POPULATION STRUCTURE AND MICROPHYLOGEOGRAPHIC PATTERNS OF DOLLY VARDEN (*SALVELINUS MALMA*) ALONG THE YUKON NORTH SLOPE

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

JAMES GARETH RHYDDERCH

In partial fulfillment of requirements

for the degree of

Master of Science

March, 2001

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ABSTRACT

POPULATION STRUCTURE AND MICROPHYLOGEOGRAPHIC PATTERNS OF DOLLY VARDEN (*SALVELINUS MALMA*) ALONG THE YUKON NORTH SLOPE

James Gareth Rhydderch
University of Guelph

Advisor:
Dr. Paul D.N. Hebert

This study investigated the genetic relationships among closely adjacent populations of Dolly Varden charr, *Salvelinus malma*, to infer recent evolutionary events on the Yukon north slope. Specifically, microphylogeographic patterns were ascertained, relationships among different life history types of charr were determined, and the potential role of dispersal and philopatry on past and present gene flow was assessed. The possibility of employing genetic markers for stock discrimination and for conservation were also examined. Microsatellite and mitochondrial D-loop data on eight populations of *S. malma* were concordant, revealing that gene flow was restricted between drainages but common among sites within drainages. Two phylogroups were observed in the Firth drainage, but the other three drainages were dominated by a single phylogroup. Furthermore, the Firth drainage contained the highest genetic diversity, while the other drainages possessed a subset of the variation found in it. These results suggest that recolonization of the Yukon north slope likely came from Alaskan populations and that the Firth drainage should receive priority for conservation.

ACKNOWLEDGEMENTS

I would like to thank Paul Hebert for giving me the fantastic opportunity to conduct research in his lab. His knowledge and enthusiasm for science has been inspirational, and also at times even a little intimidating. I began my work in Paul's lab with Dr. Junjian Chen. Not only did I get a solid introduction into molecular genetic techniques from him, but also an appreciation for the dedication required to be successful in the research field. Paul has an amazing knack at attracting diverse types of dynamic graduate students and postdoctoral fellows into his lab. I have had great pleasure in working with them all: Andrea Cox, Chad Rowe, Ryan Gregory, Rob Dooh, John Colbourne, Derek Taylor, Sarah Adamowicz, Melania Cristescu, Ed Remigio, Jeremy Deeward, Veronika Sacherova, Alina Cywinska, and Dave Hardie.

This research would not have been possible without the cooperation of Dr. Jim Reist, Department of Fisheries and Oceans. His dedication to northern research and his knowledge on northern salmonids is truly unparalleled. By extension, I would also like to thank all the people and organizations which made it possible for Dr. Reist to do his research in the arctic.

I owe virtually all of my understanding of microsatellites data collection and analysis to my time spent in Quebec. I thank Dr. Louis Bernatchez for allowing me to visit his lab and for his contagious excitement for science. I would also like to thank all of his students for making me feel so welcome, in particular, Sylvain Martin, Julie Turgeon and Vincent Castric.

I would like to thank my other committee members, Dr. Roy Danzmann and Dr. Chris Wilson, who really came through for me at the end. Also thanks to Trish

Lundrigan and Chris Jastrebski for aiding in data collection, all the fish genetics people in the Danzmann/Ferguson lab (especially Rachel Woram), and also Angela Holliss, and Ian Smith.

I would like to thank my family and friends outside of the zoology department for their understanding and perspective throughout my degree including: Mom, Dad, Sian, and Dave, the entire hairclub for men, the 35 Paisley crew (Lara, Ger, Kelly, Aaron, Alison, Dick, and Adria), also Kathleen (Philly to me!) and finally Adam, Larry and Cindy.

I owe my greatest thanks to Jonathan D.S. Witt for both his friendship and scientific stimulation. I will always savour the many (?!?) evenings spent at the Albion, the Diplomat or the Arena discussing various aspects of science, our shared interest in history, or generally solving the world's problems.

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CHAPTER 1

Evolution in the genus *Salvelinus*

Salmonid Diversity

The 30 species and 5 – 9 extant genera comprising the subfamily Salmoninae (Stearley and Smith 1993) are an important component of the Holarctic fish fauna. One of these genera, *Salvelinus*, is especially dominant in cool temperate and arctic regions where it is represented by both freshwater and anadromous forms (Scott and Crossman 1973). The genus includes six common species: *S. namaycush* (lake trout), *S. fontinalis* (brook trout), *S. confluentus* (bull trout), *S. leucomaenis* (spotted charr), *S. alpinus* (arctic charr), and *S. malma* (Dolly Varden charr) (Behnke 1972), all of which are distributed in North America except for *S. leucomaenis*. Several other narrowly endemic *Salvelinus* species have been described predominantly from western Asia (Savvaitova 1995). Behnke (1972) placed the North American species into three subgenera, two of which were monotypic. The subgenus *Baione* contained only *S. fontinalis*, while the subgenus *Cristivomer* included only *S. namaycush*, and the other species were assigned to the subgenus *Salvelinus*. These subgeneric groupings have not been seriously contested and the morphological similarities between *S. leucomaenis* and *S. namaycush* have been attributed to convergent evolution by these piscivorous predators (Behnke 1984).

Morphological studies gave little indication of the phylogenetic relationships between subgenera. Karyotyping revealed a range of chromosome numbers from 2N = 84-86 for *S. leucomanenis* to as low as 2N=78 for *S. confluentus* (Viktorovsky 1978; Cavender personal communication *in* Behnke 1984). The variance in chromosome numbers reflects Robertsonian fissions and fusions. Although higher chromosome numbers are considered ancestral, this conclusion does not fit well with zoogeographic and morphological evidence (Behnke 1984). The application of molecular techniques has now provided additional phylogenetic insights. RFLP analysis of the mitochondrial

genome supported the three subgenera proposed by Behnke (1972), but could not resolve relationships within the subgenus *Salvelinus* (Grewe *et al.* 1990).

Allozyme analyses (Crane *et al.* 1994) and rDNA sequence data (Phillips *et al.* 1994; Phillips *et al.* 1995) have also supported the existence of three subgenera. However, the nDNA data indicated that *S. confluentus* and *S. leucomaenis* were sister taxa (Crane *et al.* 1994; Phillips *et al.* 1994), while the mitochondrial data placed *S. confluentus* within the *S. alpinus* – *S. malma* complex (Grewe *et al.* 1990; Phillips *et al.* 1995). Phillips and Oakley (1997) propose that this discordance between the nuclear and mitochondrial genomes is evidence of a recent hybridization event between bull trout and the arctic charr complex. If this hypothesis is correct, the ancestral bull trout's mitochondrial genome was displaced by arctic charr mtDNA, while it retained much of its ancestral nuclear DNA. Selection on the advanced generation hybrids for bull trout nuclear DNA, or repeated backcrossing of hybrid females (carrying arctic charr mtDNA) to male bull trout, may explain these observations.

Behnke (1984) divided the subgenus *Salvelinus* into two species complexes, the arctic charr group and the Dolly Varden group (*S. leucomaenis* was left as uncertain). However, others considered arctic charr and Dolly Varden to be a single phenotypically plastic species (Savvaitova 1980; Savvaitova 1995). *S. confluentus* has also been confused with *S. malma* and convincing evidence that the two species are distinct was only achieved by detailed osteological examination (Cavender 1978; Cavender 1980).

Perhaps the most contentious issue in the subgenus *Salvelinus* relates to the taxonomic status and distribution of the *S. alpinus-malma* complex. McPhail (1961) and McCart (1980) treated populations along the north slope of Alaska and the Yukon as a

western form of arctic charr. Morrow (1980) demonstrated that these populations were, in fact, a northern form of *S. malma*. The species status of *S. malma* has been questioned (Savvaitova 1995) reflecting the low genetic divergence between *S. malma* and *S. alpinus* (Phillips *et al.* 1995). Specifically, among three subspecies of *S. alpinus*, two subspecies of *S. malma*, and the Asian stone charr (*S. albus*), ITS2 contained no phylogenetically informative sites. Three hundred base pairs at the 5' end of the external transcribed spacer (ETS) of the rDNA adjacent to the 18S coding region contained only a single base pair fixed difference between *S. a. alpinus* and the other members of the *S. alpinus – S. malma* complex. ITS1 contained 6 synapomorphies, but 5 of these were shared by *S. m. malma* and *S. albus* and a single synapomorphy was shared between *S. m. lordi* and *S. a. erythrinus*. Thus, not only are there few phylogenetically informative sites, but those present do not always group taxa thought to be conspecific (Phillips *et al.* 1999). Similarly, when genetic distance algorithms were employed, subspecies of *S. alpinus* and *S. malma* did not always group by putative species designations. Crane *et al.* (1994) report an unresolved trichotomy among Asian *S. malma*, North American *S. malma* and *S. alpinus* using allozymes. However, they argue that the fixed differences observed between these taxa at nuclear loci indicates their species status more securely than measures of genetic distance or phylogenetic trees. They cite two studies where fixed allozyme differences were observed between sympatric Arctic charr and Dolly Varden in Asia (Kartavstev *et al.* 1983) and Alaska (Gharrett *et al.* 1991). Similarly, Reist *et al.* (1997) observed a fixed difference between populations of *S. malma* and *S. alpinus* on either side of the Mackenzie delta. Over a wider geographic distribution, two fixed differences were found between this species pair (Crane *et al.* 1994). However, as Crane

et al. (1994) point out, none of the fixed allozyme differences are diagnostic across the range of these two species.

Two allopatric forms of Dolly Varden are commonly recognized in North America, a northern form (*S. m. malma*) and a southern form (*S. m. lordi*) (Figure 1-1). The Kuskokwim river in Alaska is the approximate demarcation point between the range of these forms (Craig 1989). The northern form also occurs in Asia as the type specimen for *S. m. malma* was collected in the Kamchatka river, Russia (Walbaum 1792). A southern Asiatic form (*S. m. krascheninnikovi*) occurs on the mainland south of the Amur River and also on Hokkaido and the Sakhanlin Islands in Japan (Behnke 1984).
A central theme in the literature concerning *S. malma* is its historically confused taxonomic status. As mentioned previously, *S. malma* has been confused with both *S. alpinus*, (McPhail and Lindsey 1970; McCart 1980) and *S. confluentus* (Cavender 1978; Cavender 1980). Rigorous investigations utilizing morphology, meristics, karyotypes, allozymes and nucleotide sequences have resolved much of the debate but a final consensus has yet to be achieved. It is argued here that the unresolved nomenclature of the genus reflects underlying evolutionary processes. Namely, repeated isolation events over varying temporal scales followed by secondary contact between groups or populations have resulted in varying degrees of specialization, morphological distinctiveness, genetic divergence and, most importantly, reproductive isolation. High morphological variation and low genetic divergence are not unique to the genus *Salvelinus*; the same pattern has been reported for other fish taxa inhabiting formerly glaciated areas (for example the coregonids) (Bernatchez and Wilson 1998).

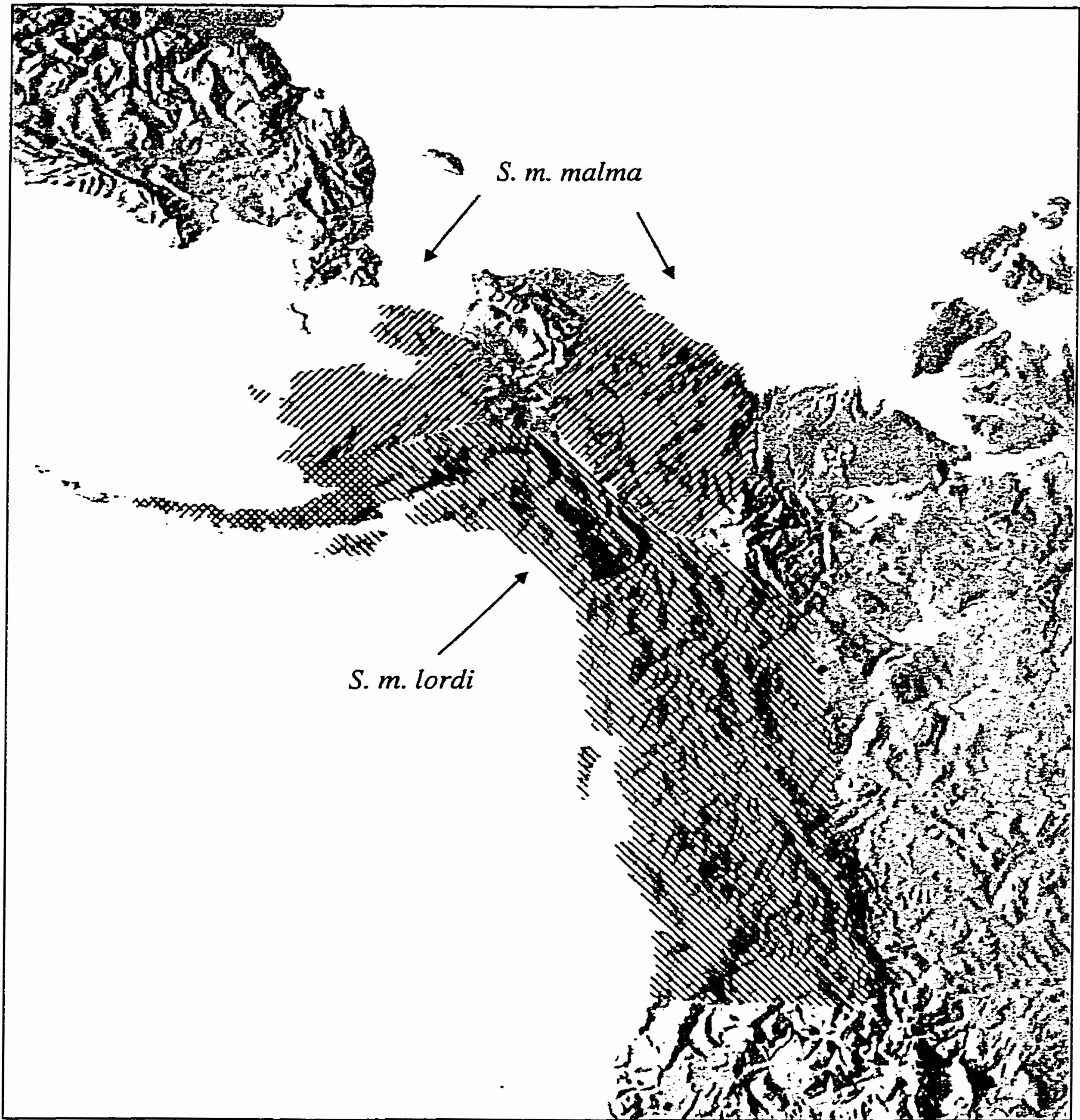

Figure 1-1. Distribution of the northern form (*S. m. malma*) and southern form (*S. m. lordi*) Dolly Varden charr in North America. Modified from Craig (1989).

Vicariance events and *Salvelinus* evolution

Taxa inhabiting areas beyond the direct influence of glaciations usually conform better to the biological species concept than those from glaciated regions. Because their habitats were less impacted by vicariance events, southern species could specialize on particular niches and develop strong pre- and post-zygotic reproductive barriers. By contrast, taxa inhabiting formerly glaciated areas have repeatedly been flushed from their habitats. As a result, divergences between clades and sister species is often low (Bernatchez and Wilson 1998) and introgression is more common (see below).

Speciation events and divergence between phylogeographic lineages usually occur under conditions of allopatry (Avise *et al.* 1998). Therefore, the glacial advances and retreats throughout the Pleistocene likely had a great influence on the evolution of species of *Salvelinus*. Glacial advances pushed the species into isolated refugia and exposed them to strong selection. As the glaciers receded, recolonization and secondary contact between previously isolated groups occurred. Whether introgression took place following secondary contact depends on factors such as the length of isolation and strength of the selection pressures against hybrids. Although species integrity appears to be maintained, hybridization between species of *Salvelinus* is common. *S. alpinus* hybridizes in the wild with both *S. fontinalis* (Hammar *et al.* 1991) and *S. namaycush* (Hammar *et al.* 1989; Wilson and Hebert 1993). Evidence for historic arctic charr – lake trout (Wilson and Bernatchez 1998) and arctic charr – brook trout (Bernatchez *et al.* 1995; Glémet *et al.* 1998) introgression has also been demonstrated. *S. malma* has similarly been shown to hybridize with *S. confluentus* (Baxter *et al.* 1997). Also, natural hybridization occurs between *S. confluentus* and *S. fontinalis* (Leary *et al.* 1983; Markle

1992). Males of *S. fontinalis* have been successfully crossed with female *S. namaycush* and introduced to the Great Lakes producing fertile F1's known as "wendigo" or "splake" (Berst *et al.* 1980). Past hybridization during unstable periods of glacial advance or retreat may have occurred with greater frequency due to the effects of bottlenecking and recolonization. Hybridization events such as these have likely played an important role in *Salvelinus* evolution. Also, these introgression events coupled with the high morphological variation within species have likely contributed to the taxonomic confusion in the group.

Despite the role which hybridization likely played in the long term evolution of *Salvelinus* species or phylogroups, species integrity has been maintained. Critical adaptations and behaviours act as prezygotic reproductive barriers between forms. Where two or more species live in sympatry, competition drives modifications of their resource and habitat utilization. For example, *S. namaycush* take over the role of top piscovore from *S. alpinus* when they co-occur in subarctic lakes (Fraser and Power 1989). Although the co-occurrence of *Salvelinus* species is not rare, it is more common for them to occur allopatrically. For example, the distribution of *S. alpinus* and those of the southern and northern forms of *S. malma* in western North America are almost entirely allopatric. This pattern probably reflects specialization to different ecozones. However, recolonization history likely also played a role where the first colonizing members were able to competitively exclude conspecifics or closely allied taxa.

Philopatry in *Salvelinus*

In anadromous fishes, population subdivision is maintained after recolonization by philopatric behaviour. Philopatry is the absence of dispersal (Shields 1982).

However, it can also be maintained by the homing of individuals to a natal area and is almost always associated with reproduction. In the case of *S. malma*, where individuals are not thought to spawn every year (McPhail and Lindsey 1970), philopatric homing may also reflect fidelity to a natal area for overwintering. Many migratory animals exhibit philopatry including marine green turtles (Bowen *et al.* 1992), whales (Baker *et al.* 1990; Gladden *et al.* 1999; Malik *et al.* 1999), birds (Eden 1986; Robertson and Cooke 1999), salmonids (Groot and Margolis 1991; Tallman and Healey 1994; Foote *et al.* 1997), lizards (DeFraipont *et al.* 2000) and insects (Roisin 1999). Philopatry is an adaptation which allows animals to range over huge geographic areas, ordinarily to increase their food uptake or to enhance their survival, while ensuring that reproduction is achieved by returning to favourable sites. Substantial research on homing has shown the variation and complexity with which migratory and philopatric species perceive their environment (Cury 1994; Dittman and Quinn 1996). Moreover, the evolutionary significance of philopatry as an isolating mechanism is receiving increased attention (Shields 1982; Lemel *et al.* 1997; Markert 1998). Philopatric taxa, despite occupying habitats with no physical barriers, often show low levels of gene flow between natal areas and evolve as if allopatric. Therefore, philopatric behaviour may have the evolutionary consequence of promoting diversification through drift and adaptation to local environments.

Although it is widely held that *S. malma* is philopatric (McPhail and Lindsey 1970; DeCicco 1997; Everett *et al.* 1997), the species occupies areas which were formerly glaciated (Figure 1-2) suggesting it also has some propensity to colonize new habitats. The observation of philopatry and recent (10k B.P.) colonization behaviour

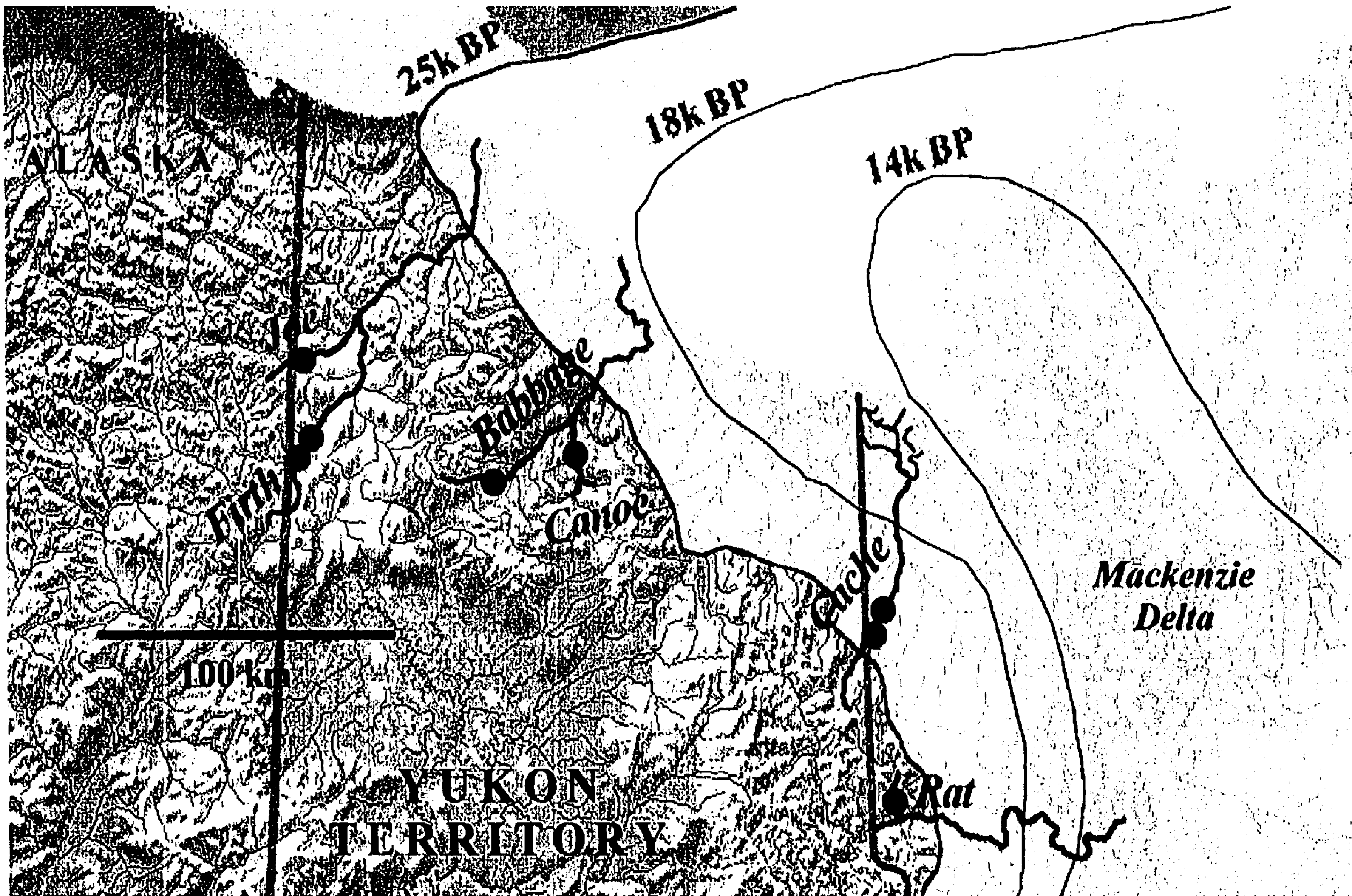

Figure 1-2. Approximate glacial margins of the Yukon north slope (25, 18, 14 k BP). Modified from Dyke and Prest (1989).

raises questions concerning the mechanisms or cues allowing both behaviours to be evident. Straying may be an alternative life history strategy (adaptive) possessed by part of the population to protect against the loss of an entire stock due to environmental catastrophe in the home stream (Sandersock 1991). Alternatively, straying may be the result of individuals 'making mistakes', and returning to a non-natal river (Avise 1994). Through time the byproduct of inaccurate homing may be a genetic structure similar to that expected through stepping stone recolonization. However, for *S. malma*, only approximately 5% of summer riverine habitat is available to for overwintering (Craig 1989) and philopatry may be an important adaptation to ensure that fish reach overwintering habitat. Fish entering new habitats (strays) may not be able to locate safe overwintering areas. A final hypothesis, which is not necessarily mutually exclusive from the other hypotheses, is that philopatry and straying are behaviours in response to the environment where periods of disturbance promote dispersal and periods of stasis promote philopatry.

Members of the genus *Salvelinus* have been the focus of much ecological and evolutionary research. Evidence for both allopatric and sympatric divergence has been shown, as well as reticulate evolution via introgression and hybridization. As such, this genus represents a model system for studying both micro- and macro-evolutionary events. One may infer through comparisons of populations, glacial races, subspecies and species, a continuum of genetic divergence and perhaps successive levels of the speciation process. The present study extends our knowledge of the population structure of an important and little studied member of the genus, *S. malma*.

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CHAPTER 2

Population structure and microphylogeographic patterns of Dolly Varden charr (*Salvelinus malma*) along the Yukon north slope

INTRODUCTION

Members of the genus *Salvelinus* have been the subject of intensive population genetic research in North America. Phylogeographic studies on arctic charr, *S. alpinus*, (Wilson *et al.* 1996) and lake trout, *S. namaycush*, (Wilson and Hebert 1998) using mitochondrial restriction fragment length polymorphisms identified multiple phylogeographic lineages in these species correlating with glacial refugia. Finer scale studies of gene diversity revealed substantial polymorphism, enabling stock discrimination of lake trout (Vitic and Strobeck 1996), arctic charr (Kornfield and Kircheis 1994; Bernatchez *et al.* 1998), brook trout (Bernatchez and Danzmann 1993; Angers *et al.* 1995; Jones *et al.* 1996; Angers and Bernatchez 1998), and bull trout (Spruell *et al.* 1999; Taylor *et al.* 1999).

The Dolly Varden charr, *Salvelinus malma*, is an important element of the freshwater fish fauna in both the north Pacific and western arctic regions (McPhail and Lindsey 1970). Two North American subspecies are recognized (Behnke 1984). The southern form, *S. malma lordi*, inhabits coastal areas from Washington state north to the Alaskan Peninsula (Haas and McPhail 1991). The northern form, *S. malma malma*, has a disjunct distribution. It occurs from the Alaskan Peninsula to Point Hope but then is absent from a 700 km stretch along the coastal plains of the central north slope (Craig 1989). Its distribution then continues from the Colville River east to the Mackenzie delta. A closely allied species, *S. alpinus*, replaces *S. malma* at most sites east of the Mackenzie (Reist *et al.* 1997), although the two species appear to co-occur in the Coppermine and Tree Rivers which are over 800 km east of this point (J.D. Reist, Dept. of Fisheries and Oceans).

Few genetic investigations of *S. malma* have been conducted. Reist *et al.* (1997) employed allozymes to distinguish between *S. malma* and *S. alpinus* in the western arctic of North America. Allozyme analyses also provided evidence of genetic divergence among populations from drainages along the Alaskan and Yukon north slope (Reist 1989; Everett *et al.* 1997). Although allozyme electrophoresis enabled successful mixed stock discrimination by broad geographic region in the Beaufort Sea area (regions defined by pooling major localized drainages) (Krueger *et al.* 1999), insufficient resolution was available to distinguish local populations. This difficulty might be due to the recent divergence of populations and the slow rate of evolutionary change at allozyme loci. However, it is also possible that gene flow has been sufficient to genetically homogenize populations in adjacent drainages.

Estoup *et al.* (1998) demonstrated that the higher levels of polymorphism at microsatellite loci (or variable number of tandem repeats – VNTR's) provide a higher power for statistical tests of population differentiation and were much more successful in assigning *Salmo trutta* to their population of origin than allozyme loci. The present study investigates the utility of two rapidly evolving DNA markers, microsatellites and noncoding mitochondrial D-loop sequence diversity, to examine the hypothesis that each spawning area of *S. malma* represents a unique breeding population.

Populations of *S. malma* on the north slope predominantly inhabit rivers draining the Brooks Range. The populations examined in this study were collected from eight sites, known to provide both spawning and overwintering habitats, distributed among tributaries from four drainages in the Yukon Territory. These rivers are fast-moving in their upper reaches, but then slow and meander on the coastal plains near sea level for

several kilometres. These rivers flow for a maximum of 7 months and freeze to a depth of 2 m in winter (Craig 1989). Populations of *S. malma* overwinter in specific tributaries which are fed by mountain springs and have a stable year-round discharge.

Considerable variation exists concerning the degree of anadromy exhibited by populations of *S. malma* and their propensity to return to natal sites. The species has been described as "almost a freshwater species" (Craig, 1989) or alternatively as a species capable of over-wintering at sea (Bernard *et al.* 1995). The extent of anadromy and subsequent movements once at sea should have important influences on the magnitude of gene flow between populations spawning in different rivers. Highly anadromous taxa are more likely to colonize both new habitats and distant regions. Various tagging studies have shown that long distance movements of *S. malma* do take place (DeCicco 1992) and that wandering between drainages occurs (McCart 1980; DeCicco 1997). However, allozyme data revealed significant genetic divergence between, and in some cases within, single drainages along the Alaskan and Canadian north slopes (including two of the drainages studied here) (Everett *et al.* 1997) suggesting high fidelity to natal areas. Thus, philopatric behaviour may oppose the effects of dispersal by sustaining genetic isolation despite anadromy.

Populations of *S. malma* on the north slope possess different life history types linked to their degree of anadromy (Reist *et al.* 1991). The first and dominant type includes anadromous fish which move to the ocean during the summer months to feed. The second type are resident fish. These are exclusively male, morphologically distinct from their sympatric anadromous counterparts, and do not journey to the ocean. The third life history type are isolates. These are morphologically similar to residents but

consist of both males and females and are allopatric to the other life history types, typically being isolated above waterfalls. This study employs genetic analyses to clarify the evolutionary relationships between these life history types.

Individuals of *S. malma* from arctic populations do not spawn every year (Armstrong and Morrow 1980). Although tagging studies have shown that fish wander between drainages, adults have never been found to spawn in different river systems (DeCicco 1997). This suggests that *S. malma* exhibits site fidelity for spawning, but not for overwintering. In order to examine site fidelity behaviour for spawning and overwintering, individuals from the Firth River system were partitioned by spawning condition and tested for genetic divergence.

The north slope of the Yukon approximately demarcates the border between the Beringian refugium and the continental ice sheet (Figure 1-2) during the height of the last glacial advance (the Wisconsinan, 125 to 18k B.P.) (Dyke and Prest 1989). The Laurentide ice sheet extended over most of the Canadian arctic west to the Mackenzie Delta, colliding to the south with the Cordilleran sheet (Prest 1973; Mayewski *et al.* 1981). Although part of the Yukon north slope was unglaciated, its biotic communities would have been directly affected by their close proximity to the ice margin.

Correlations between the extent of glaciation and patterns of gene diversity can be used to measure the effects of such historical events on modern population structure. *S. malma* may have persisted along the Yukon north slope throughout the last glaciation. Alternatively, some or all modern populations may derive from recolonization events since deglaciation. As glacial recession in the Mackenzie region proceeded in an easterly direction, recolonization, if it occurred, almost certainly followed a west to east transect

as habitats became available (Figure 1-1). It also follows that glaciation would have had a greater impact (through either local extirpation or bottlenecking) on populations closer to the glacial front than on ones distant from it. Lastly, if gene flow was low between populations in dfferent drainages after deglaciation, populations of *S. malma* closer to the glacial front should possess lower genetic diversity.

Populations of *S. malma* on the Yukon north slope and surrounding aquatic ecosystems are now threatened by environmental change (Dickson 1999). Global warming is projected to have its greatest impact on arctic environments (Kattenberg *et al.* 1996). If the climate continues to warm as forecast, arctic specialists such as *S. malma* may be displaced by their temperate adapted allies. Already, Pacific salmon have expanded their range northward and have recently been found in the Mackenzie Delta region (Babaluk *et al.* 2000). Oil and natural gas prospecting along the Yukon north slope and Mackenzie Delta in the 1970s revealed major reserves but these remain undeveloped (National Energy Board 1998). However, new initatitives propose both the extraction of natural gas and the construction of pipelines to link Alaska and the Yukon to the south (Varcoe 2000). Since these factors may impact the long-term sustainability of populations of *S. malma*, information on the patterns of genetic diversity is required to aid in the development of strategies for their conservation.

METHODS

Sampling

S. malma was collected from eight sites located on four drainages along the Yukon north slope (Figure 2-1 and Table 2-1). Six of these sites are accessible from the Beaufort Sea, but the other two sites, the upper Babbage River (Babbage-*iso*) and the upper Cache Creek (Cache-*iso*) are isolated from the ocean by waterfalls. Fish were caught by gill net, beach seining, minnow trap and electroshocking. Their fork length, weight, age, sex, and gonadal weight were determined. Also, a sexual maturity score was assigned based on the condition of their gonads (McGowan 1987). These parameters were used to assign each fish to one of five life history/cycle types (Reist 1989). Each individual was assigned to one of three life history categories: residents (RES), isolates (ISO), or anadromous (A--) (Table 2-2). Each fish with an anadromous life history was further placed in one of three life cycle stages: large juveniles (ALJ), adult resters (AAR) or adult spawners (AAS) (Table 2-2). Only anadromous individuals which had undergone at least one migration to the ocean were included, as young of the year and small juveniles cannot be discriminated from juvenile residents.

Specimens were collected in late August and early September, during spawning season. Cache, Cache-*iso*, Canoe, and Babbage-*iso* were sampled in 1988, while Rat, Firth-1, Firth-2, and Joe were sampled in 1995 (Table 2-1), which introduces a possible bias due to temporal variation. However, Everett *et al* (1997) detected no temporal variation over a 3 year allozyme survey of *S. malma* from the Alaskan north slope.

Frozen white muscle tissue was dissected from each fish and transferred to salt saturated dimethyl sulfoxide (DMSO) solution for transportation from the Freshwater

Table 2-1. Sample locations grouped by drainage and the number of *S. malma* of each life type (life history or life cycle) genetically examined.

Drainage	Location	Latitude	Longitude	Life Type[1]					
		(N)	(W)	AAS	AAR	ALJ	RES	ISO	Total
Firth R.	Firth-1	68° 39'	140° 56'	49			9		58
	Firth-2	68° 41'	140° 53'	2	4	44			50
	Joe	68° 56'	140° 59'	54	1	49	21		125
Babbage R.[3]	Babbage-*iso*[2]	68° 38'	139° 22'					26	26
	Canoe	68° 46'	138° 45'	5		39			44
Big Fish R.[3]	Cache	68° 17'	136° 21'	42		8			50
	Cache-*iso*[2]	68° 16'	136° 23'					14	14
Rat R.	Rat	67° 47'	136° 19'	48	6				54

[1] Life type categorizes each specimen by its life history type, and also for anadromous types, by its life cycle type (see methods): AAS – adult anadromous spawner, AAR- adult anadromous resting, ALJ- anadromous large juvenile, RES- large juvenile or adult male residents, ISO- large juvenile or adult isolated (above waterfalls).

[2] *iso* indicates locations isolated from potential gene flow by existing above impassable waterfalls

[3] to simplify reading of the text, the Babbage drainage is referred to as "Babbage/Canoe" and the Big Fish drainage is referred to as the "Cache drainage"

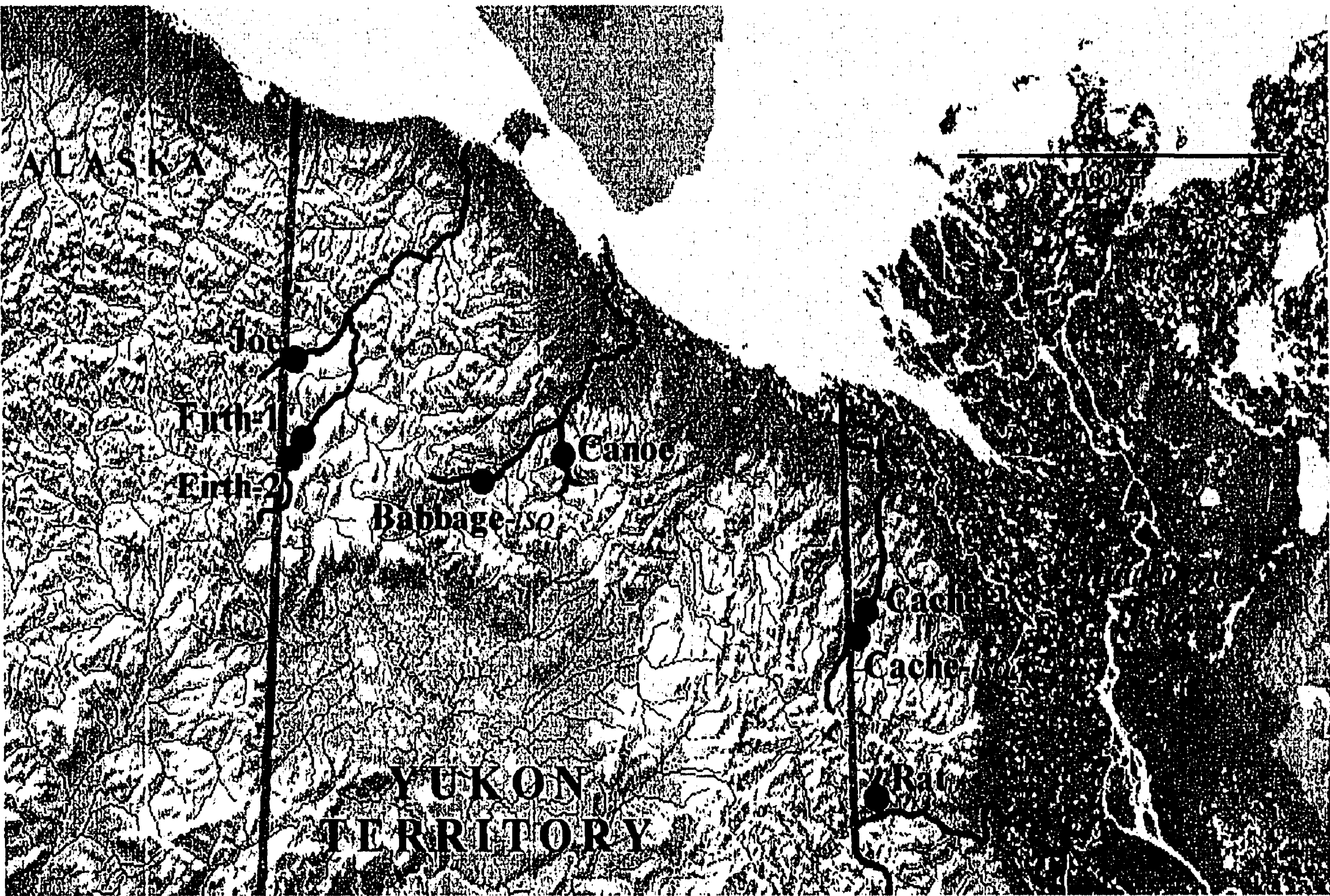


Figure 2-1. Distribution of the eight spawning sites sampled along the Yukon north slope.

Table 2-2. Key for the determination of life history and life cycle stages of *S. malma* along the Yukon north slope.

Life History / Cycle Type	**Abbreviation**	**Diagnostic Features**
Resident	RES	-small (<395 mm fork length), dark colouration -retain parr marks throughout life -spawning colouration superimposed on parr marks and retained throughout life -exclusively male -do not migrate to sea -share overwinter grounds with anadromous form
Isolate	ISO	-morphologically identical to residents (see above) -both males and females -geographically isolated from other populations by impassable waterfall barriers
Anadromous Large Juvenile	ALJ	-large size (>300 mm fork length) -silver in colour (post smolts) -immature gonads[1]
Anadromous Adult Resting	AAR	-large size (>300 mm fork length) -silver in colour -gonads mature but not ripe[1]
Anadromous Adult Spawner	AAS	-large size (>300 mm fork length) -characteristic red spawning colouration -gonads mature and ripe[1]

[1] gonad maturity index as outlined in McGowan (1987) based on visual inspection by disection

Institute, Winnipeg, to the University of Guelph (Maiers *et al.* 1998). Total genomic DNA was extracted by standard organic phenol/ chloroform methods (Brunner *et al.* 1998). DNA was quantified using a GeneQuant (Pharmacia) spectrophotometer and diluted to 50 ng/μl for all PCRs.

Mitochondrial DNA data

A 458 base pair segment of the mitochondrial DNA control region (D-loop) was amplified using the primer HN20 (5'GTG TTA TGC TTT AGT TAA GC) (Bernatchez and Danzmann 1993) and an internal primer, LD440 (5' CAG CTT GCA TAT ACA AGT GC), designed specifically for this study. An MJ PTC-100 Thermocycler was used to amplify the targeted fragment under the following conditions: initial denaturation at 94 °C for 3 min, 35 cycles of amplification at 92 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min 20 sec, and a final extension of 72 °C for 10 min. Samples were amplified under the nucleotide starvation conditions outlined by Chen and Hebert (1998) for screening using directed termination single stranded conformational polymorphism (DT-SSCP). A normal PCR reaction was initially performed to check both the yield and quality of PCR product which was run on a standard 1% ethidium bromide stained agarose mini-gel for 20 min. at 90 volts. The reagent concentrations under the normal PCR were 50 mM KCl, 10 mM Tris HCl (pH 8.3), 3 mM $MgCl_2$, 10 μM of each dNTP, 100 pmol of each primer, 1 unit of *Taq* polymerase, and 50 ng of total genomic DNA combined in a 20 μl reaction. After successful amplification under these conditions, a radioactively labelled PCR was conducted by incorporation of α-^{35}S-dCTP. The reagent concentrations for the radioactive PCR were identical to those for normal PCR except the

nucleotide concentrations were unbalanced with 10 μM of dATP, dGTP and dTTP and 2 μM of dCTP, and the incoportation of 1.5 μCi [α-^{35}S]-dCTP.

2.0 μl of radioactively labelled PCR product was combined with 2.0 μl of stop buffer (97.5% deionized formamide, 10mM EDTA, 0.3% xylene cyanol and 0.3% bromophenol blue). Samples were denatured for 3 minutes at 90 °C and then held on ice until they were loaded. 2 μl of PCR product/stop buffer were loaded in a 7% non-denaturing polyacrylamide gel (29:1 acrylamide to bis-acrylamide) in 0.5X TBE and 5% glycerol. Fifty-eight samples, plus a band pattern standard and positive control (always the same individual from Canoe River) and a negative control (containing all reagents except DNA to check for possible contamination) were loaded on a 35x43 cm gel rig. The gels were run at 15 milliamps and 6-8 watts with the current capped at 600 volts for 8 to 10 hours. After electrophoresis, the gels were lifted onto Whatman's filter paper, dried and exposed to X-ray film for 36 to 72 hours.

DT-SSCP gels were scored by grouping individuals with common banding patterns in a gel. Unique banding patterns from each autoradiograph were then cross referenced with other autoradiographs by amplifying these samples again and running side by side. Whenever uncertainty arose, samples were either rerun side by side or sequenced.

After cross referencing, unique banding patterns were sequenced to determine the number and position of mutations in each haplotype. In order to maximize the sequence information, the entire D-loop (approximately 1100 base pairs) was amplified using the primers HN20 and LN20 (5'ACC ACT AGC ACC CAA AGC TA) (Bernatchez and Danzmann, 1993). PCR amplifications were conducted in a 50 μl reaction following the

same temperature profile as for HN20/LD440 (above). The reagent concentrations were 50 mM KCl, 10 mM Tris HCl (pH 8.3), 3 mM $MgCl_2$, 200 μM of each dNTP, 200 pmol of each primer, 1 unit of *Taq* polymerase, and 50 ng of total genomic DNA. The DNA was gel purified using Qiaex II gel extraction kit (Qiagen). The purified DNA was then sequenced on an ABI 377 automated sequencer using ABI Prism Data Collection Software version 2.5XL and ABI Prism DNA Sequencing Analysis Software version 3.3. Most sequences were obtained in the HN20 primer direction. However, in order to verify that no mutations were present in the extreme 5' end of the HN20 sequence, fourteen individuals were also sequenced from the opposite direction (using LD440). DNA sequences were aligned by eye using SeqEd version 1.0.3.

Microsatellite data

Six microsatellite loci were initially screened for variation using primers developed for other salmonids. Loci *Sfo-8* and *Sfo-23* were obtained from brook trout, *S. fontinalis* (Angers *et al.*, 1995), as they had been shown to be informative in arctic charr, *S. alpinus* (Bernatchez *et al.* 1998), which is a sister taxon to *S. malma.* However, some *Sfo-8* alleles ran beyond the 350 base pair detection capacity of the TAMRA (Perkin Elmer) fluorescent ladder and *Sfo-23* did not amplify consistently. As a result, both were removed from subsequent analysis.

The locus *Cocl-3* from lake whitefish, *Coregonus clupeaformis* (Bernatchez 1996), *MST-85* from brown trout, *Salmo trutta* (Presa and Guyomard 1996), *Sco-19* from bull trout, *Salvelinus confluentus* (E. Taylor, Dept. Zoology, University of British Columbia, unpublished), and *Ots-1* from Chinook salmon, *Oncorhynchus tschawytscha* (Banks *et al.* 1999) were successfully amplified and scored. One primer for each locus

was 5' labelled with one of three colors : green (TET) for *Cocl-3, MST-85,* and *Sfo-8*, blue (6-FAM) for *Sfo-23* and *Ots-1*, and yellow (HEX) for *Sco-19*.

Sco-19, *Cocl-3* and *Ots-1* were amplified in a single 15 µl multiplex reaction containing 50 mM KCl, 10 mM Tris HCl (pH 8.3), 2 mM $MgCl_2$, 0.28 mM of each dNTP, 133 pmol of each *Sco-19* and *Ots-1* primer, 166 pmol of each *Cocl-3* primer, 50 ng of genomic DNA and 1 unit of *Taq* polymerase. The locus *MST-85* was amplified in a 15 µl reaction consisting of 50 mM KCl, 10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 66.7 pmol of each primer, 50 ng genomic DNA, and 0.5 units of Taq polymerase.

PCR amplifications were conducted in a Stratagene Robocycler Gradient 96 with an initial denaturation step of 2 min at 94 °C, 35 cycles of 1 min at 92 °C, 1 min at the annealing temperatures, and 1 min at 72 °C, ending with a 10 minute final extension at 72 °C. The annealing temperature for the multiplex reaction and for *MST-85* were 50 °C and 60 °C, respectively.

The PCR product from the four microsatellite loci were combined and diluted by adding 5 µl of the multiplex reaction and 2 µl of the *MST-85* reaction to 11 µl of ddH_2O. One µl of this diluted PCR product was added to 1.5 µl of loading buffer/internal lane standard (1.2 µl deionized formamide, 0.15 µl GeneScan 350 TAMRA size standard (Perkin Elmer), 0.15 µl loading buffer (50 mg/ml blue dextran, 25 mM EDTA). The samples were then denatured at 90 °C for 2 minutes and placed immediately on ice until loading. From 1.2 to 1.8 µl of product was loaded on a 1x TBE denaturing (7 M urea), 5% polyacrylamide (19:1, acrylamide: bis-acrylamide) gel. Data were collected on a Perkin Elmer ABI 377 automated sequencer using ABI Prism Data Collection Software

version 2.5XL and ABI GeneScan Software version 3.1. Gels were run for 2 hours at 3000 volts, 200 watts and 60 milliamperes. Electropherograms produced on GeneScan were subsequently transferred to Genotyper version 1.1 for size scoring.

Analysis

Identifying groups for analysis was carried out in two ways. First, all *S. malma* caught at a site were pooled regardless of life type for comparison to other sites. Second, each life history type within the Firth drainage sites was grouped to address site fidelity hypotheses. Some groups created from the life type criteria had low sample sizes and are identified in the analyses where appropriate (also see Appendix I and II).

Mitochondrial DNA

The level of mitochondrial DNA polymorphism was quantified by determining the total number, frequency, and divergence of haplotypes. Divergences among haplotypes were calculated using the software MTDIS (Danzmann 1998). This program has the advantage of incorporating both indels and substitutions into a simple measure of divergence by calculating the number of mutational differences between haplotypes, regardless of the type of mutation. The resulting distance matrix was then imported into MEGA (Kumar *et. al.* 1993) to construct a neighbour joining tree. One indel consisted of multiple nucleotides with positions 18 to 25 consisting of six to nine repeated Gs. In this case, pairwise differences between haplotypes were calculated to minimize the number of mutational steps. For example, haplotype *1* and haplotype *6* possessed 9 and 7 G nucleotide repeats respectively and the number of mutations at that indel was taken to be one (i.e. a single 2 base pair slippage event as opposed to two, 1 base pair slippage events). Ordinarily, indels should not be treated as equivalent mutations to substitutions

(Li and Graur 1991) and the relationship between indel and substitution mutation rates in noncoding DNA is unclear. As a result, indels are often excised before analysis in MEGA (Kumar *et. al.* 1993). However, since the divergences between geographically proximate populations of *S. malma* were small, complex mutation models for phylogenetic reconstruction are not of primary concern. Moreover, removing the indels would have resulted in the loss of much of the genetic variation detected. Of greater interest, is the geographic distribution of all haplotypes and an approximation of the number of mutational steps between them.

On the basis of haplotype divergences, two major clusters (putative phylogroups) were identified (see results). Pie diagrams showing the frequency of the two clusters within each population were constructed to illustrate their geographic distribution. To acertain whether these frequencies were significantly different between populations, pairwise differentiation tests were conducted using Arlequin 1.1 with the frequencies of cluster I and II entered as haplotype alleles. The null hypothesis tested is that the frequency of each cluster from populations i and j are the same. A non-parametric permutation procedure (10 000 permutations) available in Arlequin 1.1 was performed to obtain a P-value of the test statistic. (Note that the power of the test is reduced for populations with low sample size (i.e. Babbage-*iso* and Cache-*iso*) and as a result markedly different allele frequencies between populations may not be significant.) P-values of all population comparison analyses were adjusted by Bonferroni sequential correction for multiple simultaneous statistical tests (Rice 1989).

Populations within a drainage basin which showed non-significant differences were pooled and the test procedure was performed again. Because the Firth drainage was

the only basin to contain several anadromous populations, it was the only drainage where a chi-square test test (Nei 1987) on the cluster distribution was performed.

MTDIS was also used to calculate a genetic distance matrix among populations (Danzmann *et al.* 1991) which was then imported into MEGA to build a neighbour-joining tree. Also, a pairwise population differentiation matrix was created using Arlequin 1.1 where both the sequence divergence of haplotypes and the frequencies of haplotypes within each population were considered. The divergence of haplotypes was a simple measure of the number of mutational differences, including indels and substitutions, between haplotypes. This measure is analogous to K_{ST} (Hudson *et al.* 1992). Statistical significance was ascertained by 10 000 permutations and corrected by the sequential Bonferroni method (Rice 1989). Pie diagrams of haplotype frequencies and private alleles were also used to qualitatively examine the geographic distribution of mtDNA variation.

Microsatellite DNA

The software program, Genetic Data Analysis version 1.0 (d15) (Lewis and Zaykin 2000) was used to create summary statistics of genotype diversity for the microsatellite loci. For each population and locus, the number of individuals, number of alleles, the observed and expected heterozygosity and the inbreeding coefficient (F_{IS}) were calculated.

Genepop version 3.2 software (Raymond and Rousset 1995) was used to calculate deviations from Hardy-Weinberg expectations under the null hypothesis of random union of gametes. This probability test uses the observed data to define a rejection zone. A *P*-value is created by summing the probabilities of all distributions with the same allelic

counts with equal or lower probability to that of the observed allelic distribution. Due to the large number of alleles, a Markov chain method was used to estimate the exact *P*-value without bias (Guo and Thompson 1992) using a dememorization number of 10 000, running 1000 batches with 10 000 iterations per batch.

Tests for linkage disequilibrium were also conducted using Genepop 3.2 (Raymond and Rousset 1995), testing the null hypothesis that genotypes at one locus are independent from genotypes at the other locus. A subprogram creates contingency tables for all pairs of loci in each population and then performs Fisher's exact test on each table using a Markov chain (dememorization number = 1 000, batches = 100, iterations per batch = 1 000).

Microsatellite DNA population differentiation

A genic differentiation test was performed between all pairs of populations for all four loci with the null hypothesis of identical allelic distributions at a particular locus between two populations. In this manner, a table was constructed showing the number of loci (from 0 to 4) which departed from the null hypothesis for each population pair. Significance was calculated by the Markov chain (dememorization = 5 000, batches = 500, iterations per batch = 5 000).

Arlequin version 1.1 (Schneider *et al.* 1997) was used to calculate population pairwise fixation index comparisons (F_{ST}) analogous to Weir and Cockerham (1984). The analysis is consistent with the infinite alleles model (IAM) as each allele is assumed to be equally probable in regards to the mutational process. Another fixation index, Φ_{ST}, (Michalakis and Excoffier 1996) was computed as an analysis of molecular variance using allelic size variance entered as a matrix of Euclidian squared distances. Φ_{ST}

employs the stepwise mutation model (SMM) where alleles closer in size are assumed to be more closely related than alleles more divergent in size. Comparisons of F_{ST} and Φ_{ST} potentially clarify the relative roles of long term separation and contemporary genetic drift in population differentiation (Slatkin 1995; Goodman 1998). To be explicit, where Φ_{ST} is higher than F_{ST}, it is believed that mutations have accumulated via long term separation as the greater difference in allele sizes would have accumulated with time. Conversely, if F_{ST} is greater than Φ_{ST} then most of the divergence between populations is due to allele frequency differences and random loss of alleles likely resulting from genetic drift. The *P*-values of all Hardy-Weinberg tests and population comparison analyses were adjusted by the Bonferroni sequential corrections for multiple simultaneous statistical tests.

Two hierarchical AMOVA analyses of gene diversity were also performed using Arlequin 1.1. The first hierarchical test divided the total amount of genetic variation into three components: among individuals within a population regardless of life history type (F_{IS}), among populations within a drainage (F_{ST}), and among drainages (F_{IT}). The second test sought to partition individuals within a drainage by their life history type. The Firth river system was the only drainage with sufficient sample sizes of all life types to enable this approach. In this analysis, F_{IS} represented the variation of individuals with a particular life history type, F_{ST}, the variation among life history types within populations, and F_{IT}, the variation attributable to between population diversity within the Firth drainage. Tests of significance for the pairwise F_{ST} and Φ_{ST} analysis and the hierarchical AMOVAs were performed by a nonparametric permutation procedure with 100 000 permutations (Excoffier *et al.* 1992) available through Arlequin 1.1.

Microsatellite trees

Due to uncertainties regarding the analysis of microsatellite polymorphisms (Estoup and Angers 1998; Bernatchez *et al.* 1998), three distance measures were employed to investigate the evolutionary relationships among populations of *S. malma*: Cavalli-Sforza and Edward's (1967) chord distance (D_C); Goldstein *et al.*'s (1995) $(\delta\mu)^2$ distance; and Nei *et al.*'s (1983) D_A distance. D_A and D_C are not specific to either the IAM or the SMM, whereas $(\delta\mu)^2$ is explicitly for SMM and microsatellites. Computer simulations have shown that D_A and D_C are the best algorithms for gaining correct tree topologies under many different conditions including microsatellite loci (Takezaki and Nei 1996). The same study determined that, while the larger variance surrounding all distance measures utilizing the SMM (including $(\delta\mu)^2$) made topology reconstruction less reliable than D_A or D_C, $(\delta\mu)^2$ was the best estimator of branch lengths when using microsatellite data. However, D_A may be sufficient for branch length estimation in closely related populations where the evolutionary time span is not very long, as it fits linearity with time better than D_C (Takezaki and Nei 1996).

According to Nei (1991), the neighbour-joining alogrithm is widely applicable and thus was used for tree construction with 10 000 bootstrap resamplings of loci and individuals within samples. All trees were constructed using NJBPOP developed by J.M. Cornuet (unpublished, Institut national de la recherche agronomique, Laboratoire de neurobiologie comparée des invertébrés, Bures-sur-Yvette, France) and provided by L. Bernatchez.

Individual-based analyses

A population allocation test was performed which estimated the probability of correctly reassigning individuals to their known population of origin (Paetkau *et al.* 1995; Waser and Stroebeck 1998) utilizing the jacknife option in WHICHRUN (version 4.0 beta) software program (Banks and Eichert 2000). This analysis assumes that nuclear loci are in Hardy-Weinberg equilibrium and that all loci are independent. To assign an individual, this method calculates the probability of occurrence of the multi-locus genotype of a given individual in each population, and the individual is then assigned to the population corresponding to the largest probability of occurrence. Individuals were sampled from the baseline one at a time, recalculating allele frequencies in the absence of each individual genotype sampled before determining its most likely population of origin. In order to evaluate the strength of the reassignment for each individual, a log transformation of the probabilities was performed which identifies those individuals with less than a 1.0% chance of misassignment.

RESULTS

Sample sizes ranged from 14 (Cache Creek above falls) to 125 (Joe Creek) with a mean of 51.8 individuals (Table 2-1).

Mitochondrial DNA diversity

DT-SSCP identified 19 putative haplotypes which were subsequently confirmed by sequencing (several individuals in most cases). From the 452 to 458 base pair fragment (depending on the number of indels), 444 bases were resolved in all samples. The 47 samples sequenced in the LD440 direction and 14 samples in the HN20 direction confirmed that each of the haplotypes detected through DT-SSCP were unique (Table 2-3). In some instances, these haplotypes possessed only a single base pair substitution (e.g. haplotype *9* and *10*; *12* and *13*), demonstrating that DT-SSCP has very high resolution.

Individuals identified as sharing a particular haplotype by DT-SSCP and then sequenced were identical, with one exception. In this case, 6 samples were sequenced from DT-SSCP haplotype *6*. Five of the individuals were identical in sequence but one sequence (haplotype *4*) possessed a single extra base in a G repeat region (nucleotide position #24) (Table 2-3). Because haplotype *6* occurred in greater frequency than *4* by sequencing, all other individuals were conservatively designated as haplotype *6*. The single mutation missed by DT-SSCP screening was likely overlooked due to both its position near the 5' end of the HN20 strand and the nature of the mutation, a one base indel. The terminating fragments generated near either of the two priming sites are either very long or very short. Variation may be missed as the short terminating bands are run

Table 2-3. Polymorphic nucleotide positions in 20 haplotypes from 444 base pairs of the 3' end of the mitochondrial control region (D-loop) in *S. malma*.

Haplotype	**nucleotide position**																				
	2	**6**	**10**	**23**	**24**	**25**	**41**	**73**	**80**	**88**	**89**	**112**	**124**	**142**	**160**	**174**	**176**	**269**	**278**	**381**	**394**
1	G	G	T	G	G	G	C	A	C	A	T	A	T	T	A	T	-	C	A	T	C
2	A	C	T	G	G	G	C	A	C	A	T	A	T	T	A	T	-	C	A	T	C
3	G	G	T	G	G	-	C	A	C	A	T	A	T	T	A	T	-	C	A	T	C
4	G	G	T	G	G	-	C	A	C	A	T	A	T	T	A	T	-	C	T	T	C
5	G	G	T	G	-	-	C	A	T	A	T	A	T	T	A	T	-	C	T	T	C
6	G	G	T	G	-	-	C	A	C	A	T	A	T	T	A	T	-	C	T	T	C
7	G	G	T	G	-	-	C	A	C	A	T	A	T	C	A	T	-	C	T	T	C
8	G	G	T	G	-	-	C	A	C	A	T	A	T	T	A	T	-	C	T	C	C
9	G	G	T	-	-	-	C	A	T	A	T	A	T	T	A	T	-	C	T	C	C
10	G	G	T	-	-	-	C	A	C	A	T	A	T	T	A	T	-	C	T	C	C
11	G	G	T	-	-	-	C	A	C	-	-	A	T	T	A	T	-	C	T	C	C
12	G	G	T	G	G	-	C	A	C	A	T	G	T	T	A	T	-	T	T	C	T
13	G	G	T	G	-	-	C	A	C	A	T	G	T	T	A	T	-	T	T	C	T
14	G	G	C	G	G	-	C	G	C	A	T	G	T	T	A	T	-	T	T	C	C
15	G	G	T	G	G	G	C	G	C	A	T	G	T	T	A	T	-	T	T	C	C
16	G	G	T	G	-	-	T	A	C	A	T	G	C	T	A	T	-	T	T	C	C
17	G	G	T	G	G	G	C	A	C	A	T	G	C	T	A	T	-	T	T	C	C
18	G	G	T	G	G	-	C	A	C	A	T	G	C	T	A	T	-	T	T	C	C
19	G	G	T	G	G	-	C	A	C	A	T	G	C	T	A	A	T	T	T	C	C
20	G	G	T	G	G	-	C	A	C	A	T	G	C	T	T	T	-	T	T	C	C

off the gel and the longer fragments, migrating slowly, have little separation between adjacent bands. As other mutations were detected at both extreme ends of the 458 bp fragment, the amount of undetected variation was likely very low. Overall, the ob-served resolution of DT-SSCP was high, confirming DT-SSCP scores for 46 out of 47 individuals sequenced.

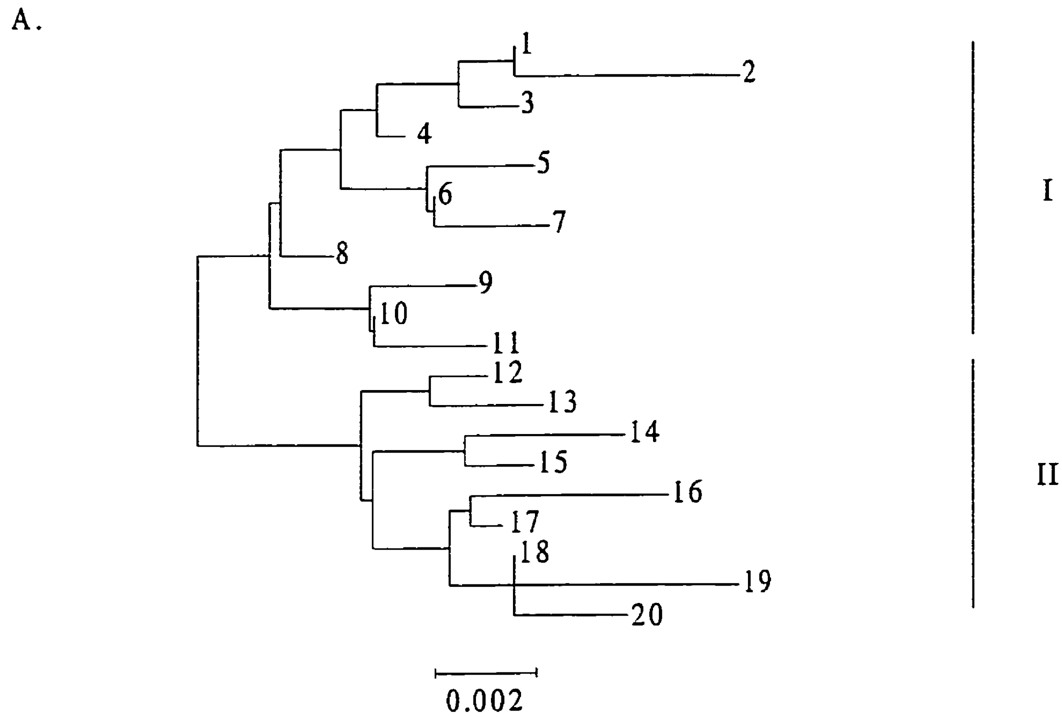
Composition and variation of D-loop haplotypes

All sequences possessed the A and T bias typical of the mitochondrial control region (e.g. Quinteiro *et al.* 2000) with an average relative nucleotide composition of 13.4% C, 32.7% T, 29.3% A, and 24.7% G. The majority of mutations were located at the 3' end of the D-loop with over half (52.4%) of the variation occurring within 89 base pairs of the primer HN20 and 81.0% within 200 base pairs (Table 2-3). These results are consistent with other studies which have shown that both ends of the control region are variable while its central core is highly conserved (Lee *et al.* 1995).

A maximum divergence of 2.02% was observed between haplotypes with a mean divergence of 1.10% (+/- 0.62%) (Figure 2-2). These divergence values derived from 21 variable nucleotide sites of which 15 were substitutions and 6 were indels (Table 2-3). As only 11 sites were parsimoniously informative and this is fewer than the number of observed haplotypes, no cladistic analysis was attempted.

Phylogroups

Phenetic analyses showed that the haplotypes formed two major clusters (Figure 2-2). Cluster I was dominant, representing over three quarters of individuals (Figure 2-3 inset). However, the diversity within each cluster was comparable as group I had 10 haplotypes and group II had 9 haplotypes. Although the divergence between cluster I and



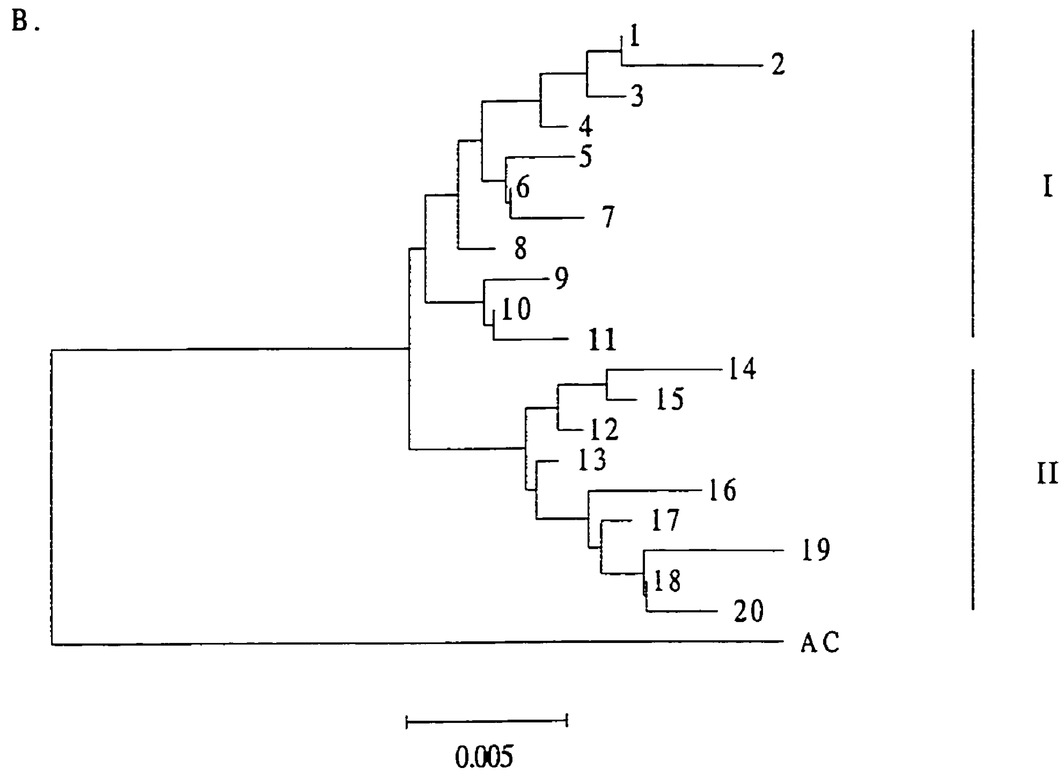
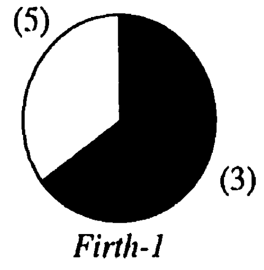
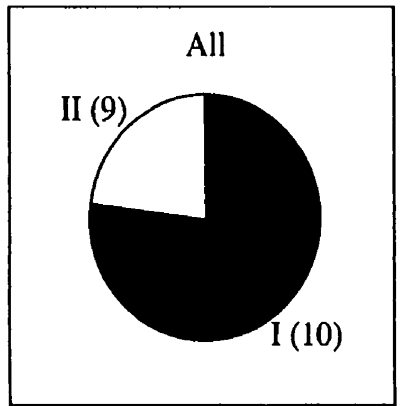
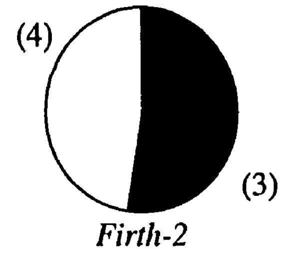


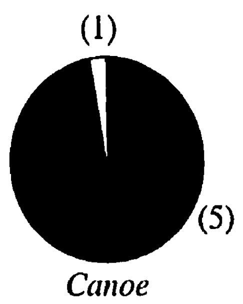
Figure 2-2A. Neighbour-joining phenogram of 20 mtDNA haplotypes in *S. malma* using proportion of nucleotide differences (including indels) distance measure (calculated in MTDIS; Danzmann 1998) from 444 base pairs of the control region. Vertical lines at right indicate 2 major clusters. Figure 2-2B includes arctic charr (AC), *S. alpinus erythrinus*, from Devon Island, Canada, as an outgroup.

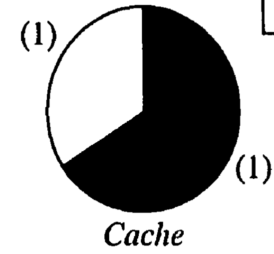
Figure 2-3. Variation in the incidence of two haplotype clusters (black – cluster I, white – cluster II) of *S. malma* by drainage (horizontal) and sampling location within drainages (vertical). The number in brackets indicates the number of unique haplotypes in each cluster. Inset (upper right box) shows the overall frequency and number of haplotypes in each cluster.

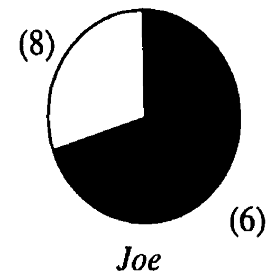

(5)
(3)
Firth-1

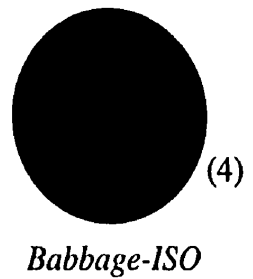

All
II (9)
I (10)

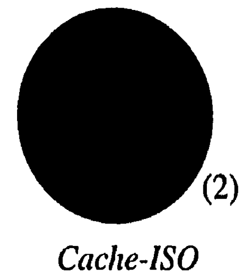

(4)
(3)
Firth-2

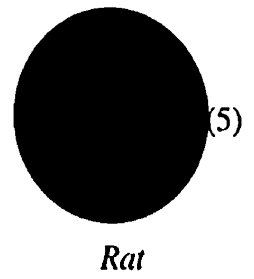

(1)
(5)
Canoe


(1)
(1)
Cache


(8)
(6)
Joe


(4)
Babbage-ISO


(2)
Cache-ISO


(5)
Rat

West
East
DRAINAGE
Firth/ Joe
Babbage/ Canoe
Cache
Rat

II was small relative to the divergence within each cluster (Figure 2-2), a general geographic pattern to their distribution was found suggesting that these clusters are phylogeographically informative (Figure 2-3). The three most easterly drainages (Babbage, Big Fish, and Rat) were almost entirely composed of haplotypes from group I. The two exceptions were (i) the Canoe River population, which had a single individual with a cluster II derived haplotype and (ii) the anadromous Cache Creek population, which had a large proportion of cluster II type haplotypes but this derived from only one unique haplotype. By contrast, the most westerly drainage, Firth, contained high proportions of both clusters and high haplotype diversity within each cluster (from 5 to 8 unique haplotypes being derived from cluster II) (Figure 2-3). Thus, the overall distribution of haplotypes showed that the haplotype diversity within cluster I was comparable across all populations (except Cache which had low diversity), but the Firth drainage populations also had high diversity within cluster II.

Both the pairwise differentiation test of cluster I and II (Table 2-4A) and a Chi-square test (χ^2=3.77, d.f.=2, p>0.6) revealed that the three Firth drainage populations were not significantly different from one another and as a result they were pooled (Table 2-4B). Despite being fixed for cluster I haplotypes, Cache-*iso* was not statistically different from Cache or FIRTH-*pooled* because of the bias introduced to permutation tests with low sample size. This result is likely not biologically meaningful as both Rat and Babbage-*iso*, which were also fixed for cluster I haplotypes and had larger sample sizes, were significantly different from Cache and FIRTH-*pooled*. The other isolated population, Babbage-*iso*, was not divergent from the downstream anadromous

Table 2-4A. Pairwise population differentiation in *S. malma* based on the frequency of the two mtDNA clusters (identified in Figures 2 & 3). * indicates a significant difference (α=0.05) derived from a non-parametric permutation procedures following adjustment for multiple tests by the sequential Bonferroni method (initial k=28). 2-3B is a repeat of the analysis with Firth drainage populations (Firth-1, Firth-2, and Joe) pooled.

A.

	Babbage *iso*	**Cache *iso***	**Cache**	**Canoe**	**Firth-1**	**Firth-2**	**Joe**
Cache-*iso*	0.000						
Cache	0.270*	0.220					
Canoe	-0.012	-0.033	0.264*				
Firth-1	0.271*	0.226	-0.021	0.266*			
Firth-2	0.412*	0.352*	0.012	0.418*	0.007		
Joe	0.192*	0.165	-0.012	0.179	-0.008	0.048	
Rat	0.000	0.000	0.352*	0.007*	0.347*	0.511*	0.232*

B.

	Babbage *iso*	**Cache *iso***	**Cache**	**Canoe**	**FIRTH-*Pooled***
Cache-*iso*	0.000				
Cache	0.270*	0.220			
Canoe	-0.012	-0.033	0.264*		
FIRTH-*pooled*	0.216*	0.196*	-0.014	0.202*	
Rat	0.000	0.000	0.352*	0.007*	0.244*

population, Canoe. FIRTH-*pooled* was significantly different from all populations except Cache (and Cache-*iso* as already noted).

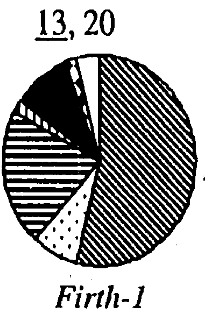
Geographic distribution and frequency of haplotypes

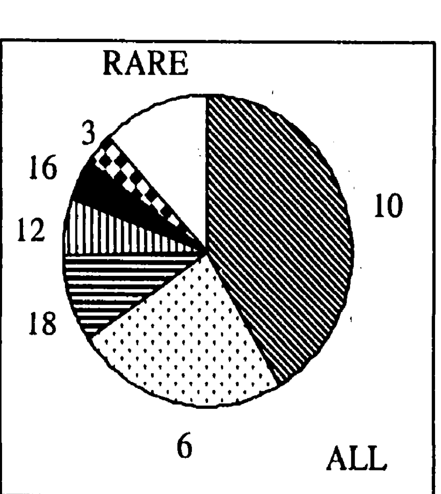
Two haplotypes (*6* and *10*) were dominant, occurring in over two thirds of the individuals screened (Figure 2-4; inset). Four other haplotypes (*3*, *12*, *16*, and *18*) were intermediate in frequency occurring in approximately one quarter of the individuals. The remaining 14 haplotypes were rare; 8 were detected in a single location and 4 of these were found in a single fish.

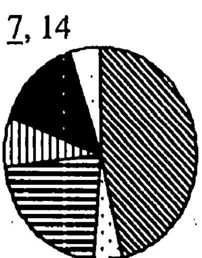
Joe Creek possessed the greatest haplotype diversity with 14 of the 20 observed haplotypes present. The Firth sites also contained considerable variation, having 8 and 7 haplotypes in Firth-1 and Firth-2 respectively. The Cache Creek drainage was least diverse, with both Cache-*iso* and Cache containing only 2 haplotypes. Generally, a drop in haplotype diversity was observed from west to east (Figure 2-4).

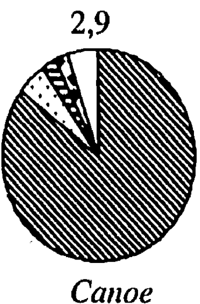
Examining the geographic distribution of mtDNA diversity revealed a division where the two western drainages (Firth and the anadromous Canoe River population) contained a high relative frequency (RF) of haplotype *10* and low RF of haplotype *6*. By contrast, the eastern Big Fish and Rat drainages were dominated by haplotype *6* and haplotype *10* was either absent (Cache Creek) or in low frequency (Rat River). While haplotype *10* was the most common overall, only haplotype *6* was present in all locations. These two dominant haplotypes (*6* and *10*) were closely allied (0.45% divergence (Figure 2-2) and thus their disjunct distribution most likely reflects low gene flow and genetic drift between the two eastern and the two western drainages. The remaining haplotypes also showed divergence between spawning sites. Haplotype *18* was confined to the west,

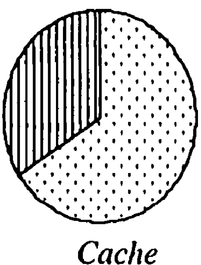
Figure 2-4. Variation in frequencies of the six dominant mtDNA haplotypes of *S. malma* by drainage (horizontal) and sampling location within drainage (vertical). Rare haplotypes (7 – 19) are indicated numerically and private haplotypes are underlined. Inset (upper right box) shows the overall frequency of the 6 dominant haplotypes (and rare haplotypes pooled).

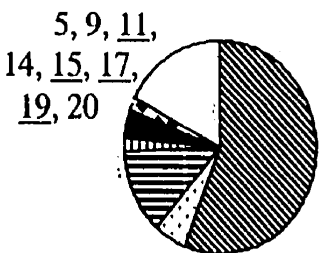

13, 20
Firth-1

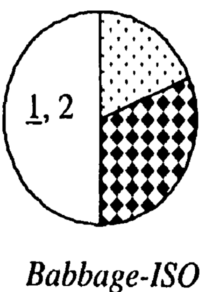

RARE
3
16
12
18
10
6
ALL

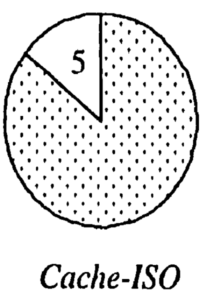

7, 14
Firth-2

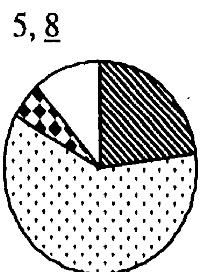

2,9
Canoe

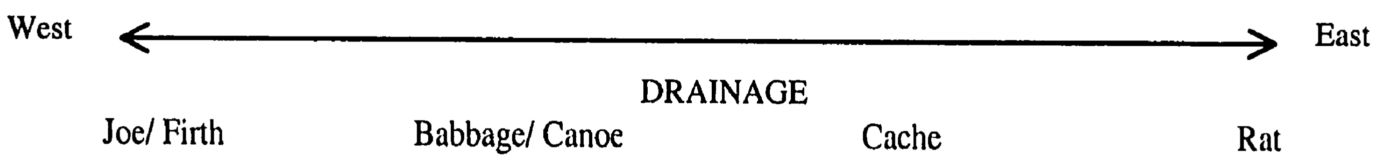

Cache


5, 9, 11,
14, 15, 17,
19, 20
Joe


1, 2
Babbage-ISO


5
Cache-ISO


5, 8
Rat


West
East
DRAINAGE
Joe/ Firth
Babbage/ Canoe
Cache
Rat

being most prevalent in the Firth and in Canoe at very low frequency. Similarly, haplotype *16* occurred only in the Firth drainage populations. Haplotype *12* was common only in the anadromous Cache Creek population, absent from the Rat and Babbage drainages, and was a minor component of the Firth populations. Only three haplotypes did not occur in the Firth drainage, two were private (*1*, in Babbage-*iso*; and *8* in Rat), and one unique to the Babbage drainage (2).

The isolated Babbage river population had an anomalous haplotype frequency distribution which did not conform to the general east – west pattern by drainage (Figure 2-4). For example, a private haplotype (*1*) occurred in 36% of individuals and haplotype (*3*), rare or absent in the other populations, was also in high frequency (36%).

The pairwise analysis of allelic variance based on the number of mutational differences between sequences (K_{ST}) revealed that all populations were significantly different (Table 2-5) except the three Firth drainage locations which were not different from each other but were significantly different from each of the other sites.

A neighbour-joining phenogram of population relationships also indicated low divergence among the Firth drainage populations (Figure 2-5). No other genetic and geographic relationships were readily apparent in the phenogram. The Babbage-*iso* population was the most divergent, likely because it was the only population not dominated by either haplotype *6* or *10*. Neither of the isolated populations clustered with downstream populations within their drainage suggesting low gene flow and divergence by drift.

Table 2-5. Pairwise population differentiation in *S. malma* based on mtDNA allele frequency (number of base pair differences including indels) variance analysis (K_{ST}) of 444 base pairs of the control region. $^{\varphi}$ indicates non-significant differences of *P* values (α=0.05) following adjustment for multiple tests by the sequential Bonferroni method (initial k=28).

	Babbage *iso*	**Cache *iso***	**Cache**	**Canoe**	**Firth-1**	**Firth-2**	**Joe**
Cache-*iso*	0.450						
Cache	0.394	0.142					
Canoe	0.547	0.718	0.619				
Firth-1	0.287	0.443	0.401	0.113			
Firth-2	0.252	0.447	0.395	0.167	-0.006$^{\varphi}$		
Joe	0.292	0.434	0.395	0.072	-0.002$^{\varphi}$	0.013$^{\varphi}$	
Rat	0.284	0.089	0.132	0.422	0.239	0.259	0.243

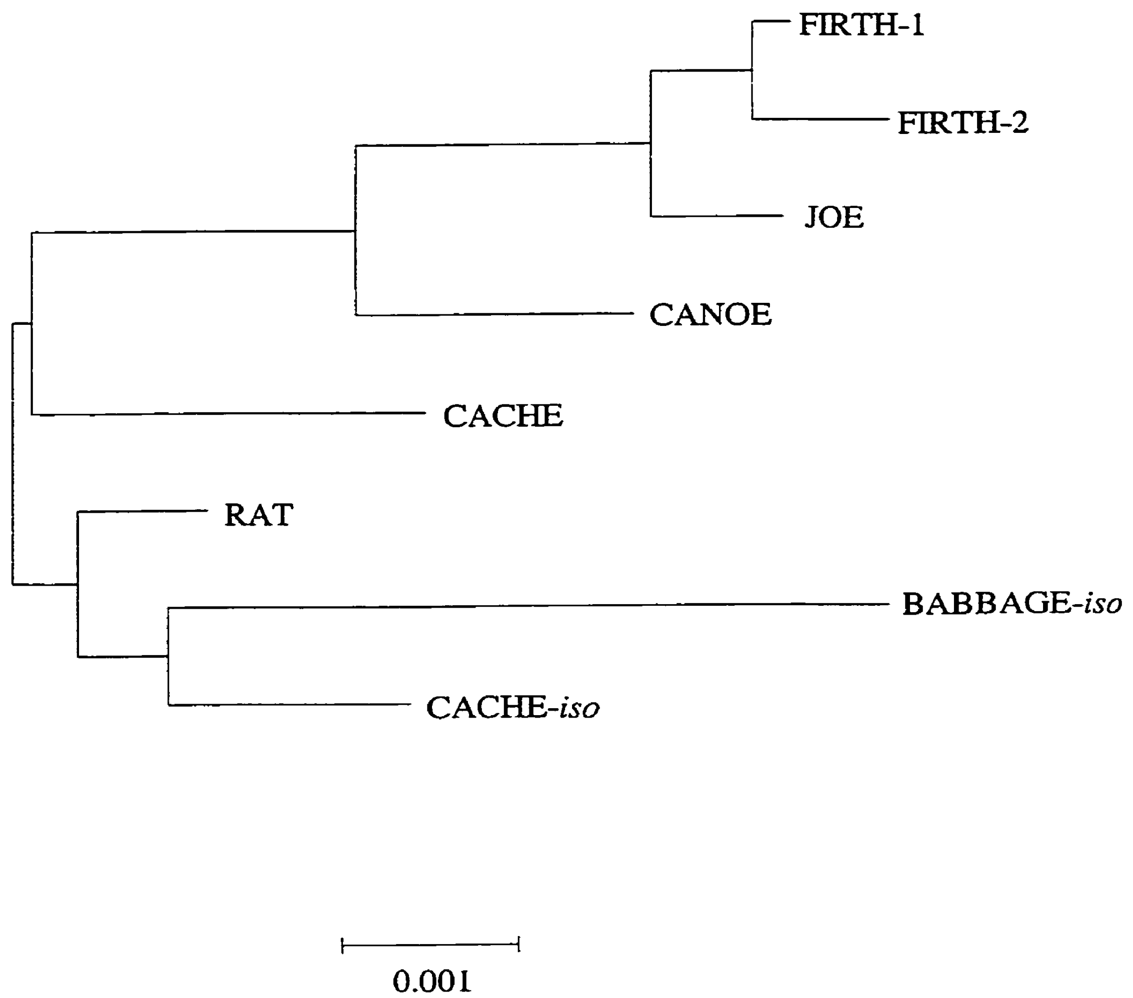


Figure 2-5. Neighbour-joining phenogram of the relationships among 8 populations of *S. malma* based upon 444 bp of the mtDNA D-loop. (Distances quantified in MTDIS.)

Intrapopulation gene diversity of microsatellites

The four microsatellite loci were highly polymorphic with 14 alleles for *MST-85*, 31 for *Cocl-3*, 37 for *Sco-19*, and 39 for *Ots-1*. When examined by population, the microsatellite loci showed more divergence in variability with just 2 alleles at *MST-85* in the population above the falls in Cache Creek, but up to 30 alleles at *Sco-19* in Joe Creek (Table 2-6). The observed heterozygosity (H_O) was typically high, averaging 0.57 across all populations and loci, but it exhibited considerable variation ranging from 0.00 to 0.95 (Table 2-6). No significant gametic phase disequilibrium was detected between locus pairs indicating an absence of linkage among the four microsatellite markers.

Following Bonferroni's sequential correction for multiple tests, significant Hardy-Weinberg deviations were detected in 11 of 36 tests, all due to an heterozygote deficit. This is more than six times the number expected by chance alone with α=0.05. Three loci showed heterozygote deficits in Joe Creek and Firth-1, while the remainder were scattered among the other populations. Nine of the 11 heterozygote deficits occurred at two loci, *Ots-1* (5) and *MST-85* (4).

Heterozyote deficits at microsatellite loci may be due to null alleles, low sample size relative to the number of alleles, or population subdivision (see discussion). To assess whether the heterozygote deficits in anadromous populations were due to pooling life cycle types (ALJ, AAR, AS), a Hardy-Weinberg analysis was run on samples partitioned by location and life history type (Data not shown – see Appendix I). Seven of the 17 life cycle/ life history groups had greater than 30 individuals (Table 2-1) and at *MST-85*, 5 of these showed significant heterozygote deficits (Appendix I). The other microsatellite loci had fewer significant heterozygote deficits (0 to 3 for groups with

Table 2-6. Microsatellite summary statistics showing the number of individuals (n), different alleles (A), observed heterozygosity (Ho), gene diversity (He), inbreeding coefficient (f) and Hardy-Weinberg equilibrium (HWE) with standard error (SE) in populations of *S. malma*. Significant (*) departures following Bonferroni corrections, $\alpha = 0.05$, $k = 8$ unbiased estimate of type-I error.

		BABBAGE *iso*	**CACHE *iso***	**CACHE**	**CANOE**	**FIRTH-1**	**FIRTH-2**	**JOE**	**RAT**	**Mean**
Cocl3	n	25	14	47	43	58	50	125	52	52
	A	9	3	16	16	20	19	24	22	16
	He	0.838	0.561	0.695	0.846	0.915	0.905	0.901	0.890	0.819
	Ho	0.920	0.500	0.660	0.791	0.793	0.840	0.816	0.808	0.766
	f	-0.101	0.112	0.052	0.066	0.135	0.073	0.094	0.093	0.066
	HWE	0.0588	1.0000	0.0458	0.1011	0.0035	0.0543	0.0003*	0.0605	
	SE	0.0010	/	0.0035	0.0033	0.0005	0.0025	0.0001	0.0034	
Ots1	n	25	14	49	43	55	48	121	54	51
	A	9	3	12	19	22	23	27	5	15
	He	0.662	0.500	0.852	0.776	0.904	0.879	0.806	0.461	0.730
	Ho	0.560	0.571	0.531	0.628	0.491	0.750	0.645	0.333	0.564
	f	0.157	-0.149	0.380	0.193	0.459	0.148	0.201	0.279	0.231
	HWE	0.19460	1.0000	0.0000*	0.0027	0.0000*	0.1404	0.0000*	0.0024	
	SE	0.00250	/	0.0000	0.0008	0.0000	0.0065	0.0000	0.0001	

Table 2-6 Con't		BABBAGE *iso*	CACHE *iso*	CACHE	CANOE	FIRTH-1	FIRTH-2	JOE	RAT	Mean
Sco19	n	25	14	49	43	58	50	125	54	52
	A	6	2	8	16	26	24	30	9	15
	He	0.651	0.198	0.604	0.821	0.926	0.922	0.945	0.796	0.733
	Ho	0.680	0.214	0.633	0.791	0.879	0.940	0.952	0.778	0.733
	f	-0.045	-0.083	-0.048	0.037	0.050	-0.020	-0.007	0.023	-0.001
	HWE	0.4506	1.0000	0.7931	0.0409	0.0325	0.9330	0.7248	0.7471	
	SE	0.0015	/	0.0021	0.0021	0.0029	0.0030	0.0066	0.0017	
MST85	n	24	14	43	43	56	49	115	53	50
	A	3	2	4	6	9	10	10	5	6
	He	0.082	0.138	0.308	0.625	0.597	0.642	0.631	0.462	0.436
	Ho	0.042	0.000	0.256	0.326	0.286	0.327	0.313	0.321	0.234
	f	0.500	1.000	0.172	0.482	0.523	0.494	0.505	0.308	0.466
	HWE	0.0213	0.0370	0.2305	0.0000*	0.0000*	0.0000*	0.0000*	0.0063	
	SE	/	/	/	0.0000	0.0000	0.0000	0.0000	0.0002	
ALL	n	25	14	47	43	57	49	122	53	51
	A	6.75	2.5	10	14.25	19.25	19	22.75	10.25	13
	He	0.558	0.349	0.615	0.767	0.835	0.837	0.821	0.652	0.679
	Ho	0.550	0.321	0.520	0.634	0.612	0.714	0.681	0.560	0.574
	f	0.015	0.082	0.156	0.175	0.269	0.148	0.170	0.143	0.157

n>30) by this partitioning of populations. Therefore, the possibility of null alleles at *MST-85* deserves consideration. To examine the effects of possible bias due to null alleles, many of the subsequent analyses were performed twice; first with *MST-85* included and subsequently with this locus excluded.

Genetic differentiation among populations

The pairwise genic differentiation analysis revealed that the distribution of allele frequencies were significantly different (global *P*-values across loci by Fisher's method) in all but one comparison (Table 2-7). The Firth-1 and Firth-2 populations were not significantly different at any loci which corresponds to their close geographic proximity. Although within the same drainage, Joe Creek was divergent from Firth-1 and Firth-2 at two and three loci respectively. While the number of loci which showed significant differences in allele frequencies ranged from 0 to 4, the majority showed significant differences at all 4 loci (mean of 3.6 over all comparisons). The removal of the *MST-85* locus did not change the global signficances or the general trend of high divergences among the populations (Table 2-7 A verses B).

Examining the distribution and frequency of alleles by locus (Figure 2-6) showed that Joe River and the two Firth River populations had the highest allelic diversity. The other populations appeared to have a subset of the total variation present in Joe and Firth and no unique alleles. There was a general reduction in allelic diversity along a west to east transect of the anadromous populations. The anadromous populations had much higher allelic diversity than the two isolated populations. For example in the Babbage River, the population above the waterfalls had six alleles at *Sco-19*, whereas the downstream anadromous Canoe River population had sixteen alleles. The Cache Creek

Table 2-7. Pairwise comparisons of the number of loci with significantly different allele frequencies (Fisher's exact test $\alpha = 0.05$) in 8 populations of *S. malma*. $^{\varphi}$ indicates the non-significant differences of the pooled *P* values (Fisher's method) following adjustment of multiple tests with the Bonferroni sequential method (k=28). A- shows the 4 loci *Cocl-3*, *Ots-1*, *Sco-19*, and *MST-85*. B- is a repeat of the analysis with *MST-85* removed to examine possible biasing effects of null alleles at that locus.

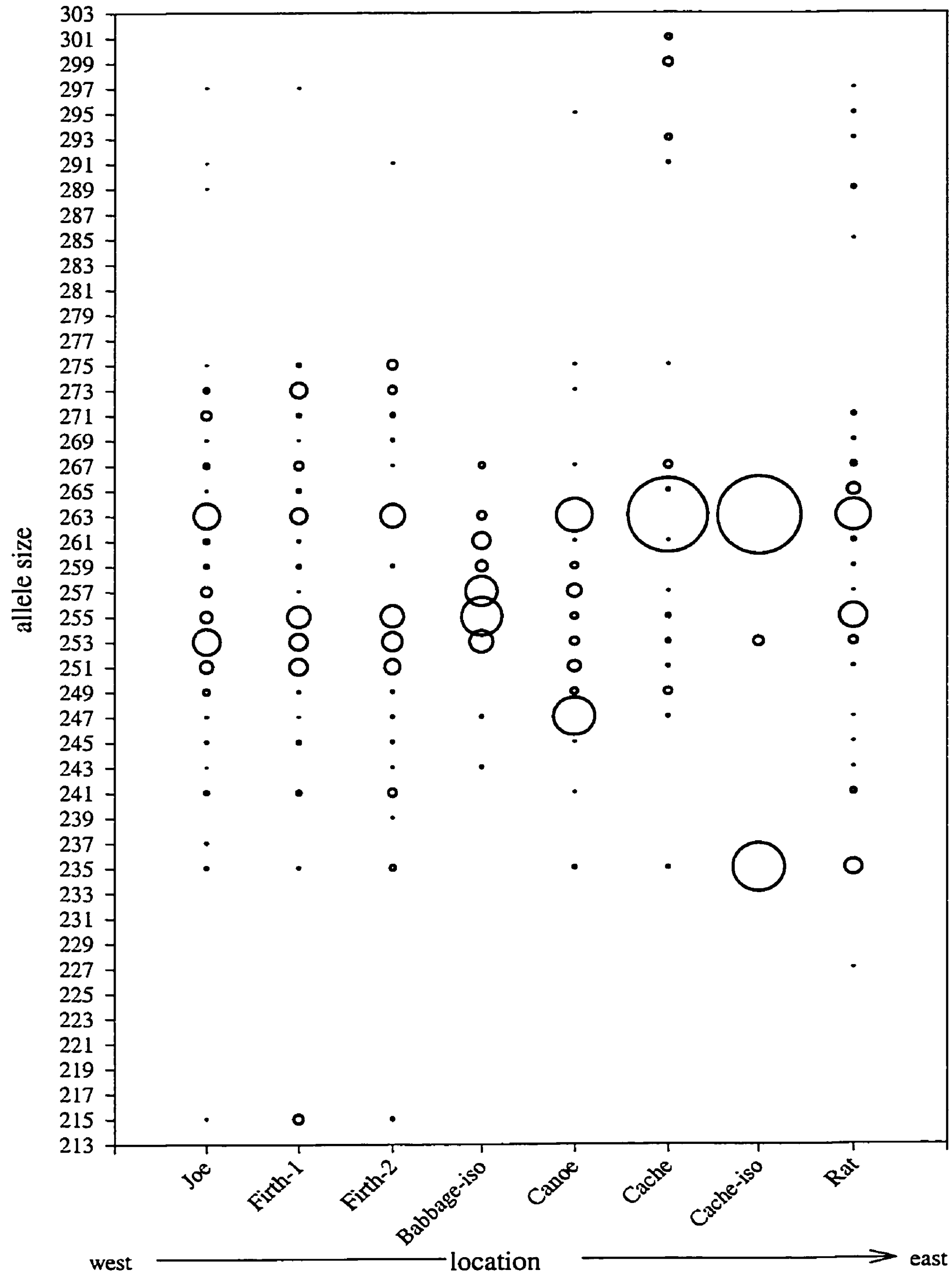
A

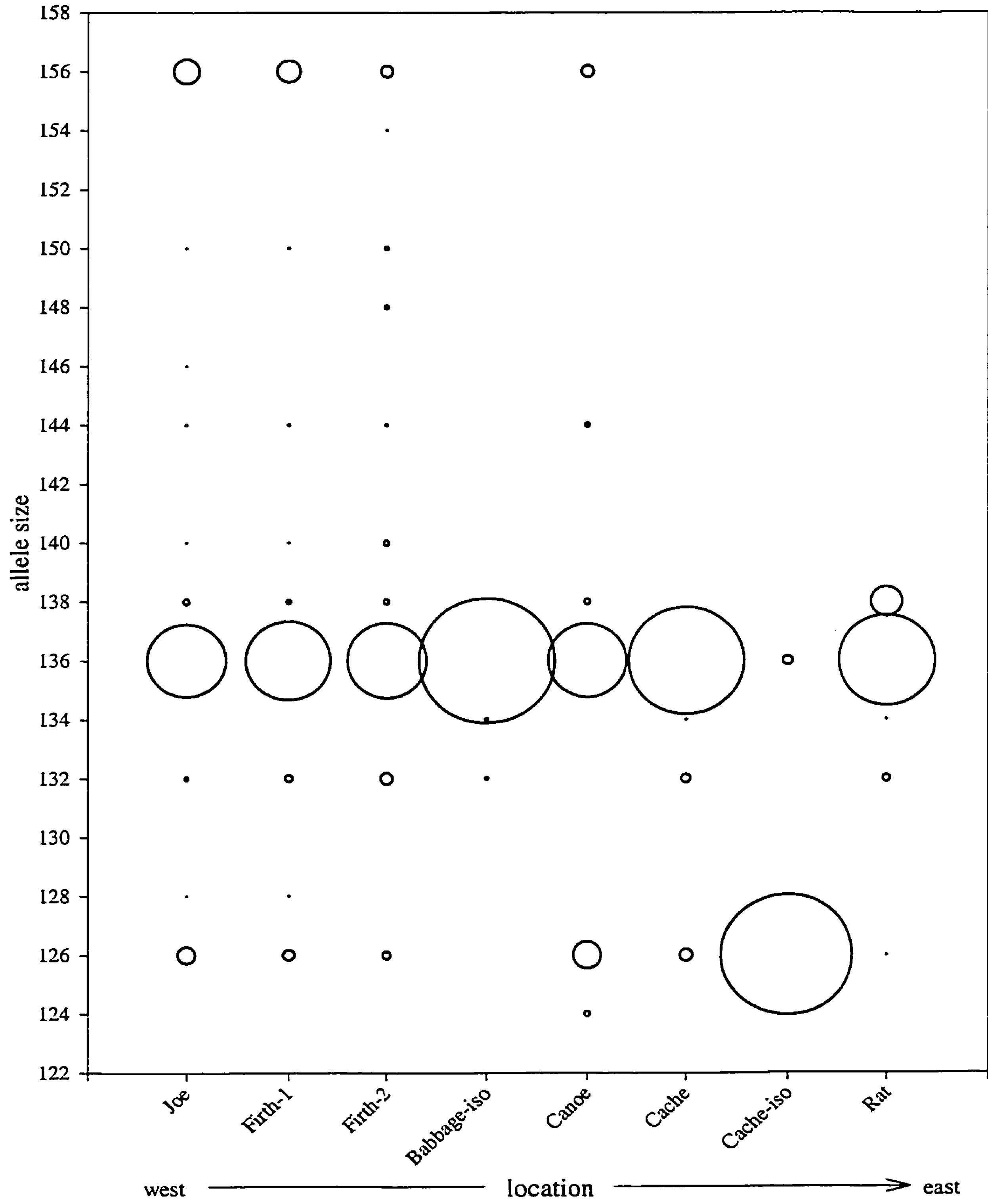
		Babbage *iso*	**Cache**	**Cache *iso***	**Canoe**	**Firth-1**	**Firth-2**	**Joe**
	n	*26*	*50*	*14*	*44*	*58*	*50*	*125*
Cache	*50*	3						
Cache-*iso*	*14*	4	3					
Canoe	*44*	3	4	4				
Firth-1	*58*	4	4	4	4			
Firth-2	*50*	4	4	4	4	0^{φ}		
Joe	*125*	4	4	4	4	2	3	
Rat	*54*	4	4	4	4	4	4	4

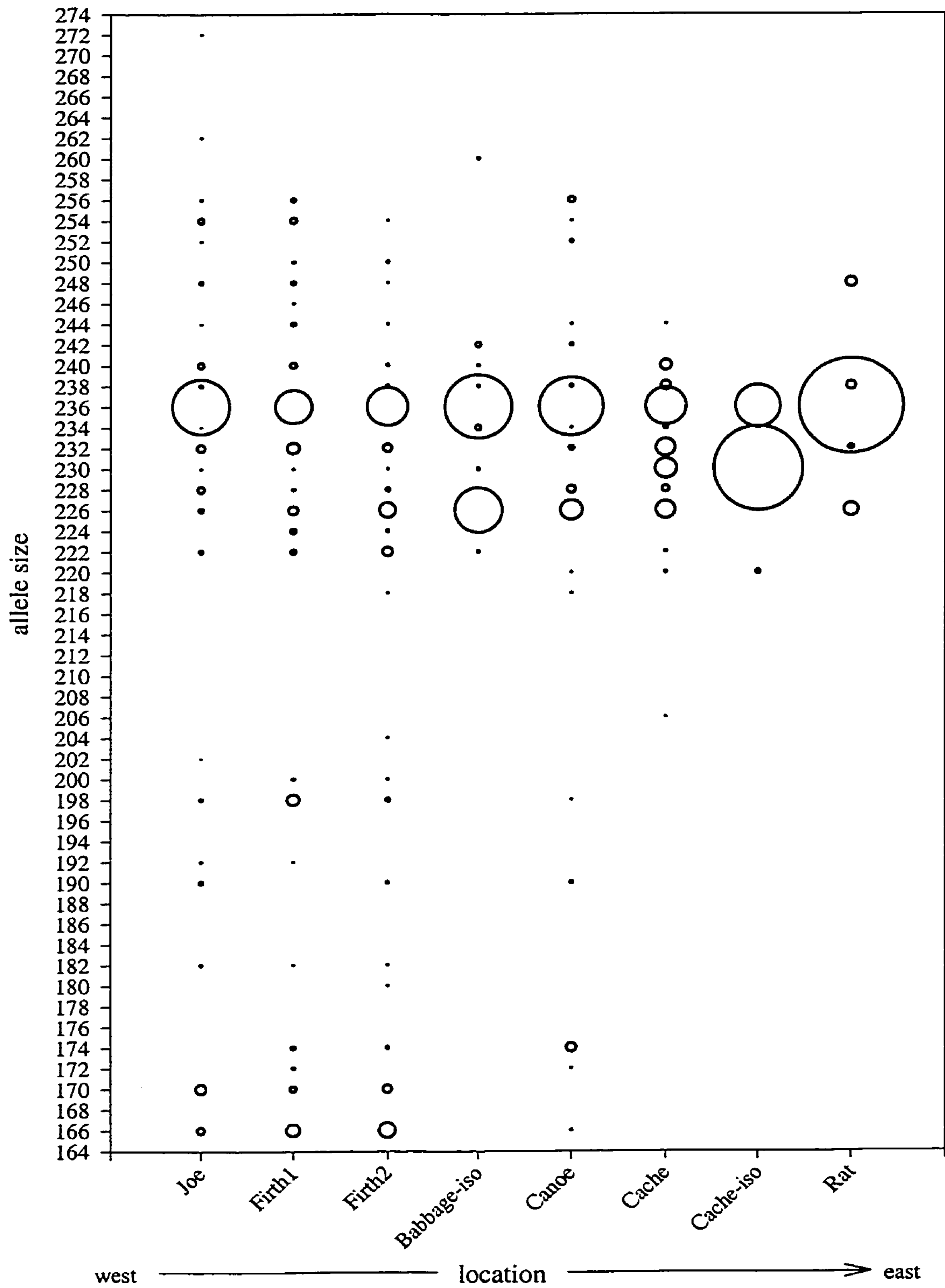
B

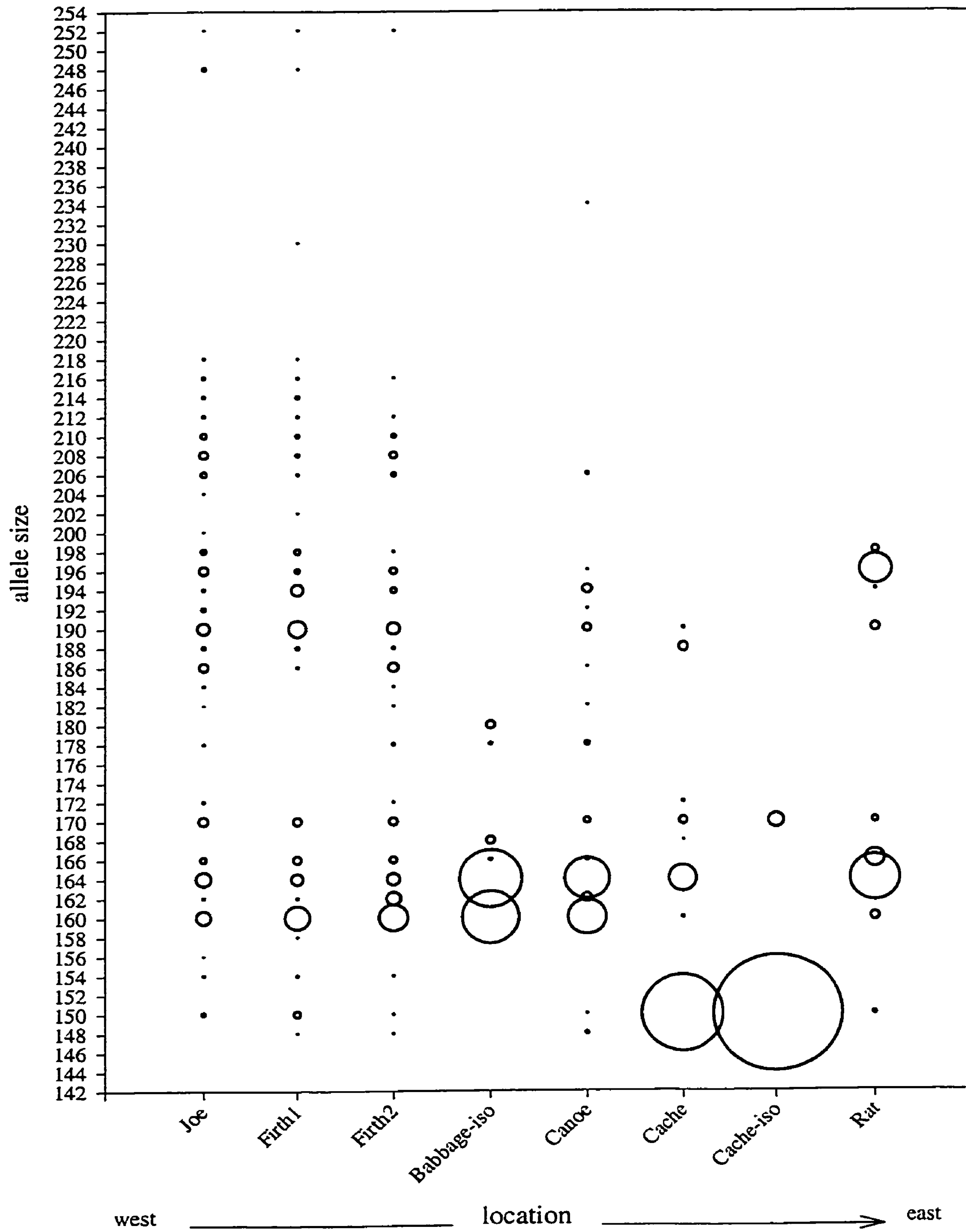
		Babbage *iso*	**Cache**	**Cache *iso***	**Canoe**	**Firth-1**	**Firth-2**	**Joe**
	n	*26*	*50*	*14*	*44*	*58*	*50*	*125*
Cache	*50*	3						
Cache-*iso*	*14*	3	2					
Canoe	*44*	2	3	3				
Firth-1	*58*	3	3	3	3			
Firth-2	*50*	3	3	3	3	0^{φ}		
Joe	*125*	3	3	3	3	2	2	
Rat	*54*	3	3	2	3	3	3	3

Figure 2-6. Microsatellite allele frequency bubble plots of A- *Cocl-3*, B- *MST-85*, C- *Ots-1*, and D- *Sco-19* for populations of *S. malma*. Each bubble represents a unique allele and the size of bubble corresponds with its relative frequency. Populations are ordered in their distribution from west to east.


Cocl-3
allele size
303
301
299
297
295
293
291
289
287
285
283
281
279
277
275
273
271
269
267
265
263
261
259
257
255
253
251
249
247
245
243
241
239
237
235
233
231
229
227
225
223
221
219
217
215
213
Joe
Firth-1
Firth-2
Babbage-iso
Canoe
Cache
Cache-iso
Rat
west
location
east


MST-85
allele size
158
156
154
152
150
148
146
144
142
140
138
136
134
132
130
128
126
124
122
Joe
Firth-1
Firth-2
Babbage-iso
Canoe
Cache
Cache-iso
Rat
west
location
east


Ots-1
allele size
274
272
270
268
266
264
262
260
258
256
254
252
250
248
246
244
242
240
238
236
234
232
230
228
226
224
222
220
218
216
214
212
210
208
206
204
202
200
198
196
194
192
190
188
186
184
182
180
178
176
174
172
170
168
166
164
Joe
Firth1
Firth2
Babbage-iso
Canoe
Cache
Cache-iso
Rat
west
location
east


Sco-19
allele size
254
252
250
248
246
244
242
240
238
236
234
232
230
228
226
224
222
220
218
216
214
212
210
208
206
204
202
200
198
196
194
192
190
188
186
184
182
180
178
176
174
172
170
168
166
164
162
160
158
156
154
152
150
148
146
144
142
Joe
Firth1
Firth2
Babbage-iso
Canoe
Cache
Cache-iso
Rat
west
location
east

isolated population, even considering its small sample size, was the most genetically depauperate. It also had the most dramatic shifts in allele frequencies compared to the other populations. For example, the dominant *Ots-1* allele was 230 in Cache-*iso* compared to allele 236 in all other populations and similarly the dominant *MST-85* allele was 126 in Cache-*iso* versus allele 136 in all other populations.

Coinciding with their high allelic diversity, the Firth and Joe populations had most of the private alleles (19 of 29; Table 2-8). However, given the high levels of polymorphism at the four loci, the number of private alleles was small and they usually occurred at low frequency. Because of their rarity these alleles may occur in other populations but escaped detection due to small sample size.

The population pairwise F_{ST} estimates showed trends similar to the genic pairwise comparisons. All populations were significantly different (by Fisher's exact test following Bonferroni sequential correction) with the exception, again, of Firth-1 and Firth-2 (Table 2-9). The pattern and magnitude of F_{ST} divergences were similar in most cases whether or not *MST-85* was included in the analysis. However, most F_{ST} estimates were lower when only 3 loci (mean 0.12) as opposed to 4 loci (mean 0.14) were analyzed. One pairwise F_{ST} was particularly affected by the locus *MST-85*: the Cache Creek anadromous and isolated populations had an estimate of 0.11 without *MST-85*, but this jumped to 0.27 when *MST-85* was included, reflecting the large difference in frequency of the two dominant alleles 126- Cache-*iso* and 136- all other populations (Figure 2-6 D).

The isolated Cache Creek population showed the greatest divergence with F_{ST} values ranging from a low of 0.27 to a high of 0.49 (mean 0.34). Its high divergence was

Table 2-8. Occurrence and frequency of private alleles of 4 microsatellite loci among 8 populations of *S. malma*.

Locus	Allele	Frequency	Location
Cocl-3	299	0.064	Cache
	301	0.043	Cache
	239	0.010	Firth-2
	237	0.016	Joe
	227	0.010	Rat
	285	0.010	Rat
Ots-1	260	0.020	Babbage-*iso*
	206	0.010	Cache
	300	0.012	Canoe
	246	0.009	Firth-1
	180	0.010	Firth-2
	204	0.010	Firth-2
	202	0.004	Joe
	237	0.012	Joe
	262	0.008	Joe
	270	0.004	Joe
	272	0.004	Joe
Sco-19	180	0.060	Babbage-*iso*
	234	0.012	Canoe
	158	0.009	Firth-1
	202	0.009	Firth-1
	230	0.009	Firth-1
	156	0.004	Joe
	200	0.004	Joe
	204	0.004	Joe
MST-85	124	0.047	Canoe
	148	0.031	Firth-2
	154	0.010	Firth-2
	146	0.004	Joe

Table 2-9. Pairwise differentiation estimates based on allelic (F_{ST}; below diagonal) and molecular (Φ_{ST}; above diagonal) variance analysis for 8 populations of *S. malma*. $^{\varphi}$ indicates non-significant differences of *P* values ($\alpha > 0.05$) following adjustment for multiple tests with the sequential Bonferroni method (initial k=28). Table A includes the 4 loci *Cocl-3, MST-85, Ots-1* and *Sco-19*. Table B repeats the analysis with *MST-85* removed due to the possibility of null alleles at that locus.

A.

	n	**Babbage *iso***	**Cache**	**Cache *iso***	**Canoe**	**Firth-1**	**Firth-2**	**Joe**	**Rat**
		26	50	14	44	58	50	125	54
Babbage-*iso*	26		0.171	0.444	0.046	0.178	0.210	0.194	0.169
Cache	50	0.183		0.275	0.183	0.285	0.309	0.297	0.286
Cache-*iso*	14	0.491	0.273		0.217	0.301	0.324	0.348	0.444
Canoe	44	0.073	0.112	0.318		0.071	0.084	0.097	0.076
Firth-1	58	0.088	0.102	0.305	0.038		-0.002$^{\varphi}$	0.010$^{\varphi}$	0.136
Firth-2	50	0.080	0.101	0.306	0.030	-0.0002$^{\varphi}$		0.038	0.190
Joe	125	0.092	0.098	0.280	0.030	0.009	0.008		0.106
Rat	54	0.093	0.141	0.391	0.066	0.079	0.069	0.058	

B.

	n	**Babbage *iso***	**Cache**	**Cache *iso***	**Canoe**	**Firth-1**	**Firth-2**	**Joe**	**Rat**
		26	50	14	44	58	50	125	54
Babbage-*iso*	26		0.172	0.348	0.051	0.183	0.215	0.201	0.170
Cache	50	0.190		0.235	0.195	0.292	0.315	0.306	0.287
Cache-*iso*	14	0.370	0.111		0.193	0.283	0.304	0.335	0.412
Canoe	44	0.051	0.123	0.283		0.071	0.086	0.099	0.080
Firth-1	58	0.079	0.112	0.241	0.043		-0.002$^{\varphi}$	0.010$^{\varphi}$	0.137
Firth-2	50	0.071	0.114	0.245	0.033	0.000$^{\varphi}$		0.040	0.193
Joe	125	0.074	0.103	0.227	0.034	0.011	0.008		0.107
Rat	54	0.087	0.154	0.307	0.063	0.082	0.074	0.052	

correlated to the presence of relatively few alleles which occurred in markedly different frequencies rather than to the presence of many private alleles (Table 2-8, Figure 2-6). Although the population above the falls on the Babbage was also isolated, it did not have as high F_{ST} estimates (mean 0.16). Comparisons among anadromous populations typically showed lower F_{ST} estimates than comparisons with the isolated populations. Of the anadromous populations, Cache Creek showed the highest F_{ST} values in pairwise comparisons. This result, once again, is likely a reflection of its lower allelic diversity rather than unique alleles. Within the Firth drainage, all three populations were most closely related to one another (4 loci F_{ST} ranged from 0.000 to 0.009, Table 2-9). Joe was significantly divergent from both Firth-1 and Firth-2, but the divergence was small.

The Φ_{ST} population pairwise estimates showed that all populations were significantly divergent except Firth-1 vs. Firth-2, and Firth-1 vs. Joe Creek (Table 2-9 – above diagonal). The general pattern of results was similar to that for the F_{ST} estimates. However, in most cases, the Φ_{ST} values were higher (21 of 28 pairwise comparisons) indicating stronger divergence when molecular variance is considered. For example, the F_{ST} estimate between the Cache anadromous and Rat populations was 0.14, but the Φ_{ST} was more than double at 0.29. As before, the pattern and magnitude of estimates was not appreciably altered by the inclusion or exclusion of *MST-85* (Table 2-9 A and B).

Population relationships

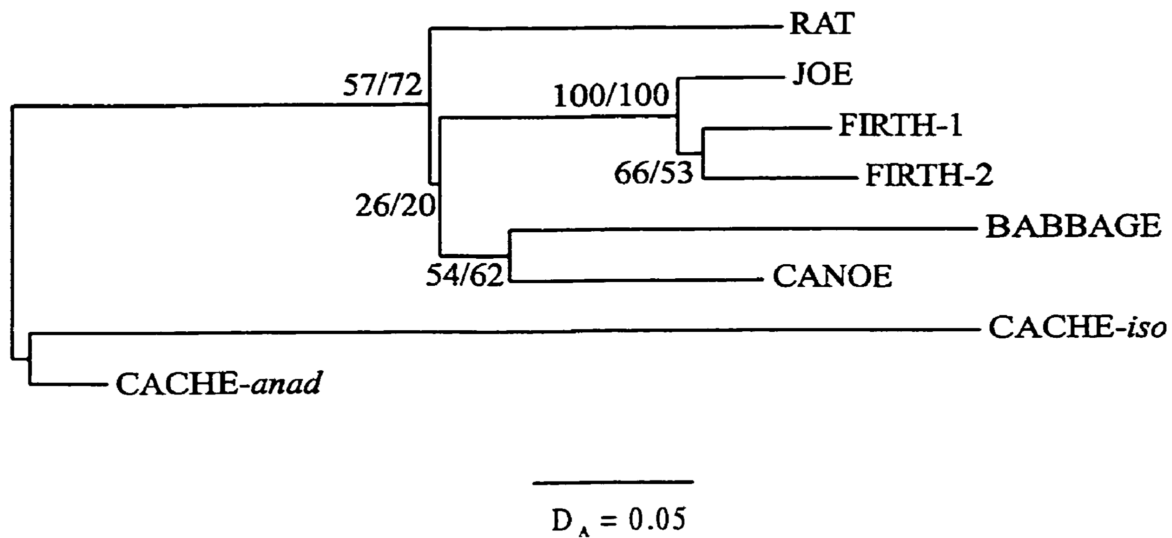
Application of the three distance measures, D_A, D_C, and ($\delta\mu^2$) produced considerable variation in population relationships (see Appendix II for a comparison and discussion of the three distance measures). The D_C distance tree was most similar to the D_A phenogram and, since D_A is thought to be more appropriate for branch length

estimation between closely related populations (Takezaki and Nei 1996), only this tree is shown (Figure 2-7). The D_A measures gave strong bootstrap support (100%) to the clustering of the three populations within the Firth drainage. Although the other nodes were supported by lower bootstrap values; all populations grouped by drainage. The branch lengths showed that the Cache Creek populations were most divergent, while the populations within the Firth drainage were most closely related.

When *MST-85* was removed from the analysis, bootstrap support for nodes usually became much stronger (Figure 2-7B). The negative impact of the *MST-85* locus on both bootstrap support and branching order likely reflects the presence of null alleles which mask true divergences. Because of the higher bootstrap support, the populations grouping by drainage, and congruence between the D_A and D_C trees (Appendix II), the phenograms constructed from the three loci likely provides the best estimates of the relationships among the populations. In contrast (and as already noted above), the pairwise comparisons of allelic variance (F_{ST}) showed a greater magnitude of differentiation when *MST-85* was included (Table 2-9).

The population structuring by drainage, although weakly supported in some cases, was also evident in the hierarchical analysis of molecular variance (Table 2-10). A small (5.5%), but significant amount of the genetic variance was found among drainages (F_{IT}). Much of this significance was likely due to the high bootstrap value grouping the Firth drainage populations. Significant variation in approximately the same proportions (4.4%) was attributable to the among populations within drainage component (F_{ST}), indicating some degree of divergence between populations within a particular river system. This is concordant with the relatively long branch lengths of the Babbage and Cache populations

A. 4 microsatellite loci



B. 3 microsatellite loci

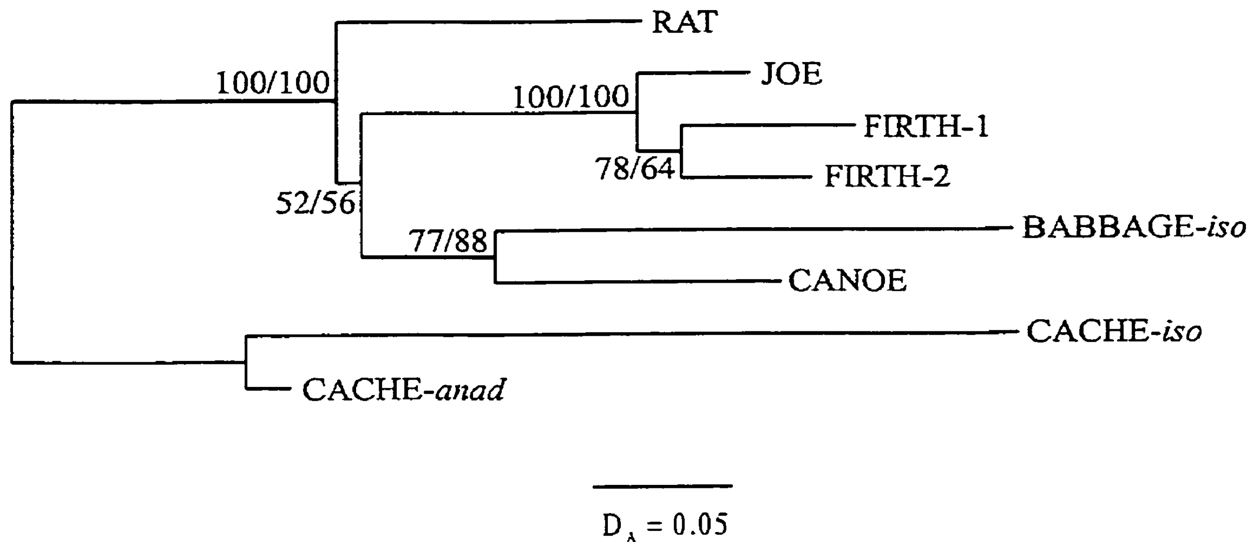


Figure 2-7. Nei *et al.* (1983) D_A distance neighbour-joining phenogram calculated from microsatellite allele frequenicies showing relationships in 8 populations of *S. malma*. Bootstrapping by loci and by individual (10 000 replicates). A- 4 loci; B- 3 loci (*MST-85* removed).

Table 2-10. Hierarchical analysis of molecular variance (equal distances among alleles) for 4 microsatellite loci (A) and 3 microsatellite loci (B) among populations of *S. malma* from four drainages; Firth, Cache, Babbage/Canoe and Rat.

A. 4 microsatellite loci:

Source of variation	d.f.	Percentage of Variation	Fixation Indices	*P*
Among drainages	3	5.46	$F_{IT} = 0.055$	0.006
Among populations within drainage	4	4.42	$F_{ST} = 0.047$	< 0.00001
Within populations	826	90.12	$F_{IS} = 0.099$	< 0.00001

B. 3 microsatellite loci (*MST-85* removed):

Source of variation	d.f.	Percentage of Variation	Fixation Indices	*P*
Among drainages	3	7.28	$F_{IT} = 0.073$	0.001
Among populations within drainage	4	2.33	$F_{ST} = 0.025$	< 0.00001
Within populations	826	90.38	$F_{IS} = 0.096$	< 0.00001

within their respective drainages (Figures 2-7). As is common with highly polymorphic microsatellite data in closely related populations, the greatest component of variation (90.1%) resided within populations.

When just three loci were considered (Table 2-10B), the among drainage component ($F_{IT} = 0.07$) became twice as important as the among population within drainage component ($F_{ST} = 0.03$). However, all the hierarchical parts remained significant in both the three and four locus analysis.

Genetic differentiation among life history/cycle types

To examine genetic structure that may be linked to differences in life history or life cycle, samples were formed by grouping individuals by their location and life type (Appendix I). This regrouping created some low sample sizes and thus only results from the Firth drainage are discussed in detail (Table 2-1).

In the allopatric population pairwise comparisons of allele frequencies (Table 2-6), Joe Creek was divergent from Firth-1 and Firth-2 at two and three loci respectively. However, when the samples were partitioned by life type no allelic pairwise differences were found within the Firth drainage (Appendix I, Table AI-3). Pairwise comparisons of allelic (F_{ST}) and molecular (Φ_{ST}) variance mirrored the allelic frequency comparison results and also were not significantly altered by *MST-85*. Within the Firth drainage, all life types from the three locations were not significantly different from one another for both F_{ST} and Φ_{ST} (Appendix I, Table AI-4).

The genetic similarity between life types within the Firth drainage was also evident in the hierarchical analysis of molecular variance (Table 2-11). The among-tributary component (F_{IT}) and among life cycle/history component (F_{ST}) of the total

Table 2-11. Hierarchical analysis of molecular variance (equal distances among alleles) for 4 microsatellite loci (A) and 3 microsatellite loci (B) among life cycle / history types and tributaries within the Firth River drainage.

A. 4 microsatellite loci :

Source of variation	**d.f.**	**Percentage of Variation**	**Fixation Indices**	**P**
Among tributaries	1	0.54	$F_{IT} = 0.005$	0.099
Among life cycle / history types within tributary	4	0.56	$F_{ST} = 0.006$	0.104
Within life cycle / history types	446	98.91	$F_{IS} = 0.011$	0.003

B. 3 microsatellite loci (*MST-85* removed) :

Source of variation	**d.f.**	**Percentage of Variation**	**Fixation Indices**	**P**
Among tributaries	1	0.69	$F_{IT} = 0.007$	0.100
Among life cycle / history types within tributary	4	0.42	$F_{ST} = 0.004$	0.105
Within life cycle / history types	446	98.89	$F_{IS} = 0.011$	0.0004

genetic variation were statistically insignificant.

Individual reassignment analysis

Reassignment of individuals back to their population of origin was conducted with the four microsatellite loci and D-loop haplotypes, three microsatellite loci (*MST-85* removed) and D-loop haplotypes (data not shown), and finally with just the four microsatellite loci (data not shown). In all cases, the analysis with all microsatellites and the D-loop haplotypes had the highest success. The two isolated populations (Babbage-*iso* and Cache-*iso*) had high reassignment success, 96% and 100% respectively, (Appendix III) indicating low gene flow. However, they were removed from the subsequent analysis due to the bias introduced from low sample size (N< 30) on correctly reassigning individuals from populations with larger sample size. Specifically, populations represented by a small sample size will inflate the relative frequency of the alleles present in the sample (and thus their importance in maximum likelihood reassignment analysis). Therefore, individuals from populations with large sample sizes may be inaccurately reassigned to populations with low sample sizes (see Appendix III).

There was variable success in reassigning individuals from anadromous populations to their home stream (Table 2-12A). Cache had the highest success with 96% of individuals being correctly reassigned. The Firth drainage showed poor reassignments with as few as 28.8% being correctly placed in Joe. However, most of the misassigned individuals were located within the Firth drainage. Due to the low divergence among Firth drainage populations, the analysis was conducted with the Firth drainage populations pooled (Firth-*pooled*) and reassignment success was modestly improved (Table 2-12B).

The ambiguity of reassignments is further illustrated by the few individuals which were assigned with less than 1.0% chance of misassignment (numbers in parantheses Table 2-12). Cache had the highest success with this stringency, 68% of individuals. Canoe and Rat had less than 50%, while the Firth drainage populations had no individuals correctly reassigned with the imposed 1% misassignment strictness. Pooling the Firth populations improved these results marginally in all cases (Table 2-12B).

Table 2-12A. Maximum likelihood population reassignments by jack-knifing individual multi-locus genotypes to the baseline population allele frequencies calculated from 4 microsatellite loci and D-loop haplotypes. Populations of *S. malma* grouped by drainage (thin horizontal line). In brackets, the number of individuals with < 1.0% chance of mis-assignment. Table 2-12B. Repeat of the analysis with Firth drainage poulations pooled.

A.

	N	Population Reassignment					
		Cache	Canoe	Firth-1	Firth-2	Joe	Rat
Cache	50	48(34) 96.00%	0	1 2.00%	0	0	1 2.00%
Canoe	44	0	36(14) 81.82%	3 6.82%	2 4.55%	2 4.55%	1(1) 2.27%
Firth-1	58	1 1.72%	6 10.34%	23(0) 39.66%	16 27.59%	7 12.07%	5 8.62%
Firth-2	50	2 4.00%	6(2) 12.00%	17 34.00%	17(0) 34.00%	4 8.00%	4 8.00%
Joe	125	5 4.00%	24 19.20%	19 15.20%	24 19.20%	36(0) 28.80%	17 13.60%
Rat	54	3 5.56%	1 1.85%	0	0	1 1.85%	49(22) 90.74%

B.

	N	Population Reassignment			
		Cache	Canoe	Firth *pooled*	Rat
Cache	50	48(34) 96.00%	0	0	2 4.00%
Canoe	44	0	38(18) 86.36%	5(1) 11.36%	1 2.27%
Firth-*pooled*	233	9 3.86%	50(3) 21.46%	137(20) 58.80%	37(1) 15.88%
Rat	54	3 5.56%	1 1.85%	0	50(24) 92.59%

DISCUSSION

Genetic characterization and population structure

The surveys of mitochondrial DNA and microsatellite diversity revealed moderate to high levels of polymorphism in the populations of *S. malma* on the Yukon north slope. The average heterozygosity (0.57) and average number of alleles (13) among the four microsatellite markers were much greater than that observed in an earler allozyme study of Alaskan *S. malma* from the north slope (Everett *et al.* 1997). In that study, 21 of 49 loci were polymorphic but individual heterozygosities averaged only 0.04. The level of microsatellite polymorphism observed here is, however, similar to that reported in other species of the genus *Salvelinus* (Angers *et al.* 1995, Bernatchez *et al.* 1998, Hébert *et al.* 2000). Also, the distribution of haplotype frequencies with a few dominant and many rare alleles is typical for fish species inhabiting formerly glaciated regions (reviewed in Billington and Hebert 1991).

Genetic differentiation was observed among populations of *S. malma* from different drainages at both mitochondrial and nuclear markers. This result suggests that gene flow among populations in different drainages is restricted, allowing diversification by genetic drift and perhaps local adaptation. Within the Firth drainage, genetic divergence among populations was slight or absent, suggesting that substantial gene flow occurs between such closely situated spawning habitats. These findings are similar to those of Reist (1989) and Everett *et al.* (1997) whose allozyme analyses indicated genetic differences between populations from different rivers but no differentiation within the Firth drainage. Therefore, although discrete spawning and overwintering habitats occur within the Firth drainage, they do not represent unique populations. These observations

are inconsistent with the member-vagrant hypothesis which predicts that the number of populations of a given species will be determined by the number of geographically stable or predictable larval retention areas (Sinclair and Iles 1988). These results contrast with a study on anadromous *S. alpinus*, where high genetic divergences between tributaries within the same drainage have been reported (Bernatchez *et al.* 1998).

To examine whether *S. malma* from the Firth drainage exhibited site fidelity for spawning but not for overwintering, individuals were partitioned by their life cycle type (see Appendix I). No genetic divergence between spawners and non-spawners was detected among the three sites in the Firth drainage. In fact, the slight divergence of the Joe Creek site when life cycle types were pooled became insignificant when the populations were partitioned by life cycle type. Thus the frequency of wandering between drainages regardless of life cycle type is low and beyond the detection capacity of the analyses employed.

The Babbage and Cache drainages both contained a population isolated above a waterfall and a downstream anadromous population. The isolated populations were in both cases differentiated from their downstream counterparts. Much of this differentiation was due to the low level of polymorphisms in the isolated populations (average number of alleles 6.8 and 2.5 Babbage and Cache respectively versus the overall average of 13 alleles). The F_{ST} and Φ_{ST} pairwise estimates showed that these isolated populations were highly divergent from each other as well as from anadromous populations in other drainages. Overall, the isolated populations were most closely allied to the downstream anadromous population of the same drainage.

Among the four drainages, the westernmost (Firth) possessed the highest mtDNA and microsatellite allelic diversity. Populations from the three more easterly drainages possessed fewer alleles and most of their alleles were not unique. Thus, these drainages contained a subset of the total genetic variation found in the Firth drainage.

Microphylogeographic reconstruction

Examining the possible role of glaciation on genetic diversity in *S. malma* is confounded by the small geographic area surveyed and the indirect (correlative) nature of genetic evidence. The underlying assumption is that the greater the distance from the glacial margin, the less influence the glacier would have on population structure. The inclusion of Alaskan drainages further west would make the following tentative observations more robust. Nonetheless, some trends were apparent.

The Firth River populations were distinguished from those in the three easterly drainages by their much higher genetic diversity at both mtDNA and nuclear DNA markers. This result suggests that populations in the three eastern drainages were either extiripated or severely bottlenecked due to glaciation. The Firth drainage, being most distant from the glacial margin, was the least influenced. Because the genetic variation of populations in the eastern drainages was largely a subset of the diversity in the Firth, fish from this river (or perhaps populations further to the west) were the most likely source for recolonization. The low allelic diversity of easterly populations suggests that recolonization involved a few founders and that subsequent gene flow from the west has been low. A competing explanation is that all the eastern drainages suffered localized bottlenecks reducing genetic diversity. Gene flow has not been great enough to build the

gene diversity up to the level observed in the Firth drainage. However, the occurence of two haplotypic phylogroups makes this latter explanation less probable.

The haplotypic variation showed a clear phylogeographic signal. The three eastern drainages were composed almost exclusively of haplotypes belonging to phylogroup I, whereas the Firth drainage was more diverse, possessing a mix of phylogroups I and II. The existence of these two phylogroups suggests the possible presence of two microrefugia or alternatively two colonization events separated in time. Several competing scenarios are plausible. Firstly, phylogroup II may be ancestral and once occurred throughout the Yukon north slope. As the Wisconsinan ice sheet advanced toward the MacKenzie delta, it extirpated populations of *S. malma* from the three eastern drainages. The subsequent retreat of the ice sheet enabled the recolonization of these habitats from a western source population (group I). The Firth river was also likely colonized by phylogroup I at this time. Perhaps the relatively rapid changes in ocean level and temperature promoted dispersal from more westerly sites. Under this hypothesis, it is unlikely that the Firth drainage was the main source for recolonization of the eastern drainages. Had it been, the expectation is that the eastern drainages would possess representatives of both phylogroups I and II. However, only one of the nine haplotypes belonging to phylogroup II in the Firth occured in the eastern drainages as oppposed to the nine (of ten possible) phylogroup I haplotypes. A second explanation is consistent with the eastern drainages being either bottlenecked or exterminated and recolonized. In this case, group I is ancestral and II is derived either from a distant (more western) population or from the Firth drainage. Again, the low levels of gene flow evident has prevented the significant spread of phylogroup II further east.

The hypothesis that the two isolated populations were established through unique colonization events was unsupported by the present study. The mitochondrial data revealed very little genetic divergence between the Cache Creek isolated population and its downstream counterpart. While the Babbage isolated population had a somewhat divergent haplotype array from the downstream Canoe river population, the haplotypes of both populations fell within phylogroup I. The divergence between these populations is more consistent with impact of genetic drift on the isolated Babbage population. Similarly, only one unique haplotype occurred among the isolated populations (haplotype *1*). This suggests that isolated populations in the Babbage and Cache were part of the same colonization event as the anadromous populations and that isolation developed subsequently.

In the pairwise comparison of microsatellite divergence among populations using both allelic (F_{ST}) and molecular (Φ_{ST}) variance, Φ_{ST} were generally higher. Therefore, the populations were differentiated not only by allele frequencies, but also by the number of mutations (assuming SMM) between alleles. One explanation is that gene flow has been restricted between these populations for a sufficiently long period of time to allow the accumulation of new mutations (Slatkin 1995; Goodman 1998). However, the recent glaciation of the region makes this improbable. These results are more likely caused by recent founder events where a few individuals from a genetically diverse source population gave rise to the extant populations. The high Φ_{ST} is thus not due to deep divergences caused by many new mutations since the populations split, but is the result of the random fixation of alleles from a more diverse ancestral metapopulation.

As a result, the extant populations appear more divergent from one another when employing the SMM due to the effects of sampling. If the three easterly drainages were colonized by just a few individuals from the more westerly drainages, they would possess the observed subset of genetic variation. If the differences in allelic compostion between populations happen to involve a large number of repeat differences (i.e. large number of mutational steps under SSM) then Φ_{ST} may be inflated relative to F_{ST}. In addition, drift will cause random loss of alleles and changes in allele frequencies, further magnifying the divergence.

Overall, these observations support the notion that historical demography may be as important as contemporary factors in explaining patterns of genetic diversity among populations of Dolly Varden, a result supported by prior work on other fishes (Bernatchez and Wilson 1998).

Reassignments

In general, the combined mtDNA and microsatellite data enabled a high probability of correctly assigning individuals to their source drainage or to a subdivision of it when there was an actual physical barrier such as a waterfall. Assignment success might have been further enhanced with larger sample sizes. Smouse and Chevillon (1998) recommend that studies examine more individuals per population than K (the total number of alleles across all loci and across all populations minus the number of loci). When examining the 4 microsatellite loci and the D-loop haplotypes, K = 135 and by this criterion, all populations examined in the current study had an insufficient sample size.

Nonetheless, the populations from the three easterly drainages had relatively high reassignment success. That individuals from the Firth drainage reassigned with high frequency to other drainages and that the converse was not true may be historically informative. As the higher genetic diversity in the Firth drainage also shows, the three easterly drainages represent a subset of the genetic variation present in the Firth. Thus some individuals from the Firth drainage share multilocus genotypes with other populations Misassignment of some individuals could also result from wandering between drainages (McCart 1980; DeCicco 1997). However, genetic divergence between drainages indicates that the extent of this wandering is low. In a mixed stock analysis of ten Alaskan drainages as well as the Firth and Babbage drainages, 87% of *S. malma* were correctly reassigned to one of three broad geographic regions using allozyme data (Krueger *et al.* 1997). However, fish could not be reassigned to their spawning site due to insufficient levels of allozyme polymorphism. Although the high allelic diversity of microsatellite markers provide an advantage over allozymes in stock identification (Estoup *et al.* 1998) they suffer from the disadvantage that high polymorphism often requires impractically large sample sizes. As microsatellites show a range in polymorphism, it is recommended that moderately polymorphic (5 – 15 alleles per locus) be identified in advance of undertaking future stock discrimination investigations on *S. malma*.

Heterozygote deficits

Analyses of genotypic arrays at the mirosatellite loci revealed more heterozygote deficits than expected by chance. Heterozygote deficits can reflect sampling error, the presence of null alleles, inbreeding or Wahlund effect. Due to the large number of alleles

relative to the sample size, sampling a single rare homozyogous individual may have a dramatic effect on conformation to Hardy-Weinberg expectations. Also, as each anadromous collection site contained a mix of spawners and residents, which are assumed to be philopatric, with non spawners, which may not be in their natal river, fundamental Hardy-Weinberg assumptions may be violated (Appendix I). Bernatchez *et al.* (1998) suggested that straying between drainages was the most likely explanation for heterozygote deficits observed in anadromous populations of *S. alpinus* from Labrador. Furthermore, Shields (1982) hypothesized that philopatry is an adaptive mechanism to promote inbreeding. The possibility that straying and alternate life history types are at least partially responsible for the heterozygote deficits observed in this study is supported by the observation that genotype frequencies in the two isolated populations were always in Hardy-Weinberg proportions.

MST-85 was the only locus which regularly showed heterozygote deficits suggesting the likely occurrence of null alleles. Such alleles have two, potentially opposing, effects on the detection of population subdivision and gene flow. First, they may act to homogenize populations if the undetected null alleles are present in high frequency and broadly distributed across populations. In this situation, gene flow between populations may go undetected. Alternatively, when specific null alleles occur in only one or a few populations (i.e. the equivalent of undetected private alleles) their presence conceals population divergence

In order to examine the potential biases introduced by null alleles at *MST-85*, the genetic differentiation analyses were performed both with that locus included and excluded. The results of these two analyses were largely concordant, indicating that the

null alleles at *MST-85* did not alter the pattern of genetic differentiation. Because most individuals amplified at least one allele at *MST-85*, no estimate of null allele frequency was performed (Markert *et al.* 1999). Microsatellite loci with suspected null alleles are often eliminated from analysis (Primmer *et al.* 1999). However, while loci with null alleles require cautious interpretation, they may still be informative. In the present case, the inclusion of *MST-85* revealed further differentiation between the Cache isolated population (dominant allele 126) and the other drainages (dominant allele 136) (Figure 2-6B).

Conservation issues

Conservation goals for fisheries management ordinarily fall into one of two classes. First there are management proposals which seek to maximize production by modeling maximum sustainable yield. Such plans require the identification of different stocks often through genetic analyses and the determination of population sizes. A second conservation philosophy aims to maintain the genetic diversity of populations so that stocks can respond to future environmental change (Moritz *et al.* 1995). Arctic fisheries, in particular, require prudent management as slow growth, complex life cycles, and extreme year to year fluctuations in recruitment (Power 1997) makes them vulnerable to overexploitation. Therefore, the conservation management goals for *S. malma* should be cautious. The following recommendations utilize criteria outlined by Moritz *et al.* (1995). They specify two types of conservation units which can be identified through genetic analysis: evolutionary significant units (ESUs) and management units (MUs). ESUs are defined as historically isolated sets of populations for which a stringent and qualitative criterion is reciprocal monophyly for mtDNA and significant divergences at

nuclear loci. MUs are one or more homogeneous populations that show statistically significant divergence of allele frequencies at nuclear or mtDNA loci from other such units. The identification of MUs aids in the short term management of the larger ESU entities. It is generally accepted that information on genetic variation should be combined with life history, morphological and behavioural information as these traits may be more relevant to current adaptation than neutral molecular markers (Waples 1995).

Although two phylogroups were identified among the Yukon north slope populations, their divergences were insufficient for cladistic analysis and thus strict reciprocal monophyly was not demonstrated. Conservatively, the Yukon north slope populations represent a single ESU. However, the existence of two phylogroups suggests that gene flow was restricted within the Beringian refuge during the last glacial advance and that microrefugia may have existed within Beringia. More broadly, these data and those of Reist *et al.* (1997) and Everett *et al.* (1997) suggests that the Alaskan north slope populations stem from the same glacial race as the Yukon populations. It is likely that the Beaufort Sea populations represent an ESU distinct from other populations of *S. malma*. This conclusion is further supported by the isolation of these populations (over 500 km) from the rest of the species' distribution (Figure 1-1).

Each population identified by spawning ground, except those in the Firth, were both genetically divergent (Waples 1991) and phylogeographically concordant (Avise and Ball 1990) in nuclear and mtDNA allele frequency distributions warranting their identification as distinct MUs. The isolation of two populations above waterfalls and their genetic divergence indicates that these populations are on separate evolutionary

trajectories from their downstream anadromous counterparts. Although examining specific local adaptations was beyond the scope of this investigation, the apparent lack of gene flow between drainages would enable such divergence. In the Firth drainage, the Joe tributary was only slightly divergent from the two Firth tributary spawning grounds and the two Firth populations were indistinguishable from one another. This evidence and that of Reist *et al.* (1997) and Everett *et al.* (1997) suggests that sufficient gene flow occurs within the Firth drainage spawning grounds to classify the Firth populations as a single MU. In terms of allelic variation, the Firth populations were the most diverse, possessing virtually all of the genetic variation found in the other drainages. Although this result does not preclude the possibility of significant local adaptations among populations in the easterly drainages, the present information suggests that the preservation of the Firth populations should have highest priority.

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APPENDIX I

Genetic differentiation among life history/cycle types

INTRODUCTION

Tagging studies along the Alaskan north slope (four studies summarized in McCart 1980) showed that although most *S. malma* return to the site where they were marked, some straying occurs. However, these strays were never found to be in reproductive condition (DeCicco 1997). DeCicco (1989) linked straying with a complex migratory pattern exhibited in *S. malma* from northwestern Alaska. Sexually mature individuals were found to overwinter in nonnatal rivers in years when they were not spawning. However, fish captured spawning at a particular site were never caught spawning elsewhere (DeCicco 1997). This suggests that *S. malma* exhibit site fidelity for spawning but not for overwintering. This result raises concerns that the pooling of fish caught at a particular site may mask the true genetic divergence of the local spawning populations.

The anadromous northern form of *S. malma* does not spawn every year (Armstrong and Morrow 1980). This is likely because the harsh conditions do not permit adequate energy to enable both growth and reproduction every year. Males, with a lower investment in gametes, may reproduce more often than females (Armstrong and Morrow 1989). Anadromous *S. malma* undertake 3 to 5 ocean migrations before reaching sexual maturity (DeCicco 1997). If maturing individuals follow food carried in marine currents, then they may become distributed over a wide geographic range (DeCicco 1997). Potentially, smolts (anadromous large juveniles) may not return to their natal spawning grounds until they first reproduce. It may be energetically advantageous to follow food patches for several years rather than to return to their natal site each year.

Sympatric life cycle types

To address the site fidelity of north slope *S. malma* of different life cycle stages, anadromous samples were assigned to one of three life types based on morphological and age characters: sexually mature spawners, sexually mature nonspawners (resting), and sexually immature nonspawners (large juveniles which had smoltified) (Table 2-2). Adult spawners are assumed to be philopatric. The genetic identity of adult spawners has been compared among sites to determine if sufficient genetic differentiation exists to enable their discrimination (and to indirectly test the assumption of spawning site fidelity). Secondly, the genetic composition of spawners has been compared to that of adult nonspawners and juveniles within sites and between sites to determine the extent of wandering by these life cycle stages.

Sympatric life history types

It is almost certain that resident and anadromous males are part of the same spawning population (Reist 1989). No record of female residents has been observed in these drainages. Behavioural studies on Alaskan populations have demonstrated that resident males display an alternate reproductive strategy from the anadromous types (Maekawa *et al* 1993). Anadromous females always paired with a large anadromous male (α-male). Generally, several other subordinate anadromous and resident males were present (satellite males) which attempted to sneak fertilizations. Females responded aggressively to resident males but did not attack anadromous males. Females spawned only once per nest when resident males were present, but up to three times when just anadromous males were present. This led Maekawa *et al* (1993) to hypothesize that

females were actively selecting to increase anadromy in their offspring under the assumption that anadromy is a heritable trait.

A preliminary question in addressing the heritability of anadromy is whether adult resident male *S. malma* will migrate to sea at a later time. Maekawa *et al* (1993) determined that on average resident males involved in spawning were significantly younger than anadromous males. However, no *S. malma* caught at sea have been reported to possess both the parr marks and spawning colours characteristic of resident males.

Regardless of the heritability of anadromy, the genetic characterization performed in this study tests the assumption that both anadromous and resident males belong to the same breeding population by monitoring allele frequency differences between them. However, the possibility that residency is a genetic trait cannot be directly tested. The relative fitness of resident males can be indirectly assessed by comparing the genetic diversity of residents to anadromous life history types.

As the resident males may represent a sink of genetic diversity (Reist 1989), the characterization of their diversity relative to anadromous life types has significant implications for the planning of conservation programs.

METHODS

To examine genetic structure that may be linked to differences in life history or life cycle types, samples were formed by grouping individuals by their location and life type. All analyses were performed as outlined in chapter 2. This regrouping created some low sample sizes: inferences are not made in these cases and are identified where relevant below. (The inclusion of low sample size life type groups are shown for completeness of the data set to illustrate all possible categories of comparison.)

RESULTS

Hardy-Weinberg tests revealed that 11 of 68 tests showed significant heterozygote deficits (following Bonferroni corrections, k=17) (Table AI-1). This represents 4 instances more than expected by chance alone (α=.05). Six of these heterozygote deficits were at the *MST-85* locus and three were at *Ots-1*. Therefore the potential for null alleles at *MST-85* exists (see Chapter 2). That the number of significant Hardy-Weinberg departures for *Ots-1* dropped from 5 to 3 when the populations were regrouped by life type suggests that population subdivision may at least be partially responsible for the heterozygote deficits (see Table 2-6).

The observed heterozyogosity among life types varied widely (Table AI-1). Most notably, the two resident life type groups were allelically diverse and had similarly high heterozygosities as their sympatric anadromous life type counterparts.

Private alleles between life types were few and occurred in low frequency (Table AI-2). Cache Creek anadromous spawners had 2 unique *Cocl-3* alleles in relatively high relative frequency (RF) of 0.05 and 0.07. Also the Babbage isolates had a single *Sco-19* allele 180 with an RF of 0.06. Virtually all other private alleles were rare (RF < 0.02). Private alleles showed no trends in their distribution by life type(s) and most occurred in life types from the Firth drainage. Thus, it is unlikely that private alleles contributed significantly to observed patterns of divergence.

Pairwise comparisons of life type allele frequencies differences by locus are shown in Table AI-3. Ignoring samples of n<14, the two isolated populations remained highly divergent from all other types. In one exception, the allele frequencies in the Cache Creek anadromous spawners were different at a single locus compared to the

Table AI-1. Number of individuals (n), different alleles (A), gene diversity (He), observed heterozygosity (Ho), inbreeding coefficient (f) and Hardy-Weinberg equilibrium (HWE) with standard error (SE) for populations grouped by life type. Significant (*) departures following Bonferroni corrections, $\alpha = 0.05$, $k = 16$ unbiased estimate of type-I error.

LOCATION	*life-type*	N		*Cocl-3*	*Ots-1*	*Sco-19*	*MST-85*	***ALL***
BAB	*iso*	26	**n**	`	25	25	24	24.75
			A	9	9	6	3	6.75
			He	0.838	0.662	0.651	0.082	0.558
			Ho	0.920	0.560	0.680	0.042	0.550
			f	-0.101	0.157	-0.045	0.500	0.015
			HW	0.058	0.196	0.443	0.021	
			SE	0.002	0.005	0.003	/	
CACHE	*iso*	14	**n**	14	14	14	14	14
			A	3	3	2	2	2.5
			He	0.561	0.500	0.198	0.138	0.349
			Ho	0.500	0.571	0.214	0.000	0.321
			f	0.112	-0.149	-0.083	1.000	0.082
			HW	1.000	1.000	1.000	0.037	
			SE	/	/	/	/	
CACHE	*alj*	8	**n**	6	8	8	6	7
			A	3	7	4	3	4.25
			He	0.318	0.892	0.617	0.591	0.604
			Ho	0.333	0.500	0.625	0.500	0.490
			f	-0.053	0.456	-0.015	0.167	0.204
			HW	1.000	0.007	0.239	1.000	
			SE	/	0.001	/	/	
CACHE	*as*	42	**n**	41	41	41	37	40
			A	16	11	8	4	9.75
			He	0.737	0.844	0.605	0.248	0.608
			Ho	0.707	0.537	0.634	0.216	0.524
			f	0.041	0.367	-0.049	0.129	0.141
			HW	0.076	0.000*	0.798	0.326	
			SE	0.008	0.000	0.004	/	
CANOE	*alj*	39	**n**	38	38	38	38	38
			A	15	19	16	6	14
			He	0.840	0.770	0.824	0.655	0.773
			Ho	0.790	0.605	0.790	0.368	0.638
			f	0.061	0.216	0.043	0.441	0.176
			HW	0.107	0.002	0.088	0.000*	
			SE	0.006	0.001	0.007	0.000	

CANOE	*as*	5	**n**	5	5	5	5	5
			A	8	7	5	2	5.5
			He	0.933	0.867	0.822	0.356	0.744
			Ho	0.800	0.800	0.800	0.000	0.600
			f	0.158	0.086	0.030	1.000	0.213
			HW	0.332	0.617	0.898	0.111	
			SE	0.006	0.004	0.001	/	
FIRTH1	*res*	9	**n**	9	8	9	8	8.5
			A	11	6	15	4	9
			He	0.915	0.842	0.980	0.642	0.845
			Ho	0.889	0.625	0.889	0.125	0.632
			f	0.030	0.271	0.099	0.816	0.263
			HW	0.799	0.451	0.019	0.001	
			SE	0.006	0.002	0.003	/	
FIRTH1	*as*	49	**n**	49	47	49	48	48.25
			A	19	22	23	8	18
			He	0.914	0.910	0.915	0.591	0.832
			Ho	0.776	0.468	0.878	0.313	0.608
			f	0.153	0.488	0.041	0.474	0.271
			HW	<0.001*	0.000*	0.036	0.000*	
			SE	0.000	0.000	0.004	0.000	
FIRTH2	*alj*	44	**n**	44	42	44	43	43.25
			A	19	22	24	9	18.5
			He	0.910	0.872	0.931	0.628	0.835
			Ho	0.818	0.738	0.955	0.372	0.721
			f	0.102	0.155	-0.025	0.410	0.138
			HW	0.010	0.124	0.984	0.000*	
			SE	0.002	0.010	0.002	0.000	
FIRTH2	*aar*	4	**n**	4	4	4	4	4
			A	5	6	6	4	5.25
			He	0.857	0.893	0.893	0.857	0.875
			Ho	1.000	0.750	1.000	0.000	0.688
			f	-0.200	0.182	-0.143	1.000	0.241
			HW	1.000	0.430	1.000	0.010	
			SE	0.000	0.004	0.000	/	

FIRTH2	*as*	2	**n**	2	2	2	2	2
			A	3	4	3	1	2.75
			He	0.833	1.000	0.833	0.000	0.667
			Ho	1.000	1.000	0.500	0.000	0.625
			f	-0.333	0.000	0.500	0.000	0.091
			HW	1.000	1.000	0.333	-	
			SE	/	/	/		
JOE	*alj*	49	**n**	49	47	49	44	47.25
			A	22	19	28	7	19
			He	0.928	0.762	0.945	0.680	0.829
			Ho	0.857	0.596	0.918	0.318	0.672
			f	0.077	0.220	0.028	0.535	0.190
			HW	0.043	0.012	0.414	0.000*	
			SE	0.005	0.003	0.015	0.000	
JOE	*aar*	1	**n**	1	1	1	1	1
			A	1	1	2	1	1.25
			He	0.000	0.000	1.000	0.000	0.250
			Ho	0.000	0.000	1.000	0.000	0.250
			f	0.000	0.000	0.000	0.000	0.000
			HW	-	-	-	-	
			SE					
JOE	*res*	21	**n**	21	21	21	21	21
			A	15	18	16	6	13.75
			He	0.912	0.909	0.944	0.588	0.838
			Ho	0.857	0.714	0.952	0.333	0.714
			f	0.061	0.219	-0.009	0.439	0.151
			HW	0.679	0.084	0.082	0.012	
			SE	0.008	0.007	0.004	0.001	
JOE	*as*	54	**n**	54	52	54	49	52.25
			A	19	20	25	9	18.25
			He	0.859	0.796	0.950	0.577	0.795
			Ho	0.778	0.673	0.982	0.306	0.685
			f	0.095	0.155	-0.033	0.472	0.140
			HW	<0.001*	0.019	0.977	0.000*	
			SE	0.000	0.004	0.003	0.000	

RAT	**aar**	6	**n**	6	6	6	5	5.75
			A	7	3	5	2	4.25
			He	0.833	0.318	0.788	0.467	0.602
			Ho	0.833	0.167	1.000	0.200	0.550
			f	0.000	0.500	-0.304	0.600	0.092
			HW	0.884	0.091	0.791	0.333	
			SE	0.002	/	0.002	/	
RAT	**as**	48	**n**	46	48	48	48	47.5
			A	22	5	9	5	10.25
			He	0.898	0.480	0.796	0.466	0.660
			Ho	0.804	0.354	0.750	0.333	0.561
			f	0.105	0.264	0.058	0.287	0.152
			HW	0.067	0.010	0.577	0.012	
			SE	0.007	0.000	0.004	0.001	
Mean			**n**	23.11	22.83	23.33	22.17	22.86
			A	11.11	10.22	11.11	4.33	9.19
			He	0.773	0.721	0.807	0.457	0.690
			Ho	0.731	0.592	0.809	0.190	0.581
			f	0.058	0.205	-0.005	0.577	0.172

Table AI-2. Occurrence and frequency of private alleles in 4 microsatellite loci among 18 groups identified by population and life-type.

Locus	Allele	Frequency	Location
Cocl-3	299	0.073	Cache-AS
	301	0.049	Cache-AS
	239	0.011	Firth2-ALJ
	227	0.011	Rat-AS
	285	0.011	Rat-AS
Ots-1	260	0.020	Babbage-iso
	206	0.063	Cache-ALJ
	300	0.013	Canoe-ALJ
	246	0.011	Firth1-AS
	180	0.012	Firth2-ALJ
	204	0.012	Firth2-ALJ
	270	0.010	Joe-AS
	272	0.010	Joe-AS
	202	0.024	Joe-res
Sco-19	180	0.060	Babbage-iso
	234	0.013	Canoe-ALJ
	158	0.010	Firth1-AS
	202	0.010	Firth1-AS
	230	0.010	Firth1-AS
	156	0.010	Joe-ALJ
	200	0.010	Joe-ALJ
	204	0.009	Joe-AS
MST-85	124	0.053	Canoe-ALJ
	148	0.035	Firth2-ALJ
	154	0.012	Firth2-ALJ

upstream Cache Creek isolates. This may suggest more recent gene flow between these populations, but the global test over all loci was significant. Due to low sample sizes, comparisons of sympatric anadromous life cycle types were only evaluated in the Firth drainage. In Joe Creek, the anadromous spawners, anadromous large juveniles and resident males collectively had no difference in allele frequencies across the 4 loci. Also, no allelic shifts were detected between Firth-1 anadromous spawners and the upstream Firth-2 anadromous large juveniles. In the allopatric population pairwise comparisons of allele frequencies (Table 2-7), Joe Creek was divergent from Firth-1 and Firth-2 at two and three loci respectively. However, when the samples were partitioned by life type, no allelic pairwise differences were found within the Firth drainage (Table AI-3).

Pairwise comparisons of allelic (F_{ST}) and molecular (Φ_{ST}) variance mirrored the allelic frequency comparison results and were not significantly altered by the exclusion of *MST-85* (Table AI-4B). Note that Weir and Cockerham's (1984) F_{ST} compensates for sample size and, generally, the low sample sizes (due to the high variance) led to nonsignificant statistical tests. The Babbage and Cache Creek isolates were significantly divergent in all their respective pairwise F_{ST} estimates where $n \geq 14$. Also, the magnitude of the significant differences were generally higher than comparisons among anadromous life-types. However, within the Babbage drainage, the Φ_{ST} comparison between Babbage isolates and Canoe anadromous large juveniles were not significantly different. The low sample size of Canoe anadromous spawners prevented their inclusion in this comparison. Within the Firth drainage, all life types from the 3 locations were not significantly different from one another for both F_{ST} and Φ_{ST}. Generally, the anadromous life-types when compared between drainages were divergent from each other. For

Table AI-3. A- 4 loci, and B- 3 loci (*MST-85* removed) pairwise comparison of life types showing the number of loci with significantly different allele frequencies (Fisher's exact test α=0.05). * indicates the significant differences of the pooled *P* values (Fisher's method^{+}).

A.

			BAB	CACHE	CACHE	CACHE	CANOE	CANOE	FIRTH1	FIRTH1
			iso	*iso*	*alj*	*as*	*alj*	*as*	*as*	*res*
		n	26	14	8	42	39	5	49	9
CACHE	*iso*	14	4*							
CACHE	*alj*	8	3*	3*						
CACHE	*as*	42	3*	1*	0					
CANOE	*alj*	39	2*	4*	1*	4*				
CANOE	*as*	5	0	4*	1*	1*	0			
FIRTH1	*as*	49	4*	4*	0*	4*	3*	0		
FIRTH1	*res*	9	4*	4*	1*	1*	1*	0	0	
FIRTH2	*aar*	4	2*	4*	1*	3*	0*	0	0	0
FIRTH2	*alj*	44	2*	4*	1*	3*	2*	0	0	0
FIRTH2	*as*	2	0	3*	0	0*	0	0	0	0
JOE	*aar*	1	0	0*	0	1*	0	0	0	0
JOE	*alj*	49	2*	4*	1*	4*	1*	0	0	0
JOE	*as*	54	3*	4*	1*	3*	3*	0	0*	0
JOE	*res*	21	4*	4*	0*	4*	3*	0	0	0
RAT	*aar*	6	2*	3*	1*	1*	0	0	0	0
RAT	*as*	48	4*	3*	2*	4*	4*	0*	4*	3*

			FIRTH2	FIRTH2	FIRTH2	JOE	JOE	JOE	JOE	RAT
			aar	*alj*	*as*	*aar*	*alj*	*as*	*res*	*aar*
		n	4	44	2	1	49	54	21	6
FIRTH2	*alj*	44	0							
FIRTH2	*as*	2	0	0						
JOE	*aar*	1	0	0	0					
JOE	*alj*	49	0	0*	0	0				
JOE	*as*	54	0	0	0	0	0			
JOE	*res*	21	0	0	0	0	0	0		
RAT	*aar*	6	0*	0	0	0	0	0	0	
RAT	*as*	48	3*	4*	1	1	4*	4*	3*	0

B.

		n	BAB *iso* 26	CACHE *iso* 14	CACHE *alj* 8	CACHE *as* 42	CANOE *alj* 39	CANOE *as* 5	FIRTH1 *as* 49	FIRTH1 *res* 9
CACHE	*iso*	14	3*							
CACHE	*alj*	8	2*	2*						
CACHE	*as*	42	3*	0*	0					
CANOE	*alj*	39	1*	3*	1*	3*				
CANOE	*as*	5	0	3*	1*	1*	0			
FIRTH1	*as*	49	3*	3*	1*	3*	3*	0		
FIRTH1	*res*	9	3*	3*	1*	1*	1*	0	0	
FIRTH2	*aar*	4	1*	3*	1*	2*	0	0	0	0
FIRTH2	*alj*	44	1*	3*	1*	3*	1*	0	0	0
FIRTH2	*as*	2	0	2*	0*	0*	0	0	0	0
JOE	*aar*	1	0	0*	0	0	0	0	0	0
JOE	*alj*	49	1*	3*	0*	3*	1*	0	0	0
JOE	*as*	54	3*	3*	1*	3*	3*	0	0*	0
JOE	*res*	21	3*	3*	0	3*	3*	0	0	0
RAT	*aar*	6	2*	2*	1*	1*	0	0	0	0
RAT	*as*	48	3*	2*	2*	3*	3*	0*	3*	2*

		n	FIRTH2 *aar* 4	FIRTH2 *alj* 44	FIRTH2 *as* 2	JOE *aar* 1	JOE *alj* 49	JOE *as* 54	JOE *res* 21	RAT *aar* 6
FIRTH2	*alj*	44	0							
FIRTH2	*as*	2	0	0						
JOE	*aar*	1	0	0	0					
JOE	*alj*	49	0	0	0	0				
JOE	*as*	54	0	0	0	0	0			
JOE	*res*	21	0	0	0	0	0	0		
RAT	*aar*	6	0	0	0	0	0	0	0	
RAT	*as*	48	2*	3*	1	0	3*	3*	2*	0

$^{+}$ individual locus pairwise comparisons and over all pooled *P*-value corrected by Bonferroni sequential adjustment for multiple tests, initial k=136.

Table AI-4. Life-type pairwise differentiation estimates based on allelic (F_{ST}; below diagonal) and molecular (Φ_{ST}; above diagonal variance analysis. *indicates overall significant difference of pooled *P*-values following adjustment for multiple tests. A- 4 loci, B- 3 loci (*MST-85* removed).

A.

			BAB	**CACHE**	**CACHE**	**CACHE**	**CANOE**	**CANOE**	**FIRTH1**	**FIRTH1**
			iso	**alj**	**as**	**iso**	**alj**	**as**	**as**	**res**
		n	26	8	42	14	39	5	49	9
BAB	*iso*	26		0.155	0.185*	0.444*	0.045	0.145	0.156*	0.558*
CACHE	*alj*	8	0.216*		-0.028	0.090	0.069	0.128	0.156	0.433*
CACHE	*as*	42	0.177*	0.007		0.295*	0.185*	0.242	0.260*	0.577*
CACHE	*iso*	14	0.491*	0.264*	0.285*		0.220*	0.311	0.283*	0.579*
CANOE	*alj*	39	0.079*	0.076	0.116*	0.316*		-0.050	0.048	0.285*
CANOE	*as*	5	0.028	0.113	0.112	0.444*	-0.015		-0.006	0.178
FIRTH1	*as*	49	0.086*	0.087*	0.104*	0.307*	0.038*	0.015		0.072
FIRTH1	*res*	9	0.135*	0.100*	0.116*	0.394*	0.045	0.034	0.004	
FIRTH2	*alj*	44	0.082*	0.076	0.097*	0.308*	0.030*	0.010	0.001	-0.005
FIRTH2	*aar*	4	0.191*	0.171	0.207*	0.449*	0.075	0.074	0.034	0.009
FIRTH2	*as*	2	0.030	0.230*	0.158	0.547*	0.058	-0.008	0.002	0.057
JOE	*alj*	49	0.097*	0.083*	0.118*	0.294*	0.024*	0.017	0.013	0.008
JOE	*aar*	1	0.338	0.411*	0.377	0.628*	0.183	0.240	0.197*	0.206*
JOE	*as*	54	0.101*	0.084*	0.103*	0.302*	0.037*	0.022	0.013	0.021
JOE	*res*	21	0.114*	0.070	0.101*	0.323*	0.043*	0.027	0.008	-0.003
RAT	aar	6	0.137	0.166	0.157*	0.462*	0.055	0.071	0.076	0.092
RAT	as	48	0.087*	0.135*	0.138*	0.392*	0.067*	0.043	0.076*	0.091*

A con't			**FIRTH2**	**FIRTH2**	**FIRTH2**	**JOE**	**JOE**	**JOE**	**JOE**	*RAT*	*RAT*
			alj	**aar**	**as**	**Alj**	**aar**	**as**	**res**	**aar**	**as**
		n	44	4	2	49	1	54	21	6	48
BAB	*iso*	26	0.214*	0.630	0.712	0.207*	0.658*	0.244*	0.323*	0.419*	0.165*
CACHE	*alj*	8	0.212	0.430	0.458*	0.237*	0.442*	0.253*	0.287*	0.380*	0.216*
CACHE	*as*	42	0.309*	0.529*	0.482	0.324*	0.301	0.341*	0.405*	0.397*	0.272*
CACHE	*iso*	14	0.337*	0.567	0.563	0.388*	0.684	0.365*	0.439*	0.571*	0.441*
CANOE	*alj*	39	0.079	0.240	0.194	0.070*	0.039	0.121*	0.155*	0.040	0.072*
CANOE	*as*	5	0.019	0.132	0.097	0.042	0.105*	0.068	0.093	0.068	0.104
FIRTH1	*as*	49	-0.008	0.053	0.037	0.019	-0.013*	0.019	0.028	0.048	0.123*
FIRTH1	*res*	9	0.069	0.107	0.158	0.116	0.139*	0.003	-0.011	0.190	0.399*
FIRTH2	*alj*	44		0.048	0.042	0.037	0.066	0.017	0.029	0.091	0.172*
FIRTH2	*aar*	4	0.032		-0.173	0.225	0.117	0.127	0.139	0.362	0.502
FIRTH2	*as*	2	0.004	0.067		0.246	0.291	0.140	0.165	0.467	0.509
JOE	*alj*	49	0.011	0.037	0.054		0.039*	0.019	0.015	0.001	0.084*
JOE	*aar*	1	0.203	0.198	0.385	0.121*		0.067	0.014	0.220	0.204
JOE	*as*	54	0.007	0.052	0.042	0.011	0.203		-0.009	0.061	0.158*
JOE	*res*	21	0.011	0.032	0.036	0.005	0.137	0.009		0.058	0.193*
RAT	aar	6	0.054	0.160	0.142	0.052	0.309	0.047	0.071		0.002
RAT	as	48	0.063*	0.179	0.082	0.057*	0.253	0.060*	0.084*	-0.013	

B.

			BAB	**CACHE**	**CACHE**	**CACHE**	**CANOE**	**CANOE**	**FIRTH1**	**FIRTH1**
			iso	***alj***	***as***	***iso***	***alj***	***as***	***as***	***res***
		n	26	8	42	14	39	5	49	9
BAB	***iso***	26		0.125	0.186*	0.348*	0.052	0.143	0.161*	0.562*
CACHE	***alj***	8	0.190*		-0.037	0.047	0.080	0.132	0.160	0.437*
CACHE	***as***	42	0.185*	-0.021		0.254*	0.197*	0.244*	0.267*	0.580*
CACHE	***iso***	14	0.370*	0.186	0.101*		0.194*	0.282	0.262*	0.567*
CANOE	***alj***	39	0.053*	0.106*	0.118*	0.284*		-0.052	0.049	0.295*
CANOE	***as***	5	0.021	0.125	0.123*	0.363*	-0.025		-0.011	0.174
FIRTH1	***as***	49	0.078*	0.105*	0.112*	0.244*	0.044*	0.015		0.075
FIRTH1	***res***	9	0.107	0.108*	0.107*	0.310*	0.049	0.020	0.007	
FIRTH2	***alj***	44	0.073*	0.095*	0.106*	0.245*	0.032*	0.008	0.002	-0.005
FIRTH2	***aar***	4	0.104	0.183	0.162*	0.387*	0.064	0.021	0.008	0.001
FIRTH2	***as***	2	0.034	0.218	0.170	0.416*	0.047	-0.006	-0.008	0.036
JOE	***alj***	49	0.061*	0.100*	0.108*	0.253*	0.028*	-0.001	0.013	0.008
JOE	***aar***	1	0.095*	0.340	0.239	0.534*	0.097	0.051	0.126*	0.160*
JOE	***as***	54	0.093*	0.099*	0.109*	0.238*	0.045*	0.024	0.017*	0.022
JOE	***res***	21	0.093*	0.075*	0.094*	0.239*	0.043*	0.014	0.009	0.002
RAT	***aar***	6	0.117	0.173	0.157*	0.368*	0.063	0.069	0.089*	0.095
RAT	***as***	48	0.082*	0.150*	0.150*	0.308*	0.062*	0.041	0.080*	0.091*

B con't			**FIRTH2**	**FIRTH2**	**FIRTH2**	**JOE**	**JOE**	**JOE**	*JOE*	*RAT*	*RAT*
			alj	***aar***	***as***	***alj***	***aar***	***as***	***res***	***aar***	***as***
		n	44	4	2	49	1	54	21	6	48
BAB	***iso***	26	0.221*	0.621	0.713	0.216*	-0.198	0.248*	0.328*	0.419*	0.166*
CACHE	***alj***	8	0.217	0.419	0.461	0.249*	-0.060	0.258*	0.291*	0.379	0.208
CACHE	***as***	42	0.3160*	0.519	0.484	0.336*	-0.026	0.346*	0.409*	0.400*	0.273*
CACHE	***iso***	14	0.320*	0.514	0.542*	0.375*	0.242*	0.356*	0.418*	0.549*	0.409*
CANOE	***alj***	39	0.082	0.235	0.214	0.073*	-0.254	0.127*	0.154*	0.054	0.075*
CANOE	***as***	5	0.015	0.104	0.098	0.036	-0.278*	0.068	0.082	0.068	0.101
FIRTH1	***as***	49	-0.008	0.049	0.043	0.020	-0.126*	0.020	0.027	0.055	0.125*
FIRTH1	***res***	9	0.070	0.111	0.161	0.125	0.102*	0.000	-0.009	0.194	0.396*
FIRTH2	***alj***	44		0.039	0.047	0.038	-0.054	0.018	0.026	0.098	0.175*
FIRTH2	***aar***	4	0.005		-0.203	0.234	0.088	0.121	0.149	0.361	0.491
FIRTH2	***as***	2	-0.005	-0.011		0.264	0.158	0.145	0.171	0.467	0.511
JOE	***alj***	49	0.006	0.027	0.022		-0.096*	0.020	0.014	0.007	0.080*
JOE	***aar***	1	0.124	0.209	0.153	0.052*		-0.032	-0.035	-0.117	-0.206
JOE	***as***	54	0.009	0.029	0.035	0.008	0.104		-0.015	0.065	0.159*
JOE	***res***	21	0.006	0.014	0.021	0.003	0.090	0.005		0.061	0.185*
RAT	***aar***	6	0.066	0.157	0.134	0.048	0.162	0.052	0.074		0.002
RAT	***as***	48	0.067*	0.153*	0.093	0.042*	0.091	0.062*	0.076*	-0.003	

example, the Rat, Cache, and Firth-1 anadromous spawners were all significantly different from each. Also anadromous large juveniles from the Canoe and Joe sites were divergent.

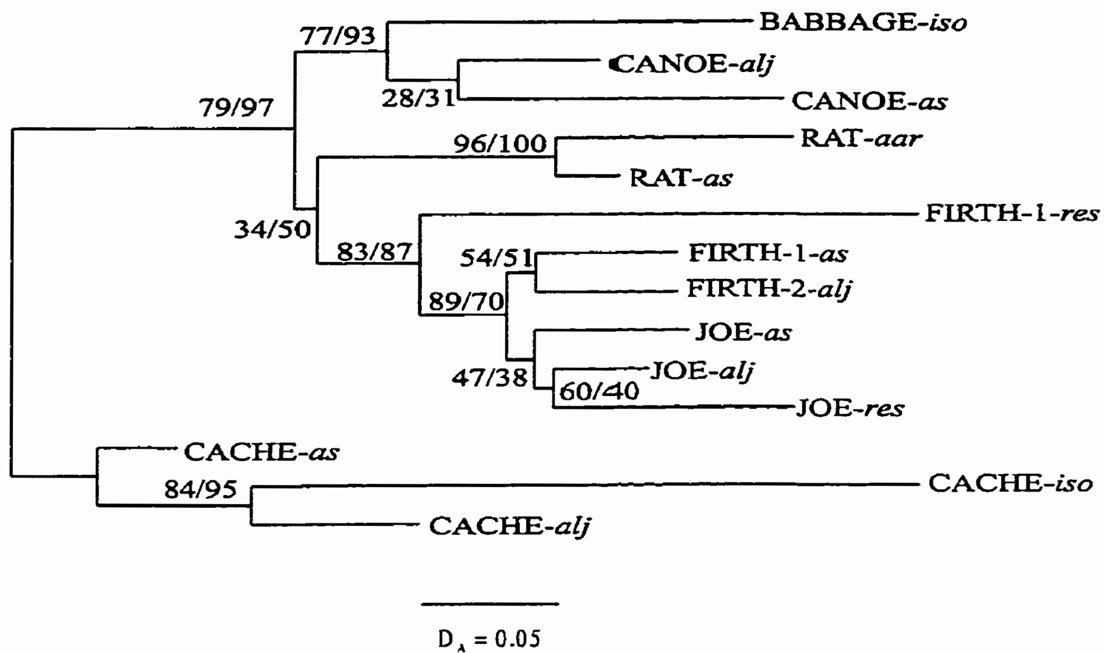
In some cases, F_{ST} was greater than Φ_{ST} but in other cases the opposite was true. Where life type comparisons had few alleles in common, Φ_{ST} was usually higher, for example JOE-*as* versus BAB-*iso* the F_{ST} was 0.101 and Φ_{ST} was 0.244.

The similarity between all life types within the Firth drainage was also evident in the hierarchical analysis of molecular variance (Table 2-11).

Evolutionary relationships between life types are shown in figure AI-1. Samples with as few as five individuals were included in constructing the neighbour-joining phenogram due to geographic clustering by drainage, correspondence with other analysis, and strong associations indicated by bootstrap support. The low sample sizes are prone to sampling bias and demand cautious interpretation. However, the appearance of logical relationships warrants them being reported.

Nei's (1983) D_A distance phenogram showed that life types of all groups clustered by drainage with high bootstrap values (Figure AI-1). Furthermore, most sympatric anadromous lifecycle types grouped together. Whether 3 (removing *MST-85*) or 4 loci were employed in the distance matrix, the phenograms constructed were very similar (Figure AI-1A vs. AI-1B). The only notable difference was the placement of the Rat drainage life types and Canoe/ Babbage life types. However both analyses had weak bootstrap support. Although the two resident life types approximately clustered by site and drainage, they had longer branch lengths than the anadromous life tyes. As with the

A. 4 microsatellite loci



B. 3 microsatellite loci

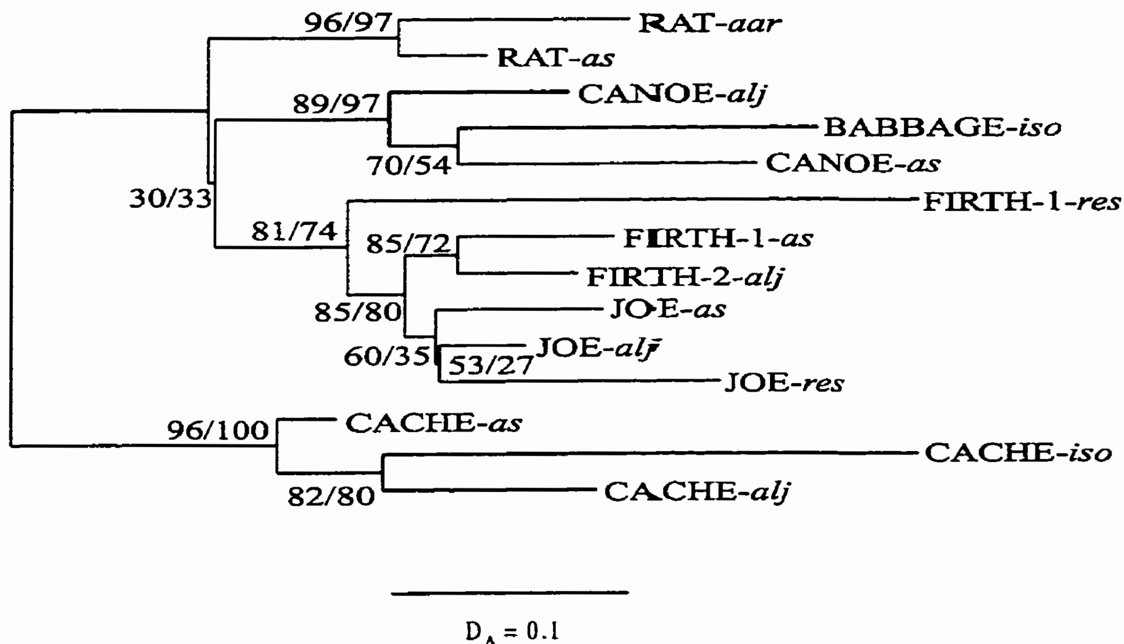


Figure AI-1. Nei's (1983) D_A distance neighbour-joining phenogram showing relationships of charr life types and location. Bootstrapping by loci and by individual (10 000 replicates). A shows the phenogram based upon all 4 microsatellite loci. B. shows the phenogram based upon just 3 loci (*MST-85* removed).

population defined by location phenogram, (Figure 2-7) Cache Creek was the most divergent.

DISCUSSION

The genetic similarity of anadromous large juveniles, residents and spawners in the Firth drainage indicates that they all derive from the same reproducing population. However, the inability to discriminate between allopatric and sympatric samples within the Firth drainage may also indicate that insufficient resolution was provided by the genetic markers.

Anadromous large juveniles from the Firth drainage were genetically different from all life types in other drainages, but were not divergent from spawners within the Firth drainage. Therefore, the majority of non-reproductives in the Firth drainage must represent individuals which have returned to their natal drainages to overwinter. Although DeCicco (1997) observed non-reproducing fish overwintering in non-natal drainages, these populations occurred southwest of the Yukon north slope. Perhaps it is the shortage of available overwintering habitat (estimated at 5% of summer riverine habitat (Craig 1989)) that prevents extensive wandering between drainages in the Yukon north slope. Wandering fish would likely have a disadvantage in finding suitable overwintering habitat compared to fish returning to their natal streams. Unequal sample sizes of sympatric anadromous life types in other drainages prevents broadening these conclusions to the entire Yukon north slope. Nonetheless, the hypothesis that smolts follow ocean currents which carry high prey densities and overwinter in distant drainages is not supported by these data.

Among all life types, the isolated populations exhibited the highest divergence. However, these isolated populations were most closely related to the downstream

anadromous life types within the same drainage. This indicates that despite waterfall barriers, isolated populations were structured by drainage.

Male residents were indistinguishable from their sympatric anadromous counterparts. Furthermore, their allelic diversity and heterozygosities were also comparable suggesting that resident males possess a significant proportion of the overall genetic diversity within the population. Therefore no correlation between life history and fitness can be drawn. Male residents may represent an important source of genetic diversity if anadromous populations come under heavy exploitation. However, if the resident life history is genetically determined, residents will contribute less to the genetic constitution of anadromous life types.

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APPENDIX II

Use of distance measures for inferring relationships with microsatellite data.

Uncertainties regarding the evolution of microsatellite loci persist (Estoup and Angers 1998) and as a result three distance measures were employed to investigate the relationships among populations of *S. malma* on the Yukon north slope: Cavalli-Sforza and Edward's (1967) chord distance (D_C), Goldstein *et al.*'s (1995) $(\delta\mu)^2$ distance, and Nei *et al.*'s (1983) D_A distance.

Population relationships

The three distance measures D_C, $(\delta\mu^2)$, and D_A showed considerable variation in population relationships when four microsatellite loci were used to construct neighbour joining phenograms (Figure AII-1A, AII-2A, Figure 2-7A). However, all three measures gave strong bootstrap support (92 to 100%) to the clustering of the 3 populations within the Firth drainage. The other nodes were generally supported by much lower bootstrap values although all 3 trees showed the Cache Creek populations as most divergent. The D_C tree was anomalous in showing little patterning by drainage. With the exception of the Firth drainage already noted above, the D_C tree strongly grouped the Firth, Babbage, and Rat drainages together. $(\delta\mu)^2$ did not group the Canoe and Babbage within the Babbage drainage as seen in the other 2 trees, but it gave strong support to the relationship of the Cache Creek populations. The branch lengths, particularly in the $(\delta\mu)^2$ tree, showed very low divergences among the Firth drainage populations with much larger differences among the other populations.

When the locus *MST-85* was removed from the analysis (for reasons as outlined in Chapter 2), bootstrap support became much stronger in virtually all cases (Figures AII-1B, AII 2B, Figure 2-7B). Also, the branch order of the D_C tree changed under the 3 locus analysis becoming identical to that of the D_A tree. The $(\delta\mu)^2$ tree changed the least

when *MST-85* was removed which most likely reflects the few dominant alleles observed at that locus being shared or close in size among all populations. The fact that the inclusion of the *MST-85* locus weakened bootstrap support and changed branch order suggests that null alleles at this locus masked true divergences. Because of the higher bootstrap support, congruence between the D_A and D_C trees, and the correlation of relationships and geographic distribution, the trees constructed from the 3 loci are most likely better estimates of true relationships than trees derived from 4 loci.

Although the $(\delta\mu)^2$ distance incorporates the stepwise mutation model (SMM) which microsatellites are widely thought to obey (e.g. Estoup and Angers 1998), it may be an inappropriate measure when either a recent bottleneck or founder event has occurred. Many of the alleles from the ancestral metapopulation are lost at random (i.e. regardless of allele size) and if insufficient time has passed for mutations to occur within the newly derived populations then the relationship between allele size and relatedness becomes misleading. Hence the weak support achieved by the $(\delta\mu)^2$ phenogram is, in itself, informative. Examining the allele frequency distributions at the 4 microsatellite loci (Figure 2-6) shows a disjunct distribution for the 3 easterly drainages (Babbage/ Canoe, Cache, and Rat). The allele frequency distribution in the Firth drainage is more diverse and relatively continuous in comparison. Thus, the north slope, and particularly the 3 easterly drainages, likely experienced either a bottleneck or founder event due to the last glaciation.

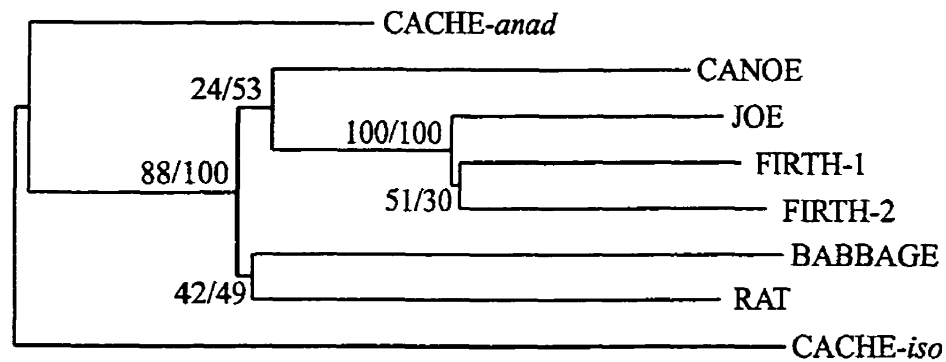
The three phenograms constructed without *MST-85* generally grouped populations by drainage lending support to these relationships. Between the D_C and D_A trees, Nei's *et al.* (1983) D_A phenogram corresponded best to geographic expectations (whether 3 or 4

loci were employed), although with weak support in some cases. Furthermore, as it is thought that the D_A distance algorithm is a better estimator of branch lengths for closely related populations than D_C (Takezaki and Nei 1996), it was deduced that the D_A phenogram was most suitable for this study.

Life type phenograms

The 3 distance measures showed diverse tree topologies when data were partitioned by life type (Figures AII-3, AII-4 and AI-1). Generally, life types grouped by population, while populations grouped by drainage. See Appendix I for details on population regrouping by life type and for interpretation of the relationships. The D_C and $(\delta\mu)^2$ trees are included here for comparison to the D_A phenogram.

A. 4 microsatellite loci



B. 3 microsatellite loci

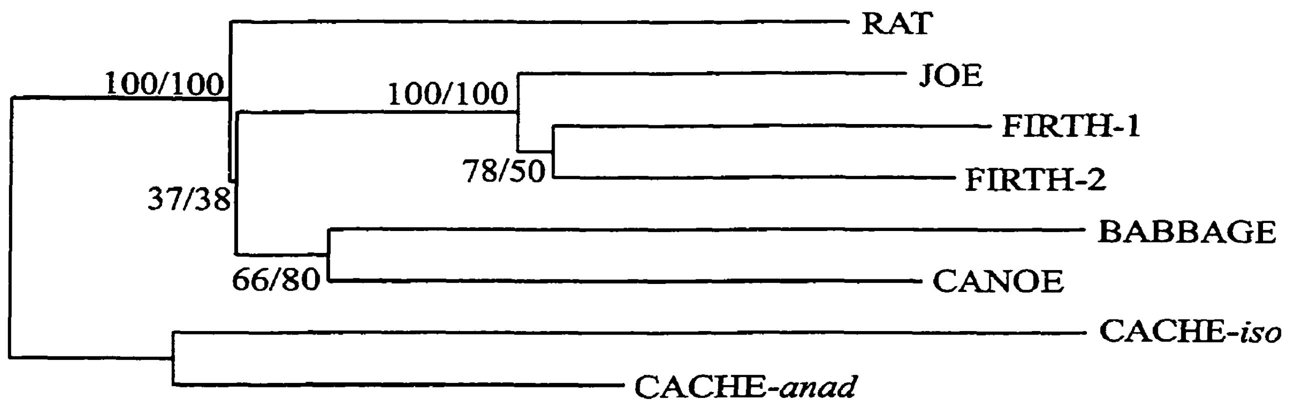
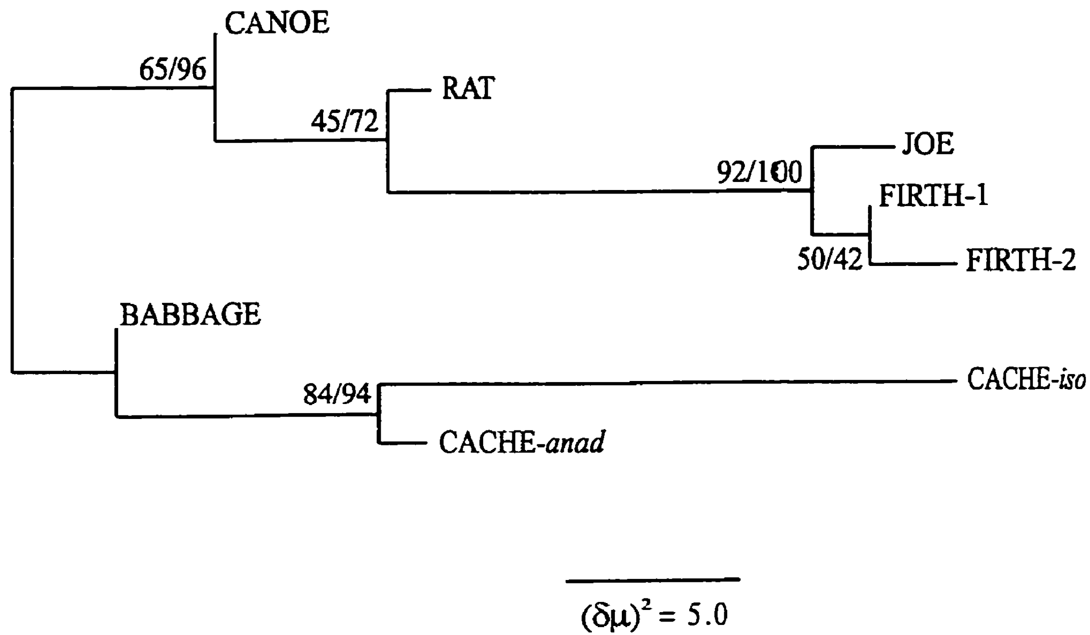


Figure AII-1. Cavalli-Sforza and Edward's (1967) chord distance neighbour-joining phenogram showing relationships of *S. malma* populations. Bootstrapping by loci and by individual (10 000 replicates).

A. 4 microsatellite loci



B. 3 microsatellite loci

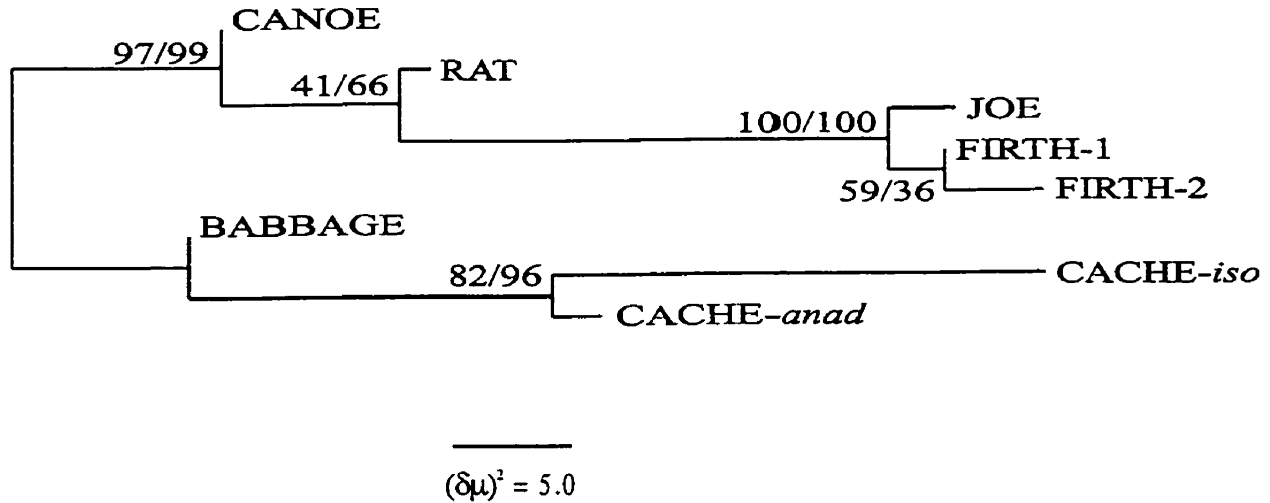
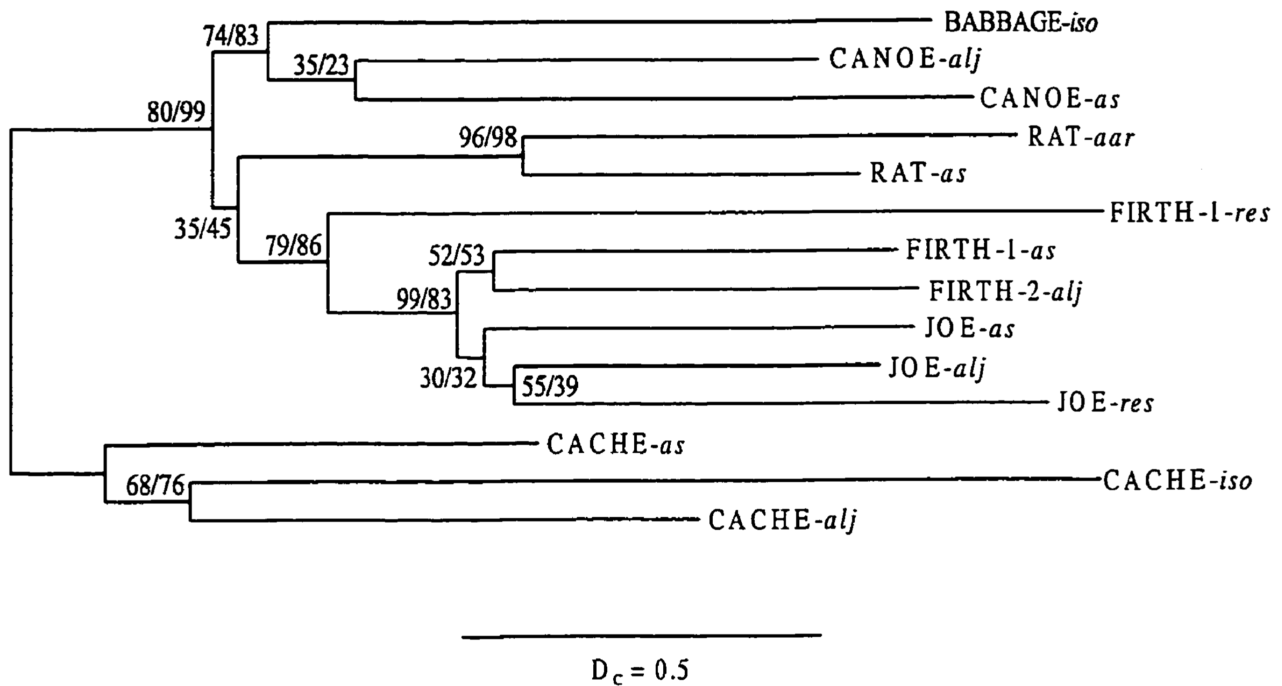
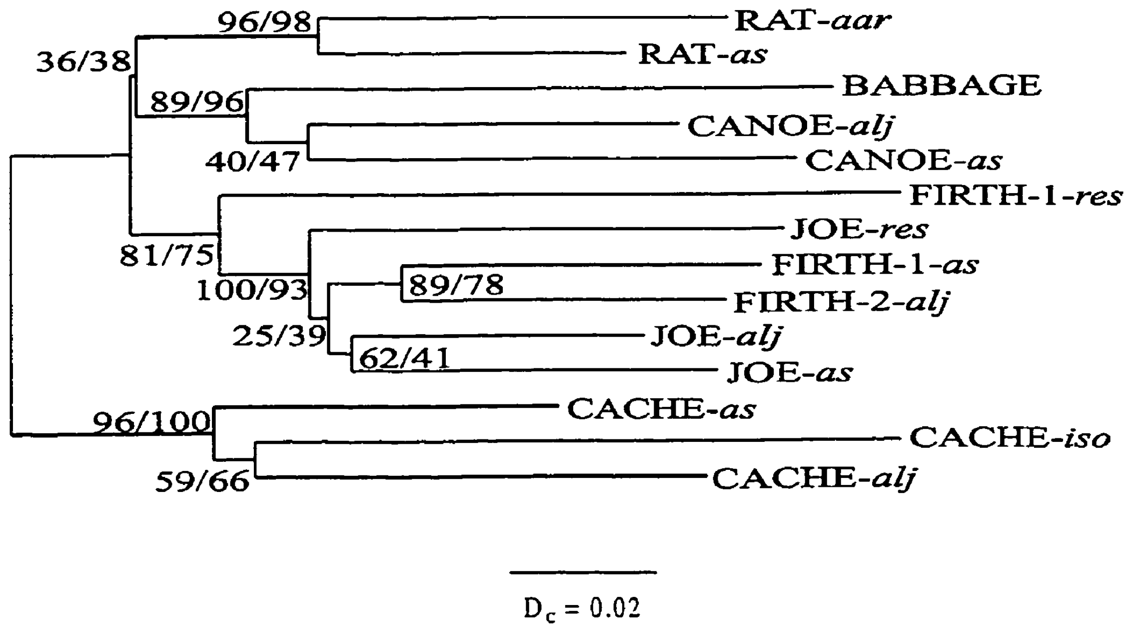


Figure AII-2. Goldstein *et al.* (1995) $(\delta\mu)^2$ distance neighbour-joining phenogram showing relationships of *S. malma*. Bootstrapping by loci and by individual (10 000 replicates).

A. 4 microsatellite loci



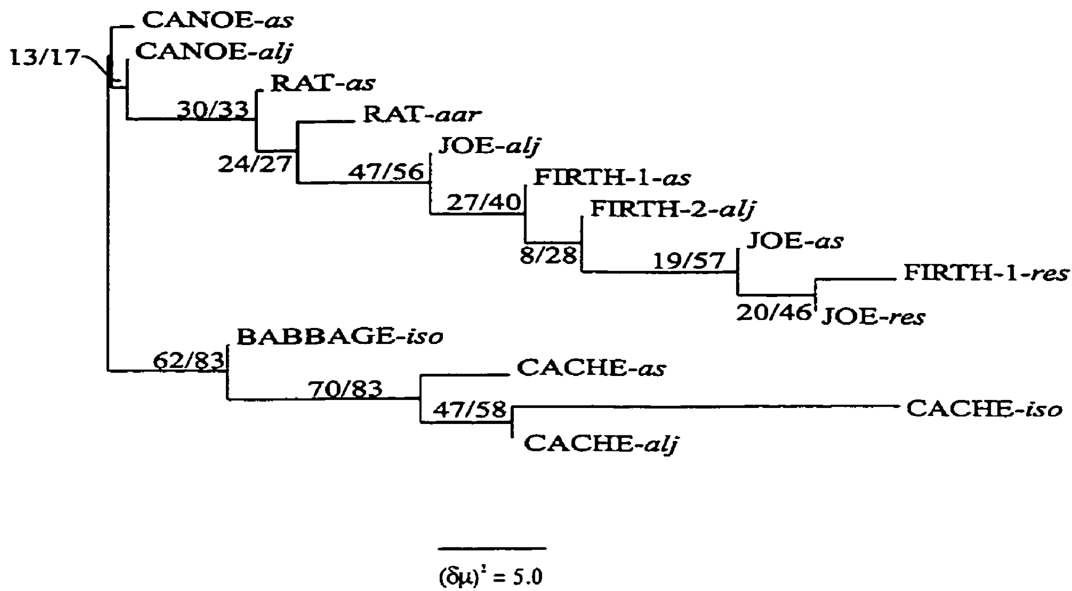
B. 3 microsatellite loci



$D_c = 0.02$

Figure AII-3. Cavalli-Sforza and Edward's (1967) chord distance neighbour-joining phenogram showing relationships of *S. malma* by life type and location. Bootstrapping by loci and by individual indicated (10 000 replicates). A – includes 4 microsatellites and B – 3 microsatellites (*MST-85* removed).

A. 4 microsatellite loci



B. 3 microsatellite loci

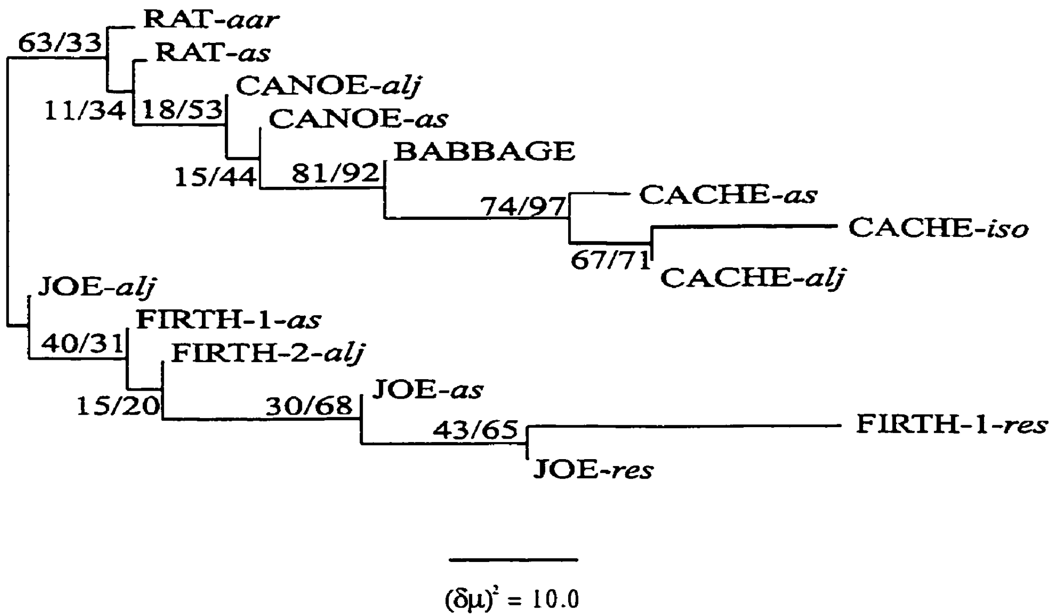


Figure AII-4. Goldstein *et al.*, (1995) $(\delta\mu)^2$ distance neighbour-joining phenogram showing relationships of *S. malma* by life types and location. Bootstrapping by loci and by individual (10 000 replicates).

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APPENDIX III. Maximum likelihood population reassignments by jack-knifing individual multi-locus genotypes to the baseline population allele frequencies calculated from 4 microsatellite loci and D-loop haplotypes. Populations grouped by drainage (thin horizontal line). In brackets, the number of individuals with < 1.0% chance of mis-assignment. (A- all populations, B- Firth drainage pooled.)

A.

		Population Reassignment							
	N	Cache *iso*	Cache	Babbage *iso*	Canoe	Firth-1	Firth-2	Joe	Rat
Cache-*iso*	14	14(13) 100.00%	0	0	0	0	0	0	0
Cache	50	8 16.00%	40(15) 80.00%	0	0	1 2.00%	0	0	1 2.00%
Babbage-*iso*	25	0	0	24(13) 96.00%	0	0	0	0	1 4.00%
Canoe	44	1 2.27%	0	2 4.55%	34(9) 77.27%	2 4.55%	2 4.55%	2 4.55%	1 2.27%
Firth-1	58	6 10.34%	0	6 10.34%	6 10.34%	20(0) 34.48%	11 18.97%	7 12.07%	2 3.45%
Firth-2	50	2 4.00%	1 2.00%	9 18.00%	6 12.00%	14 28.00%	12(0) 24.00%	4 8.00%	2 4.00%
Joe	125	12(2) 9.60%	5 4.00%	18 14.40%	15 12.00%	15 12.00%	14 11.20%	33(0) 26.40%	13 10.32%
Rat	54	0	1 1.85%	6 11.11%	1 1.85%	0	0	1 1.85%	45(16) 83.33%

B.

	N	Cache *iso*	Cache	Babbage *iso*	Canoe	Firth *pooled*	Rat
Cache-*iso*	14	14(13) 100.00%	0	0	0	0	0
Cache	50	8 16.00%	40(15) 80.00%	0	0	0	2 4.00%
Babbage-*iso*	25	0	0	24(13) 96.00%	0	0	1 4.00%
Canoe	44	1 2.27%	0	3 6.82%	35(10) 79.55%	4 9.09%	1 2.27%
Firth-*pooled*	233	26(1) 11.16%	6 2.58%	42(1) 18.03%	36 15.45%	100(7) 42.92%	23 9.87%
Rat	54	0	1 1.85%	6 11.11%	1 1.85%	0	46(16) 85.19%

APPENDIX IV. Absolute number (upper) and relative frequency (lower) of D-loop haplotypes grouped by population and life history type (see table 2-1 for life history abbreviations). Boxed regions indicate the location of unique haplotypes (private alleles). ALL- all life history types pooled where more than one type occurs.

POPULATION	LIFE TYPE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
		HAPLOTYPE																			
BABBAGE-iso	iso	5	1	5	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0.36	0.07	0.36	0.14	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CACHE-iso	iso	0	0	0	0	2	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0.14	0.86	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CACHE	alj	0	0	0	0	0	6	0	0	0	0	0	2	0	0	0	0	0	0	0	0
		0	0	0	0	0	0.75	0	0	0	0	0	0.25	0	0	0	0	0	0	0	0
	as	0	0	0	0	0	23	0	0	0	0	0	13	0	0	0	0	0	0	0	0
		0	0	0	0	0	0.64	0	0	0	0	0	0.36	0	0	0	0	0	0	0	0
	ALL	0	0	0	0	0	29	0	0	0	0	0	15	0	0	0	0	0	0	0	0
		0	0	0	0	0	0.66	0	0	0	0	0	0.34	0	0	0	0	0	0	0	0
CANOE	alj	0	1	1	0	0	2	0	0	1	30	0	0	0	0	0	0	0	1	0	0
		0	0.03	0.03	0	0	0.06	0	0	0.03	0.83	0	0	0	0	0	0	0	0.03	0	0
	as	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	ALL	0	1	1	0	0	2	0	0	1	35	0	0	0	0	0	0	0	1	0	0
		0	0.02	0.02	0	0	0.05	0	0	0.02	0.85	0	0	0	0	0	0	0	0.02	0	0
FIRTH-1	as	0	0	1	0	0	3	0	0	0	26	0	1	1	0	0	2	0	11	0	0
		0	0	0.02	0	0	0.07	0	0	0	0.58	0	0.02	0.02	0	0	0.04	0	0.24	0	0
	res	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	3	0	0	0	1
		0	0	0	0	0	0.14	0	0	0	0.29	0	0	0	0	0	0.43	0	0	0	0.14
	ALL	0	0	1	0	0	4	0	0	0	28	0	1	1	0	0	5	0	11	0	1
		0	0	0.02	0	0	0.08	0	0	0	0.55	0	0.02	0.02	0	0	0.1	0	0.22	0	0.02
FIRTH-2	aar	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0
		0	0	0	0	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0.67	0	0
	alj	0	0	0	2	0	0	1	0	0	17	0	3	0	1	0	6	0	3	0	0
		0	0	0	0.06	0	0	0.03	0	0	0.52	0	0.09	0	0.03	0	0.18	0	0.09	0	0
	as	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
		0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0.5	0	0
	ALL	0	0	0	2	0	0	1	0	0	19	0	3	0	1	0	6	0	6	0	0
		0	0	0	0.05	0	0	0.03	0	0	0.5	0	0.08	0	0.03	0	0.16	0	0.16	0	0

POPULATION	LIFE TYPE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
JOE	?	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	aar	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	alj	0	0	1	0	0	5	0	0	0	27	1	1	0	0	0	2	1	6	1	0
		0	0	0.02	0	0	0.11	0	0	0	0.6	0.02	0.02	0	0	0	0.04	0.02	0.13	0.02	0
	as	0	0	0	0	2	1	0	0	1	25	1	1	0	6	2	2	0	4	2	1
		0	0	0	0	0.04	0.02	0	0	0.02	0.52	0.02	0.02	0	0.13	0.04	0.04	0	0.08	0.04	0.02
	res	0	0	1	0	0	0	0	0	0	10	0	0	0	0	0	1	0	4	0	0
		0	0	0.06	0	0	0	0	0	0	0.63	0	0	0	0	0	0.06	0	0.25	0	0
	ALL	0	0	2	0	2	6	0	0	1	64	2	2	0	6	2	5	1	14	3	1
		0	0	0.02	0	0.02	0.05	0	0	0.01	0.58	0.02	0.02	0	0.05	0.02	0.05	0.01	0.13	0.03	0.01
RAT	aar	0	0	1	0	0	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0.17	0	0	0.67	0	0.17	0	0	0	0	0	0	0	0	0	0	0	0
	as	0	0	2	1	1	27	0	4	0	12	0	0	0	0	0	0	0	0	0	0
		0	0	0.04	0.02	0.02	0.57	0	0.09	0	0.26	0	0	0	0	0	0	0	0	0	0
	ALL	0	0	3	1	1	31	0	5	0	12	0	0	0	0	0	0	0	0	0	0
		0	0	0.06	0.02	0.02	0.58	0	0.09	0	0.23	0	0	0	0	0	0	0	0	0	0
UNKNOWN	?	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
ALL SAMPLES		5	2	12	5	5	85	1	5	2	160	2	21	1	7	2	16	1	32	3	2
		0.01	0.01	0.03	0.01	0.01	0.23	0	0.01	0.01	0.43	0.01	0.06	0	0.02	0.01	0.04	0	0.09	0.01	0.01