. If mtDNA is evolving within *G. truttaceus* at the rate of 2% per million years (BROWN, GEORGE and WILSON 1979), the amount of divergence per lineage between haplotype #4 and the remainder, 0.24%, suggests that the bottleneck occurred about 120,000 yr ago. However, there is no evidence to suggest that stream populations of *G. truttaceus* have been recently perturbed. Sea levels and climate patterns have fluctuated significantly in southern Australia during the Pleistocene and the number of freshwater habitats available to the species may have also changed with time, causing localized extinctions. The extensive southern Australian coastline available to marine larvae seeking a freshwater habitat and a high rate of gene flow between localities would ensure that rapid stream recolonization would occur without a detrimental affect on the total population heterozygosity. The life history and habitat requirements of *G. truttaceus* appear to be ideal for the survival of large-scale environmental changes which would provide an efficient buffer against bottleneck events.

The star shaped pattern of mtDNA haplotype relationships among stream populations of *G. truttaceus* could also be explained by the chance absence from that data set of intervening haplotypes which would increase the complexity of the haplotype phylogeny. As we sampled numerous fish (211) at random throughout their range, we believe that the probability of failing to collect haplotypes which would significantly alter the star shape of the haplotype phylogeny was low. The large amount of convergence among the characters describing each haplotype means that the topology of the haplotype tree presented here is not the only one which represents the phylogenetic relationships between haplotypes. However, alternate topologies are likely to be centered around haplotype #4 as it is the haplotype most similar to the other 57 haplotypes.

We believe that haplotype relationships in stream populations are being affected by weak selective forces only. If mtDNA haplotypes were subject to strong

selective forces the genome carrying the mutation would replace every other genome the population in a few generations because of the high fitness it conferred upon its "gene machine" (*sensu* DAWKINS 1976). The effect upon the mitochondrial genome diversity of the population would simulate that caused by a severe population bottleneck. Mitochondrial and nuclear genetic diversity of the lacustrine populations of *G. truttaceus* suggest that a severe bottleneck may be part of their evolutionary history. It is conceivable that the bottleneck was induced by a strong selective force upon a few mitochondrial haplotypes which may have facilitated survival in the lacustrine environment. It remains to be tested whether mitochondrial genes are in any way linked to the major adaptive shift which occurred in lacustrine populations; an alteration in life history from autumn to spring spawning.

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