Evaluation of population structure in Pacific *Lepeophtheirus salmonis* (Krøyer) using polymorphic single nucleotide and microsatellite genetic markers: evidence for high gene flow among host species and habitats

by

Amber Marie Messmer
BSc., Thompson Rivers University, 2007

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Supervisory Committee

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Abstract

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Parasitic copepods including *Lepeophtheirus salmonis* have been the focus of strong concern for the health of wild and farmed salmonids in the Pacific and Atlantic Oceans. Salmon are highly valuable species from both socioeconomic and ecological perspectives. The host-parasite dynamics of *Lepeophtheirus salmonis* and the Atlantic and Pacific salmonids have changed over evolutionary time to the point that both Atlantic and Pacific salmon and Atlantic and Pacific *Lepeophtheirus salmonis* are genetically distinct. Recent human interference with the natural population dynamics of this parasite and its hosts may have altered the population genetic structure of *Lepeophtheirus salmonis,* particularly because salmon farms may provide more stable conditions for parasite population growth. High abundance of *Lepeophtheirus salmonis* on salmon farms causes damage to the farmed salmon and leads to increased infection intensities in nearby wild hosts. Some Atlantic *Lepeophtheirus salmonis* have developed resistance to the anti-parasitic drugs they are repeatedly exposed to. No drug resistance has yet been detected within the Pacific Ocean, where only one drug is available, and heavily relied on, to treat *Lepeophtheirus salmonis* infections. Control of *Lepeophtheirus salmonis* abundance on Pacific salmon farms is important to maintain the health of farmed salmon and is also important to protect wild salmonids from increased infections originating from salmon farms.

The goal of this thesis was to characterize and employ a large suite of molecular markers to assess the population structure of *Lepeophtheirus salmonis* in the Pacific Ocean. Until this point, the primary focus of *Lepeophtheirus salmonis* population

genetics research has been limited to the Atlantic Ocean and has relied on a small number of available molecular markers. Available expressed sequence tag DNA libraries were screened to identify putative polymorphic loci, which were then experimentally evaluated. We characterized 22 novel microsatellite loci and 87 single nucleotide polymorphisms within 25 nuclear loci for *Lepeophtheirus salmonis.* We used these genetic markers, as well as 5 microsatellite loci previously developed for use in Atlantic *Lepeophtheirus salmonis* population studies, to genotype 562 *Lepeophtheirus salmonis* that were collected from12 Pacific Ocean sampling locations. We compared *Lepeophtheirus salmonis* genotypes among: (1) seven wild host populations and five farmed host populations within the Pacific Ocean; (2) geographically separated wild host populations, ranging from the Bering Sea to the southwest end of Vancouver Island, British Columbia; and (3) temporally separated cohorts of farmed Atlantic salmon from two geographically distant farm locations on the northwest coast of Vancouver Island and the Campbell River area east of central Vancouver Island. Our analyses failed to resolve significant population structure among sampled Pacific *Lepeophtheirus salmonis* and, therefore, supports a hypothesis of high gene flow throughout the Northeast Pacific Ocean.

It is important to understand the biology and population dynamics of *Lepeophtheirus salmonis* because it is a consequential parasite of wild and farmed salmonids in the Pacific Ocean. Both the molecular tools developed for this study and the population genetics information generated from this study have contributed to our overall understanding of the evolutionary history and population dynamics of *Lepeophtheirus salmonis.*

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Dedication

For my family.

Chapter 1:
Biology and population dynamics of *Lepeophtheirus salmonis*

The goal of this thesis was to characterize and employ a large suite of molecular markers in order to assess the population structure of the sea louse, *Lepeophtheirus salmonis*, in the Pacific Ocean. We accomplished this goal by comparing *L. salmonis* genotypes among: (1) seven wild host populations and five farmed host populations within the Pacific Ocean; (2) geographically separated wild host populations, ranging from the Bering Sea to the southwest end of Vancouver Island, British Columbia; and (3) temporally separated cohorts of farmed Atlantic salmon from two geographically distant farm locations on the northwest coast of Vancouver Island and the Campbell River area east of central Vancouver Island.

1.1 Introduction: the importance of salmon and sea lice

1.1.1 Socioeconomic importance of salmon in British Columbia

Salmon are important to many parts of life for people in British Columbia and Canada. Salmon provide economic benefits to BC through commercial and recreational fishing, tourism, and aquaculture. First Nations have legally-recognized rights to access salmon for food, and for social and ceremonial purposes (DFO 2013). In addition to their socioeconomic importance, Pacific salmon are critically important as members of marine and terrestrial ecosystems (e.g., Willson and Halupka 1995, Cederholm et al. 1999).

Many coastal communities and First Nations depend on salmon-related industries for employment income. For example, aquaculture provides approximately 4,550 full time jobs and $150 million CAD in labour income to the Comox-Strathcona Census Division

in BC, and the Kitasoo First Nation operates an aquaculture facility and processing plant which employs 45 band members and generates 1.5 million CAD per year (DFO 2010). The commercial salmon fishing fleet in BC provides seasonal employment of 420 "person-years," with individuals earning an annual average income of $21,100 in 2009 (Gislason 2011). In addition, commercial fisheries support an estimated 1,100 processing plant employment positions (DFO 2013). Recreational fishing is estimated to generate $689 million CAD (2010) in fishing-related consumerism in BC, with $140 million CAD of that total brought in from outside the province (DFO 2013).

Salmon aquaculture in BC produces a larger volume of fish, and generates more income than Commercial fisheries in BC. Between 2009 and 2011, the commercial salmon fishery in BC harvested an annual average of 20,800 tonnes of wild salmon, with a corresponding average landed value of $46.8 million CAD and wholesale value of $197.6 million CAD (British Columbia Seafood Industry 2011). The average annual production of farmed salmon in BC over the same period was 79,400 tonnes, with an average gate value of $443 million CAD and an average wholesale value of $535 million CAD (British Columbia Seafood Industry 2011). In contrast to commercial fisheries, which are dependent on the abundance of wild salmon, the annual salmon production in aquaculture facilities has much more temporal and economic stability (DFO 2010).

1.1.2 Ecological importance of salmon in British Columbia

Pacific salmon have diverse and important trophic relationships in both marine and terrestrial ecosystems. Salmon are prey to ecologically important predators including whales, pinnipeds, fish, birds, bears, and wolves (Willson and Halupka 1995, Hobson et al. 1997, Gende and Willson 2001, Darimont et al. 2008). Adult salmon carcasses are an

important food source for scavengers, including birds and insects, as well as an important source of nutrients for vegetative growth within riparian ecosystems (Cederholm et al. 1999, Gende and Willson 2001, Drake and Naiman 2007, Hocking et al. 2009). Pacific salmon use 1,300 − 1,500 fresh water bodies in BC and the Yukon, and 75% of these salmon pass through either the Skeena, Nass, or Fraser river systems, with some salmon, (sockeye and chinook) travelling as far as 1,500 km inland to spawn (DFO 2013).

Atlantic salmon aquaculture in BC began in the 1980s and has grown to out-produce the BC wild salmon fishery (FAO 2010). Salmonid aquaculture generates considerable socioeconomic benefit, produces a stable food source for the growing human population (FAO 2010) and reduces fishing pressure on wild fish stocks (British Columbia Seafood Industry 2011). Wild fisheries production will not be able to meet the demands of the continually expanding human population (FAO 2010). Current global fishing intensity has been linked to the decline of both commercially exploited and unexploited species (Hutchings and Reynolds 2004, Worm et al. 2006). Aquaculture can reduce fishing pressure, which may help wild fish populations recover from over-exploitation. However, salmonid aquaculture has been linked to declining health of marine ecosystems and in BC has been linked to population declines of Pacific salmonids, primarily through the transmission of disease, including parasitic copepods (Morton et al. 2004, Krkošek et al. 2006, Ashander et al. 2012). A major management concern of aquaculture in BC is the effective control of disease in farmed salmon in order to protect the health of both wild and farmed fish (Peacock et al. 2013). Understanding infection dynamics among farmed and wild salmonids is important for effective disease control (Ashander et al. 2012, Rogers et al. 2013) . Continual improvement of aquaculture management is required in

order to produce fish as a sustainable and healthy food source, as well as to reduce any potential negative impacts that aquaculture facilities have on the surrounding ecosystem.

1.2 Sea lice background

1.2.1 What are sea lice?

The term "sea louse" generally refers to various species of ectoparasitic copepods that infect marine fishes, including salmonids. The suborder Siphonostomatoida contains 75% of all species of parasitic copepods and the family Caligidae is one of 11 families found in Canada that belong to this suborder (Kabata 1988). Most members of the Caligidae are ectoparasites of marine fishes (Kabata 1988). Caligidae includes two main genera ectoparasitic copepods that infect marine salmonids: *Caligus* (approximately 200 species worldwide) and *Lepeophtheirus* (approximately 100 species worldwide). Large-scale geographic barriers separate the three most commonly reported species of *Caligus* that parasitize salmonid hosts: *Caligus elongatus* (Nordmann 1832) is found in the North Atlantic Ocean; *Caligus clemensi* (Parker and Margolis 1964) is found in the North Pacific Ocean; and *Caligus rogercresseyi* (Boxshall and Bravo 2000) is found in the South Pacific Ocean, near Chile. The geographic barriers that isolate the species of *Caligus* spp., which parasitize salmonids, do not exist to the same extent for *Lepeophtheirus*. *Lepeophtheirus salmonis* (Krøyer 1837) is found in both the North Pacific and Atlantic Oceans and is the only species known within this genus to parasitize marine salmonids at high frequency (Kabata 1988, Johnson and Albright 1991a). The species of *Caligus* that commonly parasitize salmonids infect other taxonomically diverse host fishes (but see e.g., Øines and Heuch 2005, Øines and Schram 2008), and may, therefore, be regarded more as generalist parasites than *L. salmonis* which, with few

exceptions (e.g., Jones et al. 2006), seem to exclusively parasitize salmon and their relatives (Wootten and Smith 1982).

1.2.2 Why focus on Lepeophtheirus salmonis?

Sea lice are of major global management concern for salmonid fisheries and aquaculture, as illustrated by estimates of global economic loss related to sea lice, on salmon farms of more than 430 million USD per year (Costello 2009). *Lepeophtheirus salmonis* is often the focus of research into host-parasite interactions concerning salmon because this parasite is generally reported as the more serious threat to farmed and wild salmon across the Northern hemisphere (Costello 2006, 2009). The important salmonid hosts for *L. salmonis* and *Caligus* sp. within the Atlantic Ocean include native Atlantic salmon, *Salmo salar* (L 1758), and sea trout, *Salmo trutta* (L 1758), as well as Atlantic salmon and rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792), reared in open net-pen aquaculture facilities (a.k.a. salmon farms). In the North Pacific Ocean, the main host species of *L. salmonis* and *Caligus* species include the native pink, *Oncorhynchus gorbuscha* (Walbaum 1792); chum, *O. keta* (Walbaum 1792); coho, *O. kisutch* (Walbaum 1792); sockeye, *O. nerka* (Walbaum 1792); chinook, *O. tshawytcha* (Walbaum 1792); masu salmon, *O. masou* (Brevoort 1856); and cutthroat trout, *O. clarki* (Richardson 1836). In addition, Atlantic salmon and some Pacific salmon are produced in a growing number of salmon farms in the Pacific Ocean. Salmonids are not naturally found in the southern hemisphere, however, multiple introductions have been described (Valiente et al. 2010, Riva Rossi et al. 2012). Interestingly, a sea louse native to Chilean waters, therefore evolutionarily naïve to salmonid hosts, has become a problematic parasite on

Atlantic salmon and rainbow trout farms but does not seem to infect farmed coho in Chile (Bravo 2003).

1.2.3 What are the life stages of *Lepeophtheirus salmonis*?

Lepeophtheirus salmonis development progresses through three free-swimming stages followed by five parasitic stages, each separated by a molting event (Johnson and Albright 1991b, Hamre et al. 2013). The first and second nauplius are non-feeding planktonic stages, the copepodid is the first infective stage, the first and second chalimus are attached parasitic stages, and the first and second pre-adult and final adult stages are motile parasitic stages (Johnson and Albright 1991b, Hamre et al. 2013). From the time of initial infection, a frontal filament structure cements copepodid and chalimus stages to their hosts; the frontal filament is lost in pre-adult and adult stages but temporarily re-appears during molting events (Ritchie et al. 1996). The motile pre-adult and adult stages usually congregate on particular regions of their hosts, likely motivated by increased access to mates, ease of feeding, or exposure avoidance (Ritchie et al. 1996, Todd et al. 2000).

Successful development of *L. salmonis* is highly influenced by salinity and sea water temperature (Johnson and Albright 1991b); total development time from egg to adult at 10 °C requires an average of 40 days for male lice and 52 days for female lice (Boxaspen and Naess 2000). Pelagic larval duration (PLD; first nauplius to copepodid) has important implications for the dispersal ability and population dynamics of *L. salmonis* (Selkoe and Toonen 2011). PLD is approximately 1.9 days at 15 °C and increases to 9.3 days at 5 °C (Johnson and Albright 1991b). Limited developmental success is possible in some *L. salmonis* at 2 °C with an increased PLD of 23.4 days (Boxaspen and Naess 2000).

1.2.4 What damage is caused by *Lepeophtheirus salmonis*?

The parasitic stages of *L. salmonis* feed on the surface tissues of their hosts, including mucus, epidermis and blood (Brandal et al. 1976). Adult female *L. salmonis* tend to feed on host blood, and other parasitic stages of both sexes tend to feed on skin and mucus (Fast 2013). The degree of damage done to each host is highly dependent on the susceptibility of the host species, the intensity of *L. salmonis* infection and the health of individual host fish (Fast 2013). Parasitic feeding may cause several types of direct damage to host fish, which generally include elevated physiological stress responses, osmotic imbalance and immune system impairment (Fast 2013) . *Lepeophtheirus salmonis* infection can also interfere with swimming ability, particularly in juvenile fish (Wagner et al. 2003, Nendick et al. 2011).

Accounts of *L. salmonis* feeding damage have come from field and salmon farm observations as well as from experimental exposure studies. The observations of Johnson et al. (1996) describe a sea louse epidemic in mature sockeye during their return to coastal waters in September of 1990. These sockeye carried an average of 300 lice (*L. salmonis* and *C. clemensi*) per sampled fish; tissue damage with exposed muscle was reported in 87% of fish and other damage included shallow lesions, abrasions, and missing scales (Johnson et al. 1996). Similar damage, including skin lesions, osmoregulation failure, and 100% mortality, were observed in a 34 day experimental *L. salmonis* infection including ≥ 30 lice per 40 g (post smolt) Atlantic salmon (Grimnes and Jakobsen 1996). The most severe parasite damage was associated with increased feeding activity and aggregation behaviour of motile *L. salmonis* stages, with lice concentrated on the head, dorsal and post-anal region of the salmon (Grimnes and Jakobsen 1996, Ritchie et al. 1996). A similar experimental exposure of very small pink

salmon to 50-100 *L. salmonis* copepodids resulted in 37% mortality in 0.3 g fish (approximate size at marine entry), but the mortality rate decreased to 5% in 0.7 g fish and at the size of 2.4 g no mortality was observed (Jones et al. 2008). Gene expression profiles of the 0.7 g pink salmon have given evidence that an inflammatory immune response is involved in an effective defense against *L. salmonis* infection (Sutherland et al. 2011).

The degree of tissue inflammation associated with *L. salmonis* infection varies considerably by host species (summarized by Fast 2013). In Atlantic salmon, mild to no inflammation is observed from tissue damage (Jones et al. 1990, Johnson and Albright 1992a, Jonsdottir et al. 1992, Nolan et al. 1999)**.** Tissue damage in pink, chum, chinook and coho is relatively mild to acute and these salmon are often able to shed attached lice shortly after infection (Johnson and Albright 1992a, Jones et al. 2007).

Healthy mucus and epidermal tissue form an important barrier to infection for salmon (Ingram 1980, Hjelmeland et al. 1983). Damage to this barrier, together with a stressed immune system due to *L. salmonis* feeding, may increase the susceptibility of these fish to secondary viral, bacterial or fungal infections (Johnson et al. 1996). In the sockeye epizootic example previously introduced, a high incidence of freshwater infection and pre-spawn mortality was facilitated by residual *L. salmonis* tissue damage (Johnson et al. 1996).

Generally, sea louse infections cause less damage in wild Pacific salmon than in Atlantic salmon. However, the overall effect of these infections on the health and abundance of salmon populations is not entirely resolved (e.g., Patanasatienkul et al. 2013). It is important to identify the mechanisms that influence dispersal of *L. salmonis*

between salmon farms and migrating populations of wild salmon (Krkošek et al. 2007, 2009).

1.3 Population genetics and lice

1.3.1 Dynamics of population genetics

Patterns of genetic variation can reflect the relatedness at various spatial scales within a species geographic range. These patterns of variation can be used in order to understand population dynamics and evolutionary history. The basic opposing forces that determine the population structure of a species are genetic drift, mutation, and gene flow: gene flow reduces genetic subpopulation isolation while genetic drift and mutation facilitate genetic divergence (Slatkin 1981).

Isolated mutation events accumulate over time within populations and ultimately contribute to the overall genetic variation of a species. Genetic drift and natural selection are evolutionary mechanisms that influence the frequency of occurrence of each genetic (or allelic) variant through time (Slatkin 1981). Novel mutations may first become established on a localized geographic scale and the rate at which these mutations spread throughout a species range is dependent on gene flow (Watterson 1975).

Gene flow can be described as the rate at which alleles originating in different localities become established throughout the species range (Cockerham and Weir 1993). The main mechanisms of gene flow are active migration or passive dispersal of individuals (or gametes) that successfully pass on their genetic information to following generations (Cockerham and Weir 1993, Bohonak 1999).

The mutations that give rise to novel genetic variants (in the form of new alleles) are likely unique within a species (Kimura 1969). When gene flow is very limited, the set of

alleles that accumulates within each subpopulation becomes increasingly divergent from other subpopulations (Slatkin 1985). As the level of gene flow among subpopulations increases, the degree of genetic divergence among subpopulations will decrease (Slatkin 1981). The amount and direction of gene flow that occurs will determine how well distributed these allelic variants are among different subpopulations (Slatkin 1981). When high gene flow connects subpopulations, overall genetic divergence among subpopulations is reduced because selectively neutral variation originating in one location will spread and become widespread in other subpopulations (Slatkin 1981).

Genetic drift and natural selection can influence which alleles are maintained in subpopulations over time (Feder et al. 2013). Through the progression of generations, it is likely that some allelic variation will be lost by random chance, particularly when total or effective population sizes are small (Hedgecock 1994, Hauser and Carvalho 2008). Variants that occur at very low frequencies have a higher chance of being lost than variants found at higher frequencies (Kimura and Crow 1964). The cumulative effect of these random events can lead to genetic divergence among subpopulations and is referred to as genetic drift (Beaumont 2005). If gene flow is adequately low, subpopulations can become divergent over time due to the independent outcomes of genetic drift on each subpopulation. The overall effect of genetic drift is the genome-wide random loss of low frequency alleles in different subpopulations, which can result in decreased overall genetic variability over the species range (Hedgecock 1986, Hellberg et al. 2002, Hauser and Carvalho 2008).

Natural selection has a targeted effect on genetic variation and should have the greatest effect on allele frequencies of loci under selective pressure (Beaumont and Nichols 1996,

Beaumont 2005). Selection acts at the level of the individuals within subpopulations and it is likely that differential selection pressures exist over the species range (Excoffier et al. 2009). Although the driving forces of selection can be diverse, the outcome remains the same; under a particular selection regime, selectively advantageous alleles should increase in frequency and non-advantageous alleles should decrease in frequency (Duffy and Sivars-Becker 2007, Funk and Murphy 2010).

1.2.2 What is the evolutionary history of Pacific *Lepeophtheirus salmonis*?

The Atlantic and Pacific forms of *L. salmonis* are geographically isolated and genetically distinct (Yazawa et al. 2008). Differentiation between Atlantic and Pacific *L. salmonis* was first noted in preliminary genetic comparisons using mitochondrial DNA (mtDNA) (Tjensvoll et al. 2006). The differentiation was confirmed through comparisons of Atlantic and Pacific *L. salmonis* expressed sequence tag (EST) and mtDNA sequences, which revealed a 3.2% (ESTs) to 7.1% (mtDNA) divergence (Yazawa et al. 2008). Comparisons focused on two mitochondrial genes commonly used in phylogenetic studies, cytochrome c oxidase subunit 1 (COI) and 16S ribosomal RNA (16S), indicated a 7.6% (COI) and 4.2% (16S) DNA sequence divergence between these two populations of *L. salmonis* (Yazawa et al. 2008). For reference, conspecific COI divergence in crustaceans ranges from 1.3 to 7.9% (Lefébure et al. 2006) and congeneric divergence in copepods ranges from 13 to 22% (Bucklin et al. 1999).

The 16S sequences were used to place the time of Pacific and Atlantic *L. salmonis* divergence between 2.5 and 11 million years ago (Yazawa et al. 2008). Yazawa et al. (2008) concluded that this divergence time could be used, together with our current estimates of the timing of geological events, to suggest that *L. salmonis* originally co-

evolved with Atlantic salmonids and spread from the Atlantic Ocean into the Pacific Ocean, and onto Pacific salmonids through the Bering Strait approximately 5 million years ago (Marincovich and Gladenkov 1999, Yazawa et al. 2008).

The divergence of COI and 16S among individual lice within the Pacific Ocean (0.62% COI, 0.14% 16S) is lower than divergence within the Atlantic Ocean (0.76% COI, 0.26% 16S) (Yazawa et al. 2008, Boulding et al. 2009). This indicates that Pacific *L. salmonis* have likely had less evolutionary time to accumulate variation and population structure than Atlantic *L. salmonis* (Yazawa et al. 2008). The genetic differentiation of the Pacific and Atlantic forms of *L. salmonis* must be considered in future study and management of this parasite.

1.3.3 Natural and artificial influences on population structure of Pacific *Lepeophtheirus salmonis*

Genetic population structure of Pacific *L. salmonis* may be due to differences in host susceptibility to infection (Johnson and Albright 1992a), differences in the availability of hosts for infection opportunity (Jones 1998), or the disparate selection pressures experienced by *L. salmonis* on farmed and wild hosts (Tully and Whelan 1993). Parasite genetic structure is expected to increase as the access to potential hosts becomes less predictable in time and space (Nadler 1995, Archie and Ezenwa 2011). The complexity of migration patterns and differences in the multi-year life cycles of each host species likely increase the spatial and temporal fragmentation of host populations, which could act to decrease effective population size (N_e) of *L. salmonis* through increased frequency of bottlenecks and decreased overall genetic variation (Huyse et al. 2005). Differences in host susceptibility to *L. salmonis* infection may increase selective pressure on *L. salmonis*

to overcome host immune defenses, which, in turn, could increase the overall genetic diversity of this parasite (Johnson and Albright 1992b, Adamson and Caira 1994).

Natural and artificial influences have shaped the current population structure of Pacific *L. salmonis.* The natural influences can be broadly categorized into the effects of parasite dispersal and reproductive characteristics, host behavioural complexity, and host-parasite competition (Nadler 1995). Artificial influences on *L. salmonis* population structure are likely dominated by cyclical periods of rapid parasitic population growth and decline that are specific to salmon farm environments (Dlugosch and Parker 2008, Hoberg and Brooks 2008).

1.3.3a Natural population dynamics of *Lepeophtheirus salmonis*

The population structure of free-living species is generally a product of the biological and ecological requirements and evolutionary history of each particular species. In parasitic species, population structure is additionally determined by the biology and evolutionary history of the parasite's hosts (Adamson and Caira 1994). It is therefore important to consider the effect of host-parasite interactions on the population structure of parasitic species. The main host-mediated population dynamics that affect gene flow in parasitic species include transmission mechanism, life cycle and reproductive complexity, and host specificity (Nadler 1995, Huyse et al. 2005, Archie and Ezenwa 2011). It is generally expected that parasite populations will become increasingly structured as parasite life cycles and transmission dynamics become more complex and host specificity increases (Nadler 1995, Archie and Ezenwa 2011). The life history characteristics that are often linked to complex population structure in parasites are weakly pronounced in *L. salmonis* (Adamson and Caira 1994). It is therefore likely that

population structure in *L. salmonis* will more closely resemble that of free living marine copepods (e.g., Nelson et al. 2009) than other parasites with more complex life histories (Øines and Heuch 2007).

Three important population dynamics likely influence the evolutionary history and genetic structure of Pacific *L. salmonis*:

I. Planktonic parasite larval dispersal, motile transmission capability and reproductive potential;

II. The spatial and temporal complexity of host migration behaviour; and

III. Competitive adaptation of host defenses and parasite virulence.

I. Planktonic larval dispersal, motile transmission capability and reproductive potential of *Lepeophtheirus salmonis*

In many ways, the transmission dynamics and life cycle complexity of *L. salmonis* are more comparable to the dispersal and reproductive biology of most free-living marine invertebrates than to the more complex transmission dynamics of many other parasitic taxa (Adamson and Caira 1994). *Lepeophtheirus salmonis* larval stages are free-living and planktonic, therefore, their larval dispersal potential is likely similar to that of other free-living marine invertebrates, where gene flow is generally expected to be correlated to pelagic larval duration (Selkoe and Toonen 2011). However, the planktonic larvae of many marine invertebrates, including *L. salmonis*, can influence their potential dispersal range by migrating vertically within the water column in synchrony with tidal currents or light-dark cycles (Heuch et al. 1995). These vertical movements result in a net shoreward trajectory and concentration of larvae in locations where they have a greater chance to find suitable habitat or hosts (Heuch et al. 1995). Larval retention can increase genetic divergence among populations that are expected to experience high gene flow (Burton

1997, Selkoe et al. 2008, Weersing and Toonen 2009); larvae released in coastal areas may find hosts relatively easily because host fish are often at high densities in these areas (Beamish et al. 2005). The larvae that are released in the open ocean may have a lower chance of survival due to the lower average density of potential hosts; however, larval dispersal is possible among salmon in the open ocean (Nagasawa 2001).

The motility of parasitic pre-adult and adult *L. salmonis*, together with the relatively long lifespan of adult females, likely contribute to the cumulative dispersal potential of *L. salmonis* (Jones 1998). Parasite fecundity is likely important to the population dynamics of *L. salmonis.* Individual females can produce an estimated 6-11 pairs of egg strands over an approximate 7 month adult lifespan and the number of viable eggs per strand (e.g., 55-704 eggs per strand, Heuch et al. 2000) can be strongly influenced by environmental conditions (Boxaspen and Naess 2000, Heuch et al. 2000, Orr 2007). The limited but theoretically unrestricted movements of motile *L. salmonis* stages may be comparable to the limited movements of benthic marine invertebrates, where localized movement within a small habitat zone is common (for grazing, etc.) but long range movements are likely unintentional and due to disturbances in the original habitat, or in the case of *L. salmonis*, on the surface of a salmonid host (Jones 1998). Reproductive potential and mating behaviours could also be similar to benthic invertebrates, where individual mate access is limited by the local abundance of potential mates but the local reproductive population is genetically diverse due to random settlement of planktonic larval stages (Ritchie et al. 1996, Ritchie 1997, Todd et al. 2005). It is possible that gene flow is restricted among subpopulations of *L. salmonis*, even if they have a high innate potential for dispersal, because cryptic barriers to survival or reproduction may exist that

prevent genetic introgression of migrant individuals (Boxshall 1976, Øines and Heuch 2007).

II. The spatial and temporal complexity of host migration behaviour

The migration behaviour of wild salmonids in BC is spatially and temporally complex. The complexity of host behaviour likely influences the population structure of *L. salmonis* by limiting the movement of these parasites between particular groups of fish at sea, and between generations of fish as they move between marine and fresh water environments. Different species and different populations of salmon spend various amounts of time at sea. The intensity of *L. salmonis* infections generally increases as each host remains longer at sea (Nagasawa 1987). Pink and chum fry migrate to sea almost immediately after hatching, where pinks spend two years and chum spend three to five years, before returning to fresh water (DFO 2013). Some chinook move to the marine environment immediately after hatching while others remain in fresh water for up to two years, and marine duration varies from three to five years (DFO 2013). Coho spend one year in fresh water followed by two years at sea, while sockeye spend one to three years in fresh water and one to three years at sea (Groot and Margolis 1991). Although the spawning migrations of most salmon occur from late summer to early fall, some chinook begin the return migration in the spring and coho generally migrate in the early winter (Groot and Margolis 1991).

The migration behaviour of each species varies within the marine environment. Juvenile salmon generally progress through coastal areas (neritic zone) and away from shore in May to August, and juvenile pink, chum and sockeye continue to move into the oceanic zone from August to October, while some coho and chinook remain in the neritic

zone over the winter (Beamish et al. 2007). Although most juvenile salmon have moved away from shore by the time spawning migrations bring adult fish near shore, vertical transmission of *L. salmonis* between adult and juvenile fish is still possible, particularly for those juvenile coho and chinook that remain in the coastal environment through their first year at sea. It has been estimated that approximately half of the salmon biomass in the open ocean is lost each fall as mature salmon move into freshwater environments (Beamish et al. 2007). Any *L. salmonis* that are carried by mature salmon are shed within a few weeks of these fish moving into fresh water (McLean et al. 1990, Finstad et al. 1995, Pike and Wadsworth 2000).

Lepeophtheirus salmonis abundance (number of *L. salmonis* per sample of fish, including both infected and uninfected members of the host species), intensity (number of *L. salmonis* per infected host), and prevalence (proportion of sampled fish that are infected with *L. salmonis*) vary among host species in the open ocean (Margolis et al. 1982, Nagasawa 1987, 2001). While pink and chum salmon generally have the highest north Pacific oceanic abundance (Figure 1a), the infection rate of pink salmon is disproportionately high compared to chum (Figure 1). The intensity of infection in chinook and steelhead is high, similar to pink salmon; however, because chinook and steelhead occur at lower abundance than pink salmon, these two hosts do not contribute as significantly to the overall abundance of *L. salmonis* (Nagasawa 2001). Sockeye and chum experience the lowest infection intensities, but because of the relatively high abundance of chum, a large proportion of the *L. salmonis* population affecting sockeye and chum is supported by chum. Coho support a slightly higher abundance of *L. salmonis*

than chinook and steelhead, yet coho represent a fraction of the total host abundance that is more similar to sockeye (Figure 1)

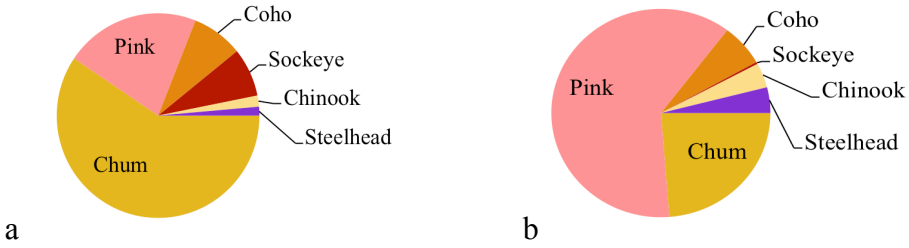

Figure 1. (a) The total abundance of oceanic Pacific salmonids captured by surface long-line in June and July of 1991-1997. Transect sampling was done in the central North Pacific Ocean and Bering Sea. (b) The total distribution of *L. salmonis* among host species captured in transect surveys in June and July of 1991-1997 (Data from Nagasawa 2001).

The annual abundance of each species of salmon follows a cyclic pattern, with some cohorts of salmon having much higher abundance than others (Groot and Margolis 1991, Nagasawa 2001, Beamish et al. 2007). The annual abundance of pink and chum is generally higher than coho, chinook and sockeye (Nagasawa 2001, Beamish et al. 2007). Pink salmon abundance fluctuates annually with large populations at sea in even years and small populations at sea in odd years (Beamish et al. 2007). Chum populations are more stable over time and have been proposed by Nagasawa (2001) to act as a stable reservoir host that supports a population of *L. salmonis* that is relatively large and constant over time so that the *L. salmonis* population is able to quickly expand in response to increased pink salmon abundance when the large "even-year" pink cohorts are at sea.

Parasitic species have complex population distributions because the movements of individual parasites are generally confined to their individual hosts. Within a larger population, the group of parasites found on one host is referred to as an infrapopulation

(Margolis et al. 1982). It may be important to consider the relatedness of individual *L. salmonis* within infrapopulations, and the size of infrapopulations, because this is the level where reproduction occurs (Huyse et al. 2005). It is unlikely that closely related lice will infect the same host fish following larval dispersal, therefore, members of an infrapopulation are not likely to be closely related (Johnson and Albright 1991a, Brooks and Stucchi 2005, Selkoe and Toonen 2011). Transfer of motile lice among hosts is possible and may further influence infrapopulation structure (Hull et al. 1998, Connors et al. 2008). Estimated among-host transfer rates of adult males is much higher than adult females (62.4% and 17.9%, respectively; Hull *et al.* 1998). Variation in infrapopulation size among host species may influence the reproductive success of *L. salmonis* by reducing the average effective population size within infrapopulations. The intensity of infection with *L. salmonis* in the open ocean is lower for some species of salmon than others. Low abundances of adult female *L. salmonis* have been reported on sockeye (1.06 lice per fish), chum (2.14 lice per fish), and coho (2.42 lice per fish), while higher abundances were found on chinook (5.27 lice per fish), pink (5.92 lice per fish), and steelhead (6.07 lice per fish; Nagasawa 2001). Similarly high abundances can be found on Atlantic salmon (7.45 motile lice per fish) in BC (Saksida et al. 2007b).

III. Competitive adaptation of host defenses and parasite virulence

Atlantic salmon and sea trout experience the highest infection rates and the most damage from *L. salmonis* infections; Pacific salmonids are generally more resistant, but still experience a wide range of susceptibility to *L. salmonis* infections (Johnson and Albright 1992a, Dawson 1997, Fast et al. 2002). Ocean surveys of *L. salmonis* distribution and abundance have revealed strong patterns of differential infection rates

among adult pink, chum, coho, chinook, sockeye, and steelhead (Nagasawa 1987, 2001). Chum and pink support the largest portion of the total *L. salmonis* population, which may be due to the relatively high abundance of these fish species, while the highest intensities of infection occur on steelhead and chinook (Nagasawa 1987, 2001).

In addition to differences in infection due to relative host abundance and parasite transmission potential, differences in host infection rates are likely dependent on the immunological ability of host species to resist parasitic infection and/or the ability of *L. salmonis* to suppress host resistance (Johnson and Albright 1992a, Fast et al. 2003). Successful host rejection of attached *L. salmonis* stages is linked to the strength of the host inflammatory response and the ability of the host to develop thickened skin (epithelial hyperplasia) at the site of *L. salmonis* infection (Johnson and Albright 1992a, Jones et al. 2007). Coho show a strong inflammatory response to *L. salmonis*, while chinook display a similar but weaker response and Atlantic salmon show little to no response to *L. salmonis* infection (Johnson and Albright 1992b). The relative response rates of coho, chinook and Atlantic salmon generally correlate to the intensities of *L. salmonis* infection naturally observed in these three species (Dawson 1997, Nagasawa 2001). Evidence of differential expression of wound healing, inflammatory response and immune response genes has been correlated to the higher resistance in juvenile pink in comparison to juvenile chum salmon (Jones et al. 2007, Braden et al. 2012). However, very small pink (0.3 g) are highly susceptible to copepodid infection and do not exhibit the same genetic response to *L. salmonis* infection as larger pink salmon (0.7 & 2.4 g; Jones *et al.* 2008, Sutherland *et al.* 2011).

Fast et al. (2003) have demonstrated that variation in the secretory response of *L. salmonis* occurs in the presence of potential host fish. Atlantic *L. salmonis* and Pacific *L. salmonis* responded similarly to the presence of Atlantic salmon and rainbow trout mucus by secreting enzymes possibly related to feeding and or avoidance of host immune responses (Firth et al. 2000, Fast et al. 2003, 2004). Pacific, but not Atlantic, *L. salmonis* produced these enzymatic secretions in the presence of coho mucus and, in general, the protease activity of Pacific *L. salmonis* was higher than that of Atlantic *L. salmonis* (Fast et al. 2003). Evidence of host immune suppression has been detected in the skin surrounding *L. salmonis* attachment sites in both susceptible (chum and Atlantic) and resistant (pink) salmon (Braden et al. 2012)

Although *L. salmonis* is adapted to survival on salmonid hosts, this louse has been documented to infect other species in the wild, most notably the threespine stickleback, *Gasterosteus aculeatus* (Jones et al. 2006). Jones et al. (2006) reported infection rates of *L. salmonis* and *Caligus clemensi* on 1,309 stickleback collected from the Broughton Archipelago in May to June 2004. *Lepeophtheirus salmonis* was found at a higher prevalence and intensity than *C. clemensi* (83.6% infected, 1-290 lice per fish; 42.8% infected, 1-34 lice per fish). A very low proportion of observed *L. salmonis* were adults (5 of 19,595; Jones *et al.* 2006). In exposure trials of Atlantic cod (*Gadus morhua*) and saithe (*Pollachius virens*) to Atlantic *L. salmonis* copepodids, low levels of infection were initially observed but no *L. salmonis* remained attached to these fish 96 hours post-exposure (Pert et al. 2009). Similarly, attempted infection of Atlantic cod, stickleback and saithe with recently mated female *L. salmonis* resulted in complete predation of lice placed with stickleback, and few successful louse settlements on Atlantic cod and saithe

(Pert et al. 2012). No egg strings were produced by *L. salmonis* on saithe, and fecundity and larval survival of *L. salmonis* on Atlantic cod were much lower than on Atlantic salmon controls (Pert et al. 2012). Non-salmonid hosts such as stickleback may be important temporary reservoirs for *L. salmonis* in near-shore waters when salmonid hosts are not abundant (Jones et al. 2006, Pert et al. 2009, 2012).

Evolutionary divergence has been described for a congener of *L. salmonis* that infects a limited number of flatfish species (Boxshall 1976). When flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*) were experimentally infected with *Lepeophtheirus pectoralis*, Boxshall (1976) found that *L. pectoralis* found on flounder produced larvae that preferred to settle on flounder, and similarly, *L. pectoralis* found on plaice produced larvae that preferred to settle on plaice. It is possible that similar patterns of host species preference could develop in *L. salmonis*, which could have important implications for the interpretation of host-parasite dynamics among different salmonids.

Geographic and genetic isolation, combined with co-evolution with phylogenetically distinct host taxa, has facilitated genetic drift and disparate evolutionary adaptation between the Atlantic and Pacific *L. salmonis* lineages (Yazawa et al. 2008) . Evidence of possible phenotypic divergence includes higher reported virulence in Atlantic *L. salmonis* than in Pacific *L. salmonis* in infections of farmed Atlantic salmon (Saksida et al. 2007b). It has also been suggested that *L. salmonis* copepodids in BC have a higher tolerance to low salinity than copepodids from Scotland (Johnson and Albright 1992a, Bricknell et al. 2006). In addition, differences have been found in the physiological response of Pacific and Atlantic *L. salmonis* to coho, a salmonid that is highly resistant to *L. salmonis* infection (Fast et al. 2003). The biological differences observed in Pacific and Atlantic *L.*

salmonis demonstrate the capacity of this parasite to adapt to different environments. Closer examination of genetic variation within the Pacific Ocean may reveal population structure and adaptive differentiation among populations of Pacific *L. salmonis.*

1.3.3b Artificial population dynamics of *Lepeophtheirus salmonis*

The introduction of Atlantic salmon farms to BC has increased the overall complexity of *L. salmonis* population dynamics and host-parasite interactions. The most obvious effects of these introductions can be divided into two categories:

I. Rapid *L. salmonis* population growth on farmed Atlantic salmon; and
II. Extreme *L. salmonis* population declines on salmon farms as a result of emamectin benzoate (EMB) treatment.

I. Rapid *Lepeophtheirus salmonis* population growth on farmed Atlantic salmon

The introduction of Atlantic salmon to the Pacific Ocean ecosystem may influence the coevolutionary trajectory of natural host-parasite dynamics between *L. salmonis* and Pacific salmonids, and may deteriorate the migratory allopatry that separates juvenile and adult Pacific salmonids (Krkošek et al. 2007, Barrett et al. 2008).

In the absence of salmon farms, adult and juvenile Pacific salmonids are geographically isolated through migratory allopatry, and this isolation prevents vertical transfer of *L. salmonis* between host generations (Krkošek et al. 2007). As mature Pacific salmonids move into fresh water in the fall, they transport *L. salmonis* into coastal areas (Beamish et al. 2007). These parasites must overwinter on alternate hosts in order to infect juvenile salmonids when these fish enter the fresh water each spring (Jones et al. 2006, Beamish et al. 2007). The introduction of salmon farms to the coastal environment

in BC has created novel overwintering habitat for *L. salmonis*, which can increase the exposure of juvenile Pacific salmonids to these parasites (Krkošek et al. 2007).

Atlantic salmon are artificially maintained at high densities on fish farms and are highly susceptible to *L. salmonis* infection (Saksida et al. 2007b). The abundance of *L. salmonis* on BC salmon farms fluctuates with the harvest schedules and EMB treatments of farmed salmon (Saksida et al. 2007a, Peacock et al. 2013). High *L. salmonis* population growth rates often precede EMB treatment, followed by negative population growth rates post-treatment (Rogers et al. 2013). The duration of this negative growth period on farms can be predicted from two environmental variables, temperature (~12°C) and salinity (>27ppb; Tucker et al. 2000, Bricknell et al. 2006, Rogers et al. 2013), in addition to the return of mature Pacific salmon to coastal waters (Orr 2007, Beamish et al. 2007).

It is important to identify whether *L. salmonis* population growth on farms is a result of bottlenecks, founder events, or continual immigration of *L. salmonis* from wild hosts. Rapid *L. salmonis* population growth is often detected on salmon farms at the same time as adult Pacific salmonids enter coastal areas near farms each fall (Orr 2007, Peacock et al. 2013). Exponential population growth of *L. salmonis* on BC farms generally continues through the fall and winter until anti-parasitic treatments are applied (Rogers et al. 2013). The rapid growth of *L. salmonis* populations on farms has been attributed to horizontal parasite transfer from wild to farmed hosts; however, environmental variables such as increased temperature and salinity may also stimulate rapid population growth of *L. salmonis* (Rogers et al. 2013). The genetic structure of *L. salmonis* on BC salmon farms may depend on whether exponential population growth is due to horizontal transfer of

lice into farms or high reinfection rates (vertical transmission) within farms. Populations of *L. salmonis* could be influenced by bottlenecks or selection pressures that are specific to farm environments if gene flow into farms from wild-sourced *L. salmonis* is restricted (Wade and McCauley 1988).

The environments *L. salmonis* encounter on farmed (mainly Atlantic salmon) and wild Pacific salmon are different in several ways: lice on farms have easy access to high densities of hosts, and Atlantic salmon have a relatively weak immune defense against *L. salmonis* (Torrissen et al. 2013). It is relatively easy for copepodids to successfully find and infect a suitable host within farms (Torrissen et al. 2013). Conversely, *L. salmonis* infecting wild hosts have a much lower likelihood of encountering a suitable host, and even if a host is found, the variation in Pacific salmonid species susceptibility to infection further reduces the chance of *L. salmonis* survival in the wild environment (Nagasawa 2001).

II. Extreme *Lepeophtheirus salmonis* population declines on salmon farms

Lepeophtheirus salmonis population control measures on salmon farms may act as strong and recurrent bottleneck events that increase genetic drift on farms and may locally reduce *L. salmonis* genetic variability (Barrett et al. 2008). Strong selection pressures may exist for *L. salmonis* in farm environments that are not present in the wild environments, and these differential selection pressures may lead to increased genetic variability across the entire Pacific *L. salmonis* population (Wade and McCauley 1988, Glover et al. 2011).

If treatment of salmon farms with EMB effectively removes *L. salmonis* from farms, subsequent growth of *L. salmonis* populations on farms must be the result of re-

colonization from external sources. If *L. salmonis* abundance is high in the external environment, then recolonization should occur quickly and founder effects should be weak or absent on farms (Dlugosch and Parker 2008). If *L. salmonis* abundance is low in external reservoirs, then re-colonization should be more gradual and founder effects may be more distinct on farms (Gandon and Michalakis 2002, Roman 2006). The potential evolution of chemotheraputant resistance in *L. salmonis* on salmon farms is likely the most important practical implication of the geographic and genetic separation of Pacific and Atlantic *L. salmonis* (Saksida et al. 2007b, 2010, Yazawa et al. 2008). The most common treatment of sea lice infection on salmon farms is EMB, which is the only licensed treatment currently available to British Columbia fish farms (Saksida et al. 2007b). Controversy exists concerning the practice of salmonid aquaculture in BC, with concerns raised about the potential capacity of salmon farms to amplify local sea louse densities and facilitate increased sea louse re-infection in wild salmonids. Particular interest has focused on the exposure of juvenile salmonids to lice as these fish migrate past salmon farms each spring (e.g. Krkošek et al. 2009, 2011). Declines have been documented for several salmon populations and correlated to the expansion of the salmon aquaculture industry in BC (Ashander et al. 2012, Rogers et al. 2013). In response to these declines, aquaculture managers have coordinated the timing of EMB treatments on salmon farms in BC and have identified that annual treatment in January or February effectively reduces local *L. salmonis* population abundance below government mandated thresholds of 3 motile *L. salmonis* per salmon through the outmigration period of wild juvenile salmonids (Rogers et al. 2013). Coordinated and optimized timing of EMB

treatments on salmon farms have been correlated with increased wild salmon abundance and decreased sea louse infection in juvenile salmon (Peacock et al. 2013).

The health of both farmed and wild salmon populations in BC are highly dependent on effective control of *L. salmonis* on farmed hosts, therefore the potential development of EMB resistance genes on BC farms would be a serious threat to the aquaculture industry as well as to wild salmon and associated fisheries (Saksida et al. 2013). In the Atlantic Ocean, *L. salmonis* have become increasingly tolerant to various drug treatments, including EMB. This resistance has been documented in Scotland (Lees et al. 2008) and in New Brunswick (Westcott et al. 2008). As of 2013, no resistance to EMB has been observed on farms in BC, but it is possible that resistance genes may develop in the future (Saksida et al. 2013).

1.4 Genetic markers

1.4.1 Genetic marker requirements for detecting population structure

The actual distributions of alleles and genotypes in a population are the product of previous mutation, genetic drift, gene flow, and natural selection. Mutation events are rare and are assumed to be equally likely to occur in all individuals of a species (Kimura 1968, 1969). The distribution and frequency of alleles among populations can be used to estimate population structure that has developed through gene flow and genetic drift (Slatkin 1981). Selection pressure can also affect the frequencies and distributions of alleles (Hedgecock 1986) but this effect should only be detectable for alleles linked to genes that have undergone selection (Excoffier et al. 2009). Selection pressure may bias allele frequencies of markers linked to traits under selection, which could be misinterpreted as the effects of gene flow and genetic drift (Cavalli-Sforza 1966,

Lewontin and Krakauer 1973). However, markers that are under selection may be informative of ecological, rather than evolutionary, history and may be useful for assigning individuals to source populations (Waples and Gaggiotti 2006). In practice, it is difficult to know how strongly selection has influenced the allele frequencies of particular genetic markers. However, it is possible to select markers that are likely to approximate neutrality by targeting DNA sequences that are variable but are unlikely responsible for phenotypic differences (Woodhead et al. 2005, Ellis and Burke 2007). Large-scale DNA sequencing projects have increased the availability of potential genetic markers for non-model organisms. Expressed sequence tag (EST) libraries contain messenger RNA derived DNA sequences. Although the primary function of EST data is to provide functional genetic information, the inclusion of non-coding genetic variation allows for ESTs to be screened for non-coding polymorphic loci that can be used for population genetics studies. One caution with this approach is that the allele frequencies of EST-derived genetic markers may be biased by physical linkage to genes with phenotypic variation and possibly selection. An additional precaution, in order to ensure approximate selective neutrality, is to compare allele frequency estimates for several loci and marker types with different mutational mechanisms (Ellis and Burke 2007). This increases genome-wide representation of genetic variation and minimizes the effects of selection on targeted genomic regions (Lewontin and Krakauer 1973). The molecular markers selected to estimate the genetic structure of natural populations should exist in two or more neutral allelic states that can be easily distinguished and are inherited following Mendelian genetic expectations (Watterson 1975, Tajima 1983). Microsatellites or simple-sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) are two

types of molecular marker that are easily identified from EST sequences and are potentially informative for population genetic structure studies (Guichoux et al. 2011).

1.4.2 Resolution and power of different marker types

Progression in our understanding of molecular genetics and corresponding technological advances have resulted in the availability of increasingly informative molecular markers that can be applied to various population genetics questions (Selkoe and Toonen 2006). SSRs and SNPs are both highly informative and commonly used markers for intraspecific comparisons of genetic structure (Coates et al. 2009, Haasl and Payseur 2011, Guichoux et al. 2011).

SSRs can generally be described as 100-300 base pair (bp) DNA sequences that contain a string of short (2-6 bp) repeated units flanked by complex sequences that can be used to amplify the targeted DNA fragment by polymerase chain reaction (PCR). SSR polymorphisms are generally thought to be a product of slipped-strand mispairing events that occur during DNA replication and result in new alleles that differ in length by one to several repeat units (Levinson and Gutman 1987, Eisen 1999). The mutation rate of SSRs is approximately 10^{-3} to 10^{-5} mutations per locus per generation (Crozier et al. 1999, Hancock and Vogler 2000). The frequency and outcome of SSR mutations are influenced by several interacting variables, including the effectiveness of cellular proofreading mechanisms, the particular repeat unit structure, and the number of repeat units present in a given allele (Chakraborty et al. 1997, Primmer et al. 1998, Neff and Gross 2001).

SNPs are point mutations that are distributed throughout coding and non-coding regions of the genome. SNP mutations can range from being selectively neutral to being directly responsible for phenotypic variation. The occurrence of each SNP mutation is

assumed to be independent. Individual SNPs are generally less informative of population structure than individual SSR loci because multiple allelic variants are possibly detected for one SSR and rarely more than two allelic variants exist for each SNP (Kalinowski 2002, Morin et al. 2009b). As an example of the usefulness of different marker types, similar levels of genetic population structure were found in chinook using 41 SNPs, 9 SSRs, and 22 allozymes (Smith et al. 2007). Despite the need for larger numbers of SNP markers for population studies, SNPs are valuable markers because it is relatively easy to generate a large number of SNP loci and these loci follow a much simpler mutation model than SSRs (Helyar et al. 2011). The allele frequencies of SNPs near genes are more likely to be affected by selection than SNPs located elsewhere. Though gene-linked SNPs may not be informative of neutral population structure, they may be useful to identify contemporary genetic divergence that is a result of adaptation to ecological variation (Waples and Gaggiotti 2006, Barreiro et al. 2008, Helyar et al. 2011).

1.4.3 What information is obtained from allelic variation?

The allele frequency distribution of neutral loci can be used to estimate the degree of population divergence that has occurred as a result of restricted gene flow and genetic drift (Slatkin 1981). Comparisons of average observed heterozygosity per individual (H_I), per subpopulation (H_S), or over the entire population (H_T), can be informative of historical population dynamics. As gene flow among subpopulations increases, the rate of differential fixation of alleles among subpopulations decreases; with increasing gene flow, alleles originating in any subpopulation have a higher chance of spreading to other subpopulations and, from a population genetic perspective, higher gene flow results in a larger effective population size (Bohonak 1999). Calculations that compare average

heterozygosity among population samples can be used to estimate the fixation index, F_{ST}, which is commonly used to indicate overall population structure (Wright 1965, Weir and Cockerham 1984). Generally, F_{ST} quantifies population divergence due to genetic drift by comparing the average heterozygosity or allele frequency variance for each subpopulation to the average taken over the total population (Cockerham and Weir 1993). F_{ST} values range from 0 to 1, with an $F_{ST} = 0$ indicating complete panmixis or unrestricted gene flow among samples (i.e., 0% fixation of different alleles in different samples) and an $F_{ST} = 1$ indicating complete isolation of samples (i.e., 100% fixation of different alleles in different samples; Wright 1951). An inverse relationship generally exists between F_{ST} values and dispersal potential: as capacity for long range dispersal increases, F_{ST} decreases (Bohonak 1999). The number of migrants per generation (Nm) can be approximated using average heterozygosity and F_{ST} estimates (Slatkin 1985).

The inbreeding coefficient, F_{IS}, is a second important fixation index used in population genetics. F_{IS} values indicate the average reduction in heterozygosity within individuals that is due to non-random mating within subpopulations (Wright 1951). F_{IS} is generally estimated by comparing the average heterozygosity within individuals to the average heterozygosity within each subpopulation and can range from $F_{IS} = -1$ (100% of individuals are heterozygous) to $F_{IS} = 1.0$ (100% of individuals are homozygous).

Analysis of molecular variance (AMOVA) is an extension of F-statistics that can be used to estimate where, within a population, most allelic variance exists by partitioning variation among hierarchically defined levels as variance among individuals, variance within subpopulations, or variance among subpopulations (Excoffier et al. 2009).

The molecular markers that are used to estimate patterns of population allele frequency variation must provide enough statistical power to be informative of actual population structure. Theoretical modeling has shown that adequate statistical power is achieved with approximately 30 neutral SNPs when estimating populations that are 1% divergent (F_{ST} = 0.01) and > 80 loci may be required to estimate more subtle, 0.5% divergence (F_{ST} = 0.005; Morin *et al.* 2009).

1.4.4 Why was it important to do a detailed study of Pacific *Lepeophtheirus salmonis* population structure?

Previous population genetics studies have attempted to identify differences among *L. salmonis* through comparing samples collected from wild and farmed hosts, geographically distant locations, and different host species (Isdal et al. 1997, Todd et al. 1997, 2004, Shinn et al. 2000, Dixon et al. 2004, Tjensvoll et al. 2005, 2006, Yazawa et al. 2008, Boulding et al. 2009, Nolan and Powell 2009, Glover et al. 2011). These studies have generally been constrained by the previously limited availability of molecular resources for *L. salmonis*. Recent and significant contributions to the molecular genetics resources that are available for *L. salmonis* have made it possible for us to efficiently identify and develop informative SSR and SNP loci that may be used to assess *L. salmonis* population structure, and to address important questions about the effective management and the evolutionary history of this important parasite (Yasuike et al. 2012).

1.4.4a What conclusions have been made from previous research of *Lepeophtheirus salmonis* population genetics?

Eleven studies have previously been published on the population genetic structure of *L. salmonis*, with an overwhelming focus on sampling within the Atlantic Ocean. One objective that has been central to previous *L. salmonis* population genetics research is the

accurate estimation of dispersal and connectivity of *L. salmonis* between farm and wild host salmonids (reviewed in Boxaspen 2006).

Early studies using allozyme and RAPD (random amplified polymorphic DNA) markers found conflicting evidence of population structure among farm samples in Norway (Isdal et al. 1997) and between wild and farm samples in Scotland (Todd et al. 1997). Isdal et al. (1997) found evidence of differentiation in *L. salmonis* comparing four allozyme loci between northern and southern farm hosts in Norway, conversely, Todd et al. (1997) found no evidence of differentiation among *L. salmonis* from wild and farmed hosts or among different host species (Atlantic salmon, sea trout and rainbow trout) from analysis of two allozyme loci. However, RAPD markers revealed significant temporal and spatial variation among the farm samples in Scotland, and evidence of unrestricted gene flow among wild samples (Global F_{ST} = 0.362; Todd et al. 1997). Evidence of genetic structure was detected in a second study using the RAPD loci developed by Todd et al. (1997) within wild and farm samples from Scotland (Global F_{ST} = 0.385), however, this genetic variation did not correlate to geographic separation of the samples (Dixon et al. 2004).

Two studies of Atlantic *L. salmonis* have used DNA sequence variation to detect population structure (Shinn et al. 2000, Tjensvoll et al. 2006). A small number of *L. salmonis* collected from wild and farmed Atlantic salmon in Scotland were sequenced at two nuclear loci (18S ribosomal RNA, n = 8; ribosomal internal transcribed spacer (ITS), n = 13). The ITS sequences revealed higher variability among lice from wild hosts (~ 86% similarity) when compared to lice from farmed hosts (~98% similarity) (Shinn et al. 2000). Tjensvoll et al. (2006) identified 10.6% to 17.5% overall genetic variability among

the DNA sequences of four mitochondrial genes (ATPase subunit 6, Cytochrome *c* oxidase subunit I, Cytochrome *b* and 16S ribosomal RNA) in *L. salmonis* samples from Atlantic salmon farms in Norway, Scotland, and Atlantic Canada, and from wild Atlantic salmon in northern Russia. Little differentiation was detected among samples from Norway, Scotland, and Russia, and significant but weak differentiation was detected from pairwise F_{ST} between European and Canadian Atlantic Ocean samples (pairwise F_{ST} = 0.009 − 0.01, $p < 0.05$). An attempt to include mitochondrial sequences from *L. salmonis* collected from chum in Japan, using PCR primers developed for Atlantic *L. salmonis,* failed to produce consistent results and, therefore, the Japanese sample was not compared to the Atlantic samples used in this study (Tjensvoll et al. 2006). Significant genetic divergence, indicative of long term genetic isolation, has been confirmed between the Atlantic and Pacific populations of *L. salmonis* by comparing complete sequences of the Atlantic and Pacific *L. salmonis* mitochondrial genomes (Tjensvoll et al. 2005).

In addition to identifying the mitochondrial genome sequence for Pacific *L. salmonis*, Yazawa et al. (2008) made preliminary comparisons of COI and 16S DNA sequences among samples collected from wild and farmed salmon in Japan (Yoichi, Hokkaido), the Bering Sea, Alaska (Port Moller, Port of Kodiak, Juneau) and British Columbia (Broughton Archipelago, Ucluelet, Sidney, Sooke) and compared these sequences to the Atlantic *L. salmonis* sequences reported by Tjensvoll et al. (2005). Fixed sequence differences (>95% identity) were found at 22 positions in 16S and 67 positions in COI between Pacific and Atlantic *L. salmonis* (Yazawa et al. 2008). Less divergence was detected among the Pacific (COI = 0.62%, 16S = 0.14%) samples than among the Atlantic samples (COI = 0.76%; 16S = 0.26%), suggesting that Pacific *L. salmonis* may

be less genetically diverse than Atlantic *L. salmonis.* However, two highly divergent individuals were detected from BC and the Bering Sea, which may indicate that possible variation within the Pacific is higher than indicated by the small samples used in this study (Yazawa et al. 2008). The most recent examination of population structure among Pacific *L. salmonis* expanded on previous COI sequence comparisons (Boulding et al. 2009). Boulding et al. (2009) compared COI sequences of *L. salmonis* collected from wild chinook (n = 51), pink (n = 32) and coho (n = 5) and farmed Atlantic salmon (n = 96) in BC and found evidence of population structure from comparing the east (Broughton Wild, n = 30) and west coast of Vancouver Island (Barkley Sound Wild, n = 50) coast wild samples, as well as from comparing farm (Broughton Farm, n = 43) and wild (Broughton Wild, n = 30) samples within the same region (pairwise F_{ST} = 0.13 − 0.29, $p < 0.00001$).

The final group of population genetics studies on *L. salmonis* used a small number of SSR loci and were focused on Atlantic sampling locations. The first study included 15 *L. salmonis* samples from wild (Atlantic salmon and sea trout) and farmed (Atlantic salmon and rainbow trout) in Scotland, with single samples from sea trout in Norway, and farmed Atlantic salmon from Atlantic and Pacific Canada (Todd et al. 2004). No population structure was detected among any of the Atlantic *L. salmonis* samples and minimal divergence was detected between Atlantic and Pacific *L. salmonis* (Global F_{ST} = 0.06, $p < 0.00001$) from the genotypes of six SSR loci (Todd et al. 2004). The final SSR-based study included only four SSR loci and compared *L. salmonis* infecting Atlantic salmon from 4 farms in Ireland, 1 farm in Norway, and 1 farm in Atlantic Canada (Nolan and Powell 2009). Nolan and Powell (2009) found evidence of low but significant genetic

differentiation among spatially and temporally separated *L. salmonis* samples (Spatial Global F_{ST} = 0.08 $p < 0.05$; Temporal Global F_{ST} = 0.07, $p < 0.05$), in contrast to the previous SSR study of (Todd et al. 2004) where no significant differentiation was found among Atlantic *L. salmonis* samples.

The various efforts that have been made to understand the population genetic structure of *L. salmonis* have generated somewhat inconsistent and even contradictory results (e.g. Boulding et al. 2009 compared to Yazawa et al. 2008 and Tjensvoll et al. 2006). However, some general trends seem to exist among the Atlantic Ocean studies: (1) high levels of overall genetic variation are detected within the Atlantic Ocean, particularly in loci with high potential for polymorphism but this variation does not seem to be consistently associated with geographically discrete samples; (2) the frequent, extreme fluctuations in population growth and mortality rates experienced by *L. salmonis* on salmon farms seem to introduce considerable temporal instability within the population structure of lice on individual farms. If different farm lice samples are collected at different times, relative to the specific stage of the population growth cycle within each farm, no clear pattern of population structure may be definable; and (3) because the majority of *L. salmonis* samples have come from farmed Atlantic salmon hosts, the usefulness of these samples, given their potential temporal instability, may not provide results that are representative of large scale population dynamics or the evolutionary history of gene flow and genetic drift experienced by *L. salmonis.*

1.4.4b What evidence exists to suggest that Pacific and Atlantic *Lepeophtheirus salmonis* populations are different?

Very few population genetics studies have focused on *L. salmonis* from the Pacific Ocean (Yazawa et al. 2008, Boulding et al. 2009). The few studies that have quantified

the genetic variation of Pacific and Atlantic *L. salmonis* indicate that significant genetic differences very likely exist between these divergent lineages (Todd et al. 2004, Tjensvoll et al. 2006, Yazawa et al. 2008, Boulding et al. 2009). The preliminary studies of Pacific *L. salmonis* provide insufficient information for us to fully understand the population dynamics of *L. salmonis.* These studies included relatively small sample sizes and molecular markers that give a limited sense of the overall genetic variation among and within Pacific *L. salmonis* (Yazawa et al. 2008, Boulding et al. 2009).

It is possible that the evolutionary histories of the genetically distinct Atlantic and Pacific *L. salmonis* lineages have produced different population structures within the Pacific and Atlantic Oceans because Pacific and Atlantic *L. salmonis* have co-evolved with separate host species and in separate marine environments over evolutionary time (Yazawa et al. 2008). It is, therefore, important to evaluate patterns of gene flow and genetic drift within the Pacific Ocean, rather than assuming that the population structure of Pacific *L. salmonis* is the same as the population structure of Atlantic *L. salmonis* (Yazawa et al. 2008).

The genetic markers characterized through our research will contribute greatly to the available genetic resources that can be, and already have been (Glover et al. 2011), used for future evaluation of population structure in *L. salmonis*.

Chapter 2:
Assessment of population structure in Pacific *Lepeophtheirus salmonis* (Krøyer) using single nucleotide polymorphism and microsatellite genetic markers

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Abstract

The ectoparasitic sea louse, *Lepeophtheirus salmonis* (Krøyer), has caused great concern for both wild salmon fisheries and the salmon aquaculture industry. Identifying the population structure of this parasite is important for better understanding its dispersal capabilities and controlling louse infections. Most of the sea lice population studies carried out to date have been focused on Atlantic Ocean *L. salmonis* where host parasite interactions may be quite different from those in the Pacific Ocean. In this study we examined the genetic population structure of sea lice from 12 Pacific Ocean samples ranging from the Bering Sea to southern Vancouver Island using 27 microsatellite and 87 single nucleotide polymorphisms (SNPs) from 25 loci. Louse samples were analyzed for genetic differentiation among farmed and wild host salmon in addition to temporal differentiation from 2007 to 2009 and spatial differentiation over the entire sampling range. Our analyses failed to resolve significant population structure in *L. salmonis* for any of these three comparisons. Our results therefore support a hypothesis of high migration and panmixis of *L. salmonis* within the studied area of the Pacific Ocean.

2.1 Introduction

The sea louse, *Lepeophtheirus salmonis* (Krøyer), is an ectoparasitic copepod of marine salmonids in the northern hemisphere (Kabata 1979). Sea louse infections are common in adult Pacific salmonids (Nagasawa 2001 , Beamish et al. 2005, Costello 2006) as well as in farmed Atlantic salmon (*Salmo salar*) in British Columbia. The introduction of salmon farms to the Pacific Ocean may affect infection rates of wild fish through increased *L. salmonis* abundance and exposure of juvenile salmonids to these parasites (Krkošek et al.

2005, 2007). Sea louse infections cause host tissue damage, stress and immune dysregulation, which can lead to secondary infection and increased likelihood of mortality, conditions which may be magnified in juvenile fish (Reviewed in Costello 2006, Wagner et al. 2008). In addition to negative effects on wild hosts, louse infections on farmed Atlantic salmon are detrimental to the aquaculture industry, resulting in large economic losses (Johnson et al. 2004, Costello 2009).

An important part of understanding and controlling *L. salmonis* infections is identifying the dispersal capabilities of this species. Dispersal may be possible during all 10 *L. salmonis* life stages; larval stages are planktonic, juvenile through adult stages are mobilized through host migration, and eggs are likewise distributed through maternal release on migrating salmon (Kabata 1979, Johnson and Albright 1991b, Boxaspen 2006). This high potential for dispersal, coupled with the significant rate of *L. salmonis* infection in large populations of wild Pacific salmon (Beamish et al. 2005) may result in considerable gene flow and little population structure over the natural range of *L. salmonis* in the Pacific Ocean. The introduction of Atlantic salmon farms to the coast of BC may facilitate rapid, localized increases in louse population density which could affect parasite population dispersal and alter the population structure of this parasite in the areas surrounding farms; if the migration rate into these areas is low, louse populations should remain relatively isolated (Todd et al. 1997). If farm populations are reproductively isolated from lice on wild salmon, then reduced gene flow may be detected through the use of genetic markers that compare louse samples collected from wild and farmed salmon.

Most studies that have examined the population structure of *L. salmonis* have focused on the Atlantic Ocean, particularly the Northeastern Atlantic, where dispersal mechanisms and selective pressures on lice may differ from those experienced by Pacific *L. salmonis*. These Atlantic Ocean studies have produced contradictory conclusions, with results ranging from little to no detectable structure across the Atlantic (Todd et al. 2004, Tjensvoll et al. 2006) to significant structure within a relatively small coastal region (Dixon et al. 2004, Nolan and Powell 2009). In Ireland, significant temporal structure has been detected between lice collected from the same site sampled multiple times throughout a single year (Nolan and Powell 2009). These conclusions might be affected by small sample sizes and the previously limited availability of molecular markers. This, in addition to the genetic divergence between Atlantic and Pacific *L. salmonis* (Yazawa et al. 2008), warrants further investigation, particularly in the Pacific, where currently only one study by Boulding et al. (2009) has addressed population structure. This study has reported strong population structure in the Pacific, even between samples collected from wild and farmed hosts in the same archipelago; however, only one mitochondrial locus and few louse samples were analyzed, which may have resulted in poor representation of the *L. salmonis* genome.

The farming industry employs several strategies to manage *L. salmonis* infestations, including fallowing and spatial separation of farms from wild hosts. In addition to the use of these non-chemical treatment methods, chemical theraputants remain an important control method (Denholm et al. 2002, Read and Fernandes 2003, Costello 2006). As dependence on delousing drugs has increased, the efficacy of these drugs in treating *L. salmonis* infections has declined, particularly in Atlantic Ocean farms (Jones et al. 1992,

Denholm et al. 2002, Sevatdal and Horsberg 2003, Fallang et al. 2004, Lees et al. 2008). Although Pacific and Atlantic forms of *L. salmonis* are genetically distinct (Todd et al. 2004, Tjensvoll et al. 2006, Yazawa et al. 2008), independent development of drug resistance may still be possible in Pacific lice (Denholm et al. 2002). Development of drug resistance has been reported in a closely related species, *Caligus rogercresseyi*, on *S. salar* farms in Chile (Bravo et al. 2008). However, if large wild pacific salmonid populations act as a mechanism for dispersal of *L. salmonis*, resistant louse strains may be less likely to develop due to the homogenizing effects of migration and disparate selective pressures found in wild and farm environments (Sevatdal et al. 2005).

In the present study, we present the most comprehensive population genetic survey of *L. salmonis* to date. We employ the largest set of microsatellite markers and the first reported use of nuclear single nucleotide polymorphisms (SNPs) in population structure analysis of *L. salmonis*. Further, linked SNPs were combined into haplotypes in addition to being treated independently in an effort to increase statistical power (Morin et al. 2009a, Haasl and Payseur 2011). A total of 114 markers from 52 nuclear loci (27 microsatellites and 25 SNP loci) were used in the evaluation of 562 sea lice from 12 sample collections that range from the Bering Sea to southern Vancouver Island. Our analysis was conducted to determine if *L. salmonis* population structure exists in the Pacific Ocean. Understanding the population structure of Pacific *L. salmonis* is important for the successful management of this pest on salmon farms and evaluation of the impact of infections of wild salmonids.

2.2 Materials and methods

2.2.1 Sample collection

Lepeophtheirus salmonis were collected from 9 sites in 2009 along the central coast of BC and Vancouver Island (Figure 2). These collections included 3 *S. salar* farm sites, 2 of which were sampled in 2007 in addition to 2009. Each of these 9 sites was within 675 km of the other sites. One out-group from the Bering Sea was also sampled in 2007; this site was approximately 3160 km from the closest neighboring site. Between 38 and 56 individuals from each site were included in the analysis (see Table 1 for collection details and Figure 2 for collection locations).

Table 1. Collection details for *L. salmonis* samples used in the study.

Collection Site	Number of Individuals	Wild/ Farm	Host Species	Collection Date
Bering Sea (Bs)	56	Wild	*Oncorhynchus* sp.	November 2007
Quatsino (Qs07)	47	Farm	*S. salar*	November 2007
Quatsino (Qs)	48	Farm	*S. salar*	Summer/Fall 2009
Klemtu (Kt)	47	Farm	*S. salar*	July 22, 2009
Donegal (Dg)	44	Wild	*O. gorbuscha*	July 28/29, 2009
Ahta (At)	47	Wild	*O. gorbuscha*	July/August 2009
Kakweken (Kw)	47	Wild	*O. gorbuscha*	July/August 2009
Glendale (Gd)	47	Wild	*O. gorbuscha*	August 2009
Nodales (Nd07)	38	Farm	*S. salar*	November 2007
Nodales (Nd)	48	Farm	*S. salar*	August 24/27, 2009
Ucluelet (Uc)	46	Wild	*O. tshawytscha*	July 31/August 1, 2009
Pedder Bay (Pb)	47	Wild	*O. gorbuscha/ O. tshawytscha*	August 22, 2009

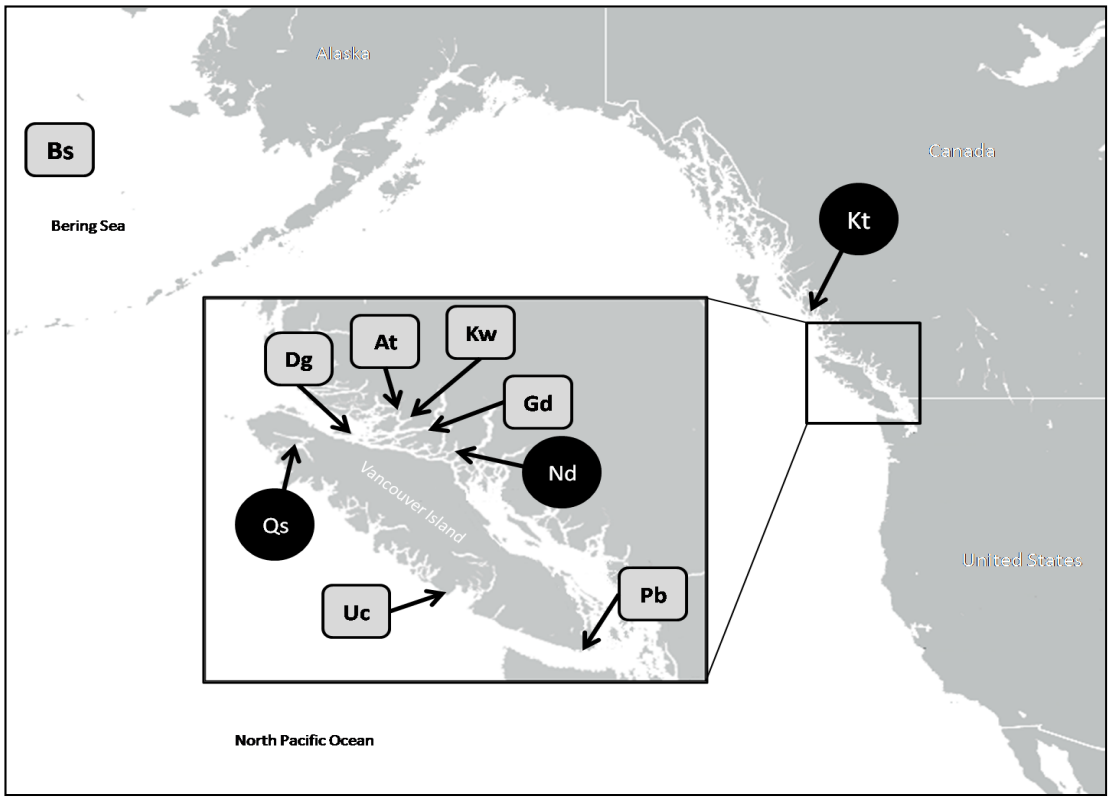

Figure 2. Map of the collection locations for the *L. salmonis* groups. Maps redrawn from OpenStreetMap; Map data (c) OpenStreetMap (and) contributors, CC-BY-SA, http://www.openstreetmap.org/.

2.2.2 DNA extractions

DNA was extracted from ethanol-preserved tissue using a Chelex extraction buffer protocol in a 96 well format (adapted from Nelson et al. 1998). Approximately 2 mm^3 of tissue from the anterior of each louse, avoiding mouthparts and reproductive region, was placed in 200 µl solution of 5% Chelex 100® (Biorad), 0.2% SDS and 0.27 mg/ml proteinase K (Invitrogen). Samples were incubated at 55 °C for 2 h followed by 95 °C for 10 min. A 1:10 dilution of extracted DNA in DNase/RNase free H_2O (Gibco) was used for all further work. Each plate contained 1–2 negative controls, where tissue was not added to the extraction buffer.

2.2.3 Locus characterization and amplification

Marker identification and primer design

A combined Pacific and Atlantic *L. salmonis* expressed sequence tag (EST) library of 76,642 EST sequences (Benson et al. 2005, Yazawa et al. 2008) was clustered into 19,122 transcripts using CAP3 assembly under default parameters (Huang 1999, Koop et al. 2008). This library was screened to identify potentially useful SNP and microsatellite loci. Microsatellites were detected using Repeat Finder software (Benson 1999, Table 8). SNP primer design was targeted to the 3′ end of the EST sequences. Primers were designed for these loci using Primer3 software (Rozen and Skaletsky 2000) and tested using 11 individual *L. salmonis* from geographically distinct locations to maximize polymorphism detection. We included 5 additional microsatellite loci described by Todd et al. (2004) in our study. These loci were identified from anonymous genome regions and are therefore less likely than EST derived microsatellites to be influenced by selection (Ellis and Burke 2007).

SNP amplification and sequencing

The primer pairs designed for SNP detection were evaluated for PCR specificity through agarose gel electrophoresis and sequencing accuracy through quality of sequence electropherograms. In addition, electropherograms were examined for polymorphism and only primer pairs that produced high quality sequence with at least one polymorphic site of a minor allele frequency (MAF) 15% were utilized in subsequent work. PCRs were conducted using 1 µl DNA template with each primer pair under the following conditions: 320 µM each dNTP (Promega), 0.5 µM each forward and reverse primer (IDT), 0.625 U Taq DNA polymerase (Promega), 1X GoTaq Flexi Colorless PCR buffer

(Promega), 2.5 mM $MgCl_2$ to a final volume of 20 µl with DNAse/RNAse free H_2O (Gibco). Reactions were performed using a PTC-225 Peltier Thermalcycler (MJ Research) or TC-412 (Techne) under the following reaction profile: 3 min at 95 °C followed by 42 cycles of 30 s at 95°C, 30 s at 52°C and 30 s at 72°C, with a final extension of 72 °C for 10 min. Following amplification, the reactions were purified using the MinElute 96 UF PCR purification kit (Qiagen) and eluted in 30 µl of DNAse / RNAse free H_2O (Gibco). Average DNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer. Approximately 20 ng of each sample was used for each 5 µl sequencing reaction which contained 0.5 µl BigDye Terminator v3.1 (ABI) sequencing buffer, 0.5 µl BigDye Terminator v3.1 (ABI) ready reaction mix and 0.64 uM forward or reverse primer (IDT) (see Table 2). Sequencing reactions were conducted with a 1 min 95 °C initial denaturation followed by 30 cycles of 30 s at 95 °C, 15 s at 50°C and 90s at 60°C with a final extension of 5min at 72°C. The samples were ethanol precipitated and re-suspended in 25µl of DNAse / RNAse free H_2O (Gibco) and run on an ABI 3730 DNA Analyzer. All low quality sequence, either due to insertion/deletion, microsatellite slippage or sequencing reaction failure, was removed from the data set using the trim function in Geneious 4.7.6 (error probability limit of 0.01) (Drummond et al. 2009). Sequences were aligned in Geneious with default parameters and trimmed so that ~75% of the sequences contained only high quality base calls. Short sequences were elongated to match the size of full-length sequences using the ambiguous base code N. All ambiguous bases found in high quality regions were scored manually using the IUB/IUPAC ambiguous base code. Any base with an MAF 5% relative to the consensus in any one population was retained for further analysis. The SNP haplotype of each

individual was identified using the program PHASE 2.1.1 using default parameters; these haplotypes included all observed SNPs (Stephens et al. 2001, Stephens and Donnelly 2003)

Table 2. Primer pairs used in the amplification and sequencing of DNA fragments that contain SNPs used in the study. All primer pairs use an annealing temperature of 52 °C. a) alternative locus designation, b) indicates whether the forward or reverse primer was used for sequencing, c) expected PCR product size, d) number of SNPs with greater than 5% minor allele frequency (MAF).

Locus #/ Alt. Des.[a]	Forward Primer	Reverse Primer	Seq. Primer[b]	b.p.[c]	>5% MAF[d]
1174/1,A06	CTGAACATTCCTGGAACTACG	GACGCAAACAACACTTTTCC	Rev	500	4
6222/1,A09	AGTGTGACCTCTATTTAACGGG	GTTATCCGAAAATCCCTTCC	For	506	8
8997/1,A10	TCCATGAGAATGAATGAGGG	GGGGACTGACAAGAAGAAGG	Rev	564	6
9854/1,B01	TTGGAAATTGGATAGGAGGG	CATTTGAACAATACACGGGC	For/Rev	530	3
5757/1,B05	AATTCTCCTACTATGCCCGC	GATCCATGTATTTGCTTGCC	For	528	2
8588/1,B06	TCATTGTGACCAATTTGACG	GTTCAAAGCAATCTTGAGGC	For	544	3
9860/1,B12	TGTTCTGGAGAGGATTGTCC	ACATGGGATTGTCAATTTGC	For	533	1
168/1,C02	GGGCATCAATATTTCATTCG	TAACGAATGATCTTACAATGAGG	Rev	628	2
7006/1,C03	TCAAAGAATTAACCTCTGATGG	AGGATACCAGCTCCTTCTGG	Rev	518	3
3147/1,C04	TCATGGCTCCAACTCTTAGC	TTTGGGTATATTAATCCGGC	Rev	618	2
80/1,C06	CTGCTGGAGATCGACTTAGG	AACAGTAACTGAGGGCAAGG	For	555	8
8283/1,C07	TGTTACAATGTTGGCTAAGGC	GACAGCCAAGATGAGAAACC	Rev	589	4
8590/1,C11	TTCATCCAAATCCTCAATTAGC	TTCATCATAGACCCTCTGCC	For	527	2
930/1,C12	TGGATGAGGGTATTCGTAGG	TTGCGTGACATAGAAGAATCC	Rev	522	2
8241/1,D04	TCGGAGTAGGAGAAATCACG	AATCCGTTTTCATTCGTTCC	Rev	513	2
10458/1,D05	TTCTGTGAAGTGACAGCAGC	TCGTAGAAAACCATTCACCC	Rev	582	3
7499/1,D06	TCTCTTCTTGGGTTGTGAGG	AGGATTCCTCAGGCAGTAGC	For	630	2
8731/1,D08	TAAAGGCTGTGAAAACTGGG	CAAAGAATATTTGGAGTAAAGCC	For	611	3
234/1,D09	AAATACAAAAATCCGGGAGG	AAATCTTGACGTTGCTGTCC	For	515	3
10103/1,D10	TTATACATTTTTCCGGAAGGG	TCATGTGTTGTGCTTCTTGC	For	516	3
2055/1,D12	ATGCGGATCAATGATGTACC	TTTCCCAAAATGTAAAACGC	For	527	3
396/1,E01	AAAGGAATCCTCCATTTTCG	ATGTCCTTAGGGGACACTCC	Rev	581	5
7844/1,E04	GTGGAGTAGGAGGGGATAGC	CTCTCTCTCTCTCAACATCACG	For	512	6
5803/1,E08	CCTTCATCTTCTCAAACCCC	TTACTTCCATTTTCGTTGGC	For	601	2
10295/1,E10	ATCTCGAGGTCTTCAAATCG	AGCGAAATATGGATTAGGTCC	Rev	522	5

Microsatellite amplification and scoring

Microsatellite loci were PCR amplified using 22 newly reported primer pairs and 5 primer pairs that have been used in previous population studies on Atlantic *L. salmonis* (Table 8) (Todd et al. 2004). PCRs were conducted using 1 µl DNA template with each primer pair under the following conditions: 320µM each dNTP (Promega), 0.48µM each forward and reverse primers with one primer 5′ labeled with 6FAM or HEX fluorescent dyes (IDT), 0.5 U Taq DNA polymerase (Promega), 1X GoTaq Flexi Colorless PCR buffer (Promega), 2.5 mM $MgCl_2$ to a final volume of 12 µl with DNAse/ RNAse free H_2O (Gibco). Reactions were performed using a PTC-225 Peltier Thermalcycler (MJ Research) or TC-412 (Techne) under the following reaction profile: 3 min at 95 °C followed by 40 cycles of 30 s at 95 °C, 30 s at the optimal annealing temperature and 30 s at 72 °C (Table 8). Some loci required a final extension step of 72 °C for 10 min (Table 8). Genotyping cocktails consisted of 0.5 µl PCR product, 9.9 µl Hi-Di™ Formamide (ABI) and 0.1 µl GeneScan™ 500-ROX™ Size Standard (ABI). Samples were denatured at 95 °C for 3 min and immediately chilled on ice for 5min before fragment size separation on an ABI 3730 DNA Analyzer. Electropherograms were scored using GeneMapper V4.0 (ABI). Allele bins of 0.8 base pairs (bp) in width were identified manually; scoring was automated and reviewed for errors in each individual. Any samples in which the peaks could not be clearly identified were removed from the data set.

2.2.4 Data analysis

The three data sets: microsatellite loci, SNP loci scored independently and SNP loci scored as haplotypes, were treated independently for each analysis. Locus summary

information was collected for all microsatellite and haplotyped SNP loci. Micro-Checker (van Oosterhout et al. 2004) was used to screen microsatellite loci for null (unamplified) alleles. Each data set was evaluated for linkage disequilibrium (LD) using Genepop 4.0 with default parameters (Raymond and Rousset 1995, Rousset 2008). MSA 4.05 (Dieringer and Schlötterer 2003) was used to collect microsatellite allele counts while for the SNP data Convert 1.31 (Glaubitz 2004) was used to determine allele frequencies. Arlequin 3.11 (Excoffier et al. 2005) was used with default settings to test the genotype data for Hardy-Weinberg Equilibrium (HWE), observed heterozygosity and Nei's expected heterozygosity (H_o and H_e) for each locus, population specific F_{IS}, mean H_e per locus over all populations, and pairwise F_{ST}. Private alleles (alleles observed in only one sample) were identified with Convert 1.31 and the frequency of private alleles was used to estimate the number of migrants (Nm) using Genepop V4.0. Global F_{ST}s were determined using Genepop V4.0 with default settings. Global and pairwise F_{ST} values were compared between EST-microsatellites and anonymous microsatellites. All analyses of molecular variance (AMOVA) partitioning variance among populations, among individuals within populations and within individuals were calculated under default parameters for each data set in Arlequin 3.11. Correlation between genetic and geographic distance was determined with a Mantel test in Arlequin 3.11 with 100,000 permutations. Population structure was assigned independently of sample sites using Structure 2.3 (Pritchard et al. 2000, Falush et al. 2003). These analyses were done using a Burnin of 100,000 steps followed by 100,000 Markov-chain Monte Carlo steps. We ran these analyses with and without considering the sample sites and assumed the admixture model with allele frequencies correlated among populations. This simulation was run

expecting that all individuals cluster into 1–5 groups (K=1–5) at 3 iterations for each value of K.

2.3 Results and discussion

2.3.1 SNP detection and primer design

An EST database and clustered contiguous sequences were used to identify 118 putative transcripts from which primer pairs in the 3′ regions were identified. Of these primers, 94 pairs produced strong PCR amplified DNA segments of approximately 500-650 bp which contained at least 1 possible SNP. In the 11 individuals screened, 500 suspected SNPs were detected. Sequences that did not possess a site with a MAF >15% were discarded, leaving 57 loci, of which 35 were used in further analysis. Not all of the 35 primer pairs produced useable data for all of the 12 populations in this study. Three loci contained simple sequence repeats and 4 loci produced low quality electropherograms for >50% of the individuals. Two of the amplified products contained bases that appeared as SNPs, but produced peaks of unequal height in >50% of the ambiguous bases, which made base calling difficult and unreliable. One locus contained a single SNP at the end of the fragment, resulting in this base being trimmed for quality in >25% of individuals. The remaining 25 primer pairs (Table 2) produced clear sequences with scorable SNP sites. All sequences for each individual were concatenated and separated by a short linker sequence (WMDDMW) to construct a sequence string that included all amplified fragments for each individual (Table S2). A total of 87 SNPs of >5% MAF were found distributed in the 25 loci, with 1 to 8 SNPs per locus. SNP names and sequence locations are provided in Table 9.

All SNPs from the same locus were combined, and haplotypes were inferred using the program PHASE2.1.1. The inclusion of one marker, LsaSP0708UVic, in determining the likely haplotype of locus E01, produced an ambiguous result in >15% of the individuals, and was removed from the haplotype analysis. Thus, 85 markers grouped into 24 sets of 2–8 SNPs and a single un-linked SNP from locus B12 were used in the subsequent haplotype analysis.

2.3.2 Descriptive statistics

Allele summaries

Two alleles were observed in most SNP sites, while only 9 of 87 SNPs had 3 observed alleles (Table 10a). In 2 of these 9 instances, all 3 alleles occurred frequently; in the other 7 instances, the 3rd allele was found very infrequently and always represented a private allele. None of these private alleles were detected in more than one individual (Table 3). Each SNP haplotype marker possessed between 2 and 16 haplotypes (Table 10b) and 24 private haplotypes were observed in the entire data set (Table 3). When private alleles are prevalent in a population they can be indicative of restricted gene flow (Slatkin 1985). Private alleles were detected in all samples in this data set; the highest frequency of private alleles was observed in the Quatsino 2007 sample, with 4 private alleles including one that was observed in 2 individuals.

The number of alleles observed at each microsatellite locus ranged from 5 to 39 with an average of 15.8 alleles per locus (Table 10c). The number of alleles observed ranged from 5 to 29 for the EST-microsatellites and 17 to 39 for the anonymous microsatellites (Table 10c). Private alleles in this data set were observed~1% of the time; 96% of all private alleles were observed in one individual and none were observed more than twice

(see Table 3). Overall, few private alleles were detected and their prevalence was not high enough to provide evidence of restricted gene flow.

Table 3. Summary of private alleles detected in analysis of the 12 *L. salmonis* collections using each of the three data sets. PA = private alleles, see Table 1 for sample abbreviations.

		Bs	Qs07	Qs	Kt	Dg	At	Kw	Gd	Nd	Nd07	Uc	Pb	Total
Individual SNPs	PA seen 1X	1	0	0	0	0	2	1	1	1	0	1	0	7
	PA seen 2X	0	0	0	0	0	0	0	0	0	0	0	0	0
Haplotyped SNPs	PA seen 1X	2	3	1	3	2	3	2	1	1	1	3	1	23
	PA seen 2X	0	1	0	0	0	0	0	0	0	0	0	0	1
Microsatellites	PA seen 1X	6	8	7	13	6	8	5	10	7	7	3	8	88
	PA seen 2X	0	2	0	0	0	0	0	0	0	0	0	1	3

Table 4. A determination of the average number of migrants into each collection of *L. salmonis* using a private allele method from GenepopV4.0.

		Individual SNPs	SNP haplotypes	Micro-satellites
Mean sample size	$\bar{n}$	45.35	45.18	46.47
Mean private allele frequency	$\bar{p}$ (1)	0.0107	0.0115	0.0114
Number of migrants (Nm) for	$\bar{N} = 10$	121.91	105.14	107.43
	$\bar{N} = 25$	31.26	27.57	28.07
	$\bar{N} = 50$	17.52	15.57	15.84
Nm corrected for sample size		19.32	17.23	17.04

Hardy–Weinberg equilibrium

The probability of departure from HWE was calculated for each locus in each sample to ensure that the assumptions of HWE were generally met before further tests were run. The 1044 tests for HWE in the individual SNP dataset revealed 28 individual loci that significantly deviated from HWE at $\alpha = 0.05$ and no tests were significant at a more stringent $\alpha = 0.001$ (Table 10a). This more stringent significance value was used as an

alternative to Bonferroni corrections. Some individual SNP samples deviated from HWE more frequently than others at α=0.05 (Table 10a). The HWE test for the SNP haplotype data indicated that 28 loci in individual samples were out of HWE at α = 0.05, which was reduced to 1 sample at α = 0.001 (Table 10b). The test for HWE in the microsatellite data indicated that 12 loci in individual samples were out of HWE; however, none were out of HWE at α = 0.001 (Table 10c). In agreement with tests for HWE, all F_{IS} values were small and did not suggest the occurrence of inbreeding in our samples (Table 10).

Linkage disequilibrium

Linkage disequilibrium (LD), the non-random assortment of alleles in separate loci, can be caused by physical linkage on a chromosome or by epistatic effects resulting in instances where certain alleles are frequently associated with each other. Of the 44,892 pairwise comparisons of individual SNP site LD, 1982 were significant at α = 0.05; however, after removal of the 579 that suggested linkage between SNPs already known to be found in the same locus, the number was lowered to 1403. Of these, only 12 remain significant at α=0.001 and none of these occur between SNPs from the same PCR fragment. When SNP haplotypes were screened for LD, 178 of 3600 pairwise comparisons were significant at α = 0.05 and only 4 were significant at α = 0.001. Analysis for LD among the microsatellite loci indicated that at α = 0.05, 185 of 4212 pairwise comparisons of were likely to be linked; this was reduced to 27 at α=0.001.

2.3.3 Population structure in Pacific *Lepeophtheirus salmonis*

F_{ST} values indicate the degree of structure among subpopulations through comparing H_e of the entire population to the H_e of each subpopulation that is included in the analysis. As the F_{ST} value approaches 1, it indicates stronger reproductive isolation

among subpopulations. Global F_{ST} values average the individual F_{ST} values obtained for each locus and each sample in the data set. In our study, global F_{ST} values indicate no significant overall structure, with $F_{ST} = 0.0$ for all 3 data sets. Pairwise F_{ST} analysis between samples in each data set revealed only one significant but small result of $F_{ST} = 0.006$ ($P = 0.018$) for the comparison between Bering Sea and Donegal using the individual SNP data set (Table 4).

An extension of F-statistics is an AMOVA, which uses a genetic distance matrix to make hierarchical comparisons among samples to determine where most variation occurs in a population (e.g. among individuals or among groups of individuals; Excoffier et al. 1992). AMOVA results of this study indicate that essentially all (98–99%) observed variation is due to differences in allele frequency at the level of the individual (Table 5a), a result that again suggests that no population structure is present. For the entire data set, no significant F_{ST} values were detected, aside from the single pairwise F_{ST} value in the individual SNP analysis. None of the hierarchical sample combinations revealed significant population structure (Table 5b–d).

Regional reproductive isolation has been indicated in studies of Irish (Nolan and Powell 2009), Scottish (Todd et al. 1997, Dixon et al. 2004), Norwegian and British Columbian sea lice (Boulding et al. 2009), while other studies have reported no differentiation (Todd et al. 2004, Tjensvoll et al. 2006; Kevin Glover, personal communication). In our analysis there was no indication of structural division among samples collected in BC (excluding the Bering Sea out-group). When all sites were included no significant global F_{ST} or AMOVA was observed and only one significant but low pairwise F_{ST} result was observed between Donegal and the Bering Sea (Table 4b).

These results suggest that gene flow is strong enough to maintain panmixis in the Northeastern Pacific Ocean and some slight population structure may exist between the Bering Sea and the coast of British Columbia.

Table 5. Pairwise F_{ST} statistics (below diagonal) and associated P-values (above diagonal) for the comparison of 12 *L. salmonis* samples, as determined by Arlequin 3.11. The table is separated by data set into a) Haplotyped SNP analysis, b) Individual SNP analysis and c) Microsatellite analysis. See Table 1 for sample abbreviations.

		KT	QS	UC	PB	ND	GD	KW	AT	DG	QS07	BS	ND07
a)	KT	*	0.75676 ±0.0364	0.90991 ±0.0253	0.60360 ±0.0526	0.81982 ±0.0439	0.79279 ±0.0485	0.99099 ±0.0030	0.30631 ±0.0454	0.54054 ±0.0359	0.98198 ±0.0096	0.86486 ±0.0389	0.98198 ±0.0096
	QS	0.000	*	0.99099 ±0.0030	0.72973 ±0.0287	0.77477 ±0.0310	0.30631 ±0.0388	0.95495 ±0.0203	0.99099 ±0.0030	0.98198 ±0.0096	0.99099 ±0.0030	0.22523 ±0.0434	0.95495 ±0.0151
	UC	0.000	0.000	*	0.97297 ±0.0184	0.98198 ±0.0096	0.99099 ±0.0030	0.99099 ±0.0030	0.85586 ±0.0312	0.99099 ±0.0030	0.99099 ±0.0030	0.81081 ±0.0304	0.87387 ±0.0334
	PB	0.000	0.000	0.000	*	0.79279 ±0.0354	0.98198 ±0.0096	0.69369 ±0.0526	0.38739 ±0.0273	0.89189 ±0.0287	0.94595 ±0.0205	0.37838 ±0.0227	0.23423 ±0.0511
	ND	0.000	0.000	0.000	0.000	*	0.54955 ±0.0438	0.72973 ±0.0479	0.36036 ±0.0489	0.88288 ±0.0228	0.90090 ±0.0272	0.90090 ±0.0333	0.60360 ±0.0576
	GD	0.000	0.001	0.000	0.000	0.000	*	0.93694 ±0.0203	0.18018 ±0.0359	0.79279 ±0.0327	0.99099 ±0.0030	0.54955 ±0.0613	0.84685 ±0.0244
	KW	0.000	0.000	0.000	0.000	0.000	0.000	*	0.79279 ±0.0379	0.77477 ±0.0279	0.99099 ±0.0030	0.53153 ±0.0345	0.97297 ±0.0184
	AT	0.001	0.000	0.000	0.001	0.001	0.002	0.000	*	0.49550 ±0.0280	0.95495 ±0.0151	0.36937 ±0.0628	0.88288 ±0.0228
	DG	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*	0.98198 ±0.0096	0.63964 ±0.0516	0.47748 ±0.0305
	QS07	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*	0.44144 ±0.0459	0.89189 ±0.0345
	BS	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*	0.16216 ±0.0326
	ND07	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.002	*
b)	KT	*	0.95495 ±0.0203	0.97297 ±0.0125	0.70270 ±0.0485	0.88288 ±0.0184	0.93694 ±0.0203	0.99099 ±0.0030	0.72072 ±0.0636	0.29730 ±0.0576	0.99099 ±0.0030	0.95495 ±0.0203	0.80180 ±0.0516
	QS	0.000	*	0.99099 ±0.0030	0.81982 ±0.0287	0.93694 ±0.0244	0.90991 ±0.0287	0.95495 ±0.0151	0.99099 ±0.0030	0.89189 ±0.0253	0.99099 ±0.0030	0.56757 ±0.0360	0.98198 ±0.0096
	UC	0.000	0.000	*	0.81081 ±0.0429	0.97297 ±0.0125	0.99099 ±0.0030	0.99099 ±0.0030	0.91892 ±0.0184	0.90090 ±0.0236	0.99099 ±0.0030	0.83784 ±0.0196	0.79279 ±0.0228
	PB	0.000	0.000	0.000	*	0.90991 ±0.0165	0.98198 ±0.0096	0.51351 ±0.0471	0.49550 ±0.0475	0.97297 ±0.0125	0.89189 ±0.0318	0.14414 ±0.0411	0.13514 ±0.0474
	ND	0.000	0.000	0.000	0.000	*	0.81081 ±0.0272	0.69369 ±0.0471	0.29730 ±0.0408	0.97297 ±0.0125	0.88288 ±0.0403	0.15315 ±0.0305	0.58559 ±0.0413
	GD	0.000	0.000	0.000	0.000	0.000	*	0.94595 ±0.0154	0.70270 ±0.0466	0.89189 ±0.0287	0.99099 ±0.0030	0.18919 ±0.0394	0.72973 ±0.0395
	KW	0.000	0.000	0.000	0.000	0.000	0.000	*	0.95495 ±0.0203	0.34234 ±0.0485	0.96396 ±0.0142	0.62162 ±0.0459	0.74775 ±0.0471
	AT	0.000	0.000	0.000	0.000	0.002	0.000	0.000	*	0.24324 ±0.0333	0.99099 ±0.0030	0.59459 ±0.0578	0.53153 ±0.0459
	DG	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.002	*	0.91892 ±0.0228	**0.01802 ±0.0121**	0.33333 ±0.0360
	QS07	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*	0.57658 ±0.0411	0.66667 ±0.0364
	BS	0.000	0.000	0.000	0.003	0.002	0.001	0.000	0.000	**0.006**	0.000	*	0.08108 ±0.0344
	ND07	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.001	0.000	0.004	*
c)	KT	-	0.11712 ±0.0408	0.44144 ±0.0417	0.72072 ±0.0333	0.91892 ±0.0298	0.36937 ±0.0344	0.38739 ±0.0471	0.95495 ±0.0203	0.91892 ±0.0266	0.64865 ±0.0522	0.31532 ±0.0434	0.37838 ±0.0446
	QS	0.002	-	0.55856 ±0.0466	0.51351 ±0.0490	0.30631 ±0.0454	0.09910 ±0.0163	0.30631 ±0.0338	0.36036 ±0.0383	0.84685 ±0.0389	0.55856 ±0.0227	0.36036 ±0.0606	0.53153 ±0.0459
	UC	0.000	0.000	-	0.37838 ±0.0379	0.89189 ±0.0253	0.65766 ±0.0360	0.69369 ±0.0360	0.49550 ±0.0412	0.60360 ±0.0622	0.79279 ±0.0446	0.65766 ±0.0543	0.98198 ±0.0096
	PB	0.000	0.000	0.000	-	0.90991 ±0.0214	0.40541 ±0.0595	0.68468 ±0.0546	0.11712 ±0.0273	0.94595 ±0.0281	0.72072 ±0.0606	0.97297 ±0.0125	0.60360 ±0.0576
	ND	0.000	0.000	0.000	0.000	-	0.41441 ±0.0454	0.96396 ±0.0142	0.80180 ±0.0439	0.99099 ±0.0030	0.91892 ±0.0298	0.47748 ±0.0637	0.54054 ±0.0489
	GD	0.000	0.002	0.000	0.000	0.000	-	0.11712 ±0.0305	0.52252 ±0.0647	0.18919 ±0.0417	0.47748 ±0.0471	0.15315 ±0.0490	0.49550 ±0.0475
	KW	0.000	0.001	0.000	0.000	0.000	0.002	-	0.48649 ±0.0529	0.62162 ±0.0598	0.45045 ±0.0525	0.51351 ±0.0385	0.45946 ±0.0515
	AT	0.000	0.000	0.000	0.001	0.000	0.000	0.000	-	0.59459 ±0.0529	0.93694 ±0.0279	0.63964 ±0.0459	0.61261 ±0.0485
	DG	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	-	0.93694 ±0.0203	0.54054 ±0.0621	0.62162 ±0.0613
	QS07	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	0.62162 ±0.0723	0.23423 ±0.0364
	BS	0.000	0.001	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	-	0.36036 ±0.0742
	ND07	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	-

2.3.4 Analysis of data using the Structure program

The program Structure groups individuals based on genotype without considering collection site information. This is useful when attempting to elucidate population structure in a species without clear population boundaries, such as *L. salmonis*. Our simulations in Structure failed to reveal any population groupings for either the SNP haplotype data set or the microsatellite data set (data not shown). This was true when between 2 and 5 groupings (K-values) were imposed on the data sets. It is therefore unlikely that the sample site comparisons we have made in other analyses overlook any cryptic population structure in the entire data set. The individual SNP data set was not included because Structure will not work properly with linked loci.

2.3.5 Host habitat as a source of population structure

We compared samples from farmed and wild salmon to determine whether unique louse populations exist on farms. Farm specific population structure may exist if the rate of gene flow between wild and farm environments is low enough to allow unique allele frequencies to persist in *L. salmonis* on farms. This type of population structure is of particular interest when considering the development and spread of delousing treatment resistance in lice, and when trying to identify the source of louse infections on juvenile Pacific salmon. In order for resistant louse strains to become prevalent they would likely require an environment with constant selective pressure for resistance and isolation from non-resistant migrants, which would slow the establishment of the resistant phenotype. If farm lice are genetically distinct from wild lice, it may also be possible to determine whether louse infections on wild juvenile salmon originate on Atlantic salmon farms. Our results fail to find any evidence of this type of population structure through pairwise F_{ST}

analyses, as none of the comparisons between samples collected from wild and farmed hosts, or between 2 collections from farmed hosts in separate locations were significant. A hierarchical AMOVA, where all wild samples were grouped together and each farm sample was treated as an independent group, indicates that less than 0.025% of observed genetic variance can be attributed to differences among these groups (Table 5). The F-statistic that reports structure among hierarchical groupings in AMOVA (F_{CT}) was 0.0 for each of the 3 datasets (Table 5b P = 0.348–0.936). In addition, the private allele method of Nm estimation indicates that 17–19 individuals migrate among sample sites per generation (Table 6). This number of migrants is well above the minimum estimates required to counter the effects of genetic drift (Slatkin 1985).

Evaluation of farm vs. wild louse populations has been previously analyzed in both the Atlantic and the Pacific Oceans. Todd et al. (2004) were unable to identify significant structure in analysis of six microsatellite loci using lice collected from wild hosts (Atlantic salmon and sea trout) and from farmed hosts (Atlantic salmon and rainbow trout). A study of cytochrome c oxidase subunit 1 (COI) sequences in samples from the Pacific Ocean produced large pairwise F_{ST} values between lice from farmed and wild hosts (Boulding et al. 2009). The result of Boulding's Pacific *L. salmonis* study strongly contrasts with our own; however, this may be due in part to differences in sample sizes and marker types used in these studies. Our study did not detect any significance in pairwise comparison among sites that are in similar locations to many of those used by Boulding et al. (2009). No pairwise F_{ST} comparisons of farm to wild samples in our study revealed significant population structure. Our data suggest that no barrier to gene flow

exists between farms and wild host habitats, as indicated by the lack of population structure detected between wild and farm sea lice samples.

Costello (2006) has estimated that sea lice larvae typically disperse 10–50 km from their point of origin, a distance which is likely sufficient for lice to move between wild and farmed hosts in the channels and inlets where farms are often located. While the large Pacific salmon runs where fish migrate from the open ocean through near shore waters are likely significant contributors to the homogenous population structure we have observed in *L. salmonis*, additional influences may include the presence of juvenile salmonids that over-winter near shore (Beamish et al. 2007) and non-salmonids such as stickleback that act as temporary hosts to *L. salmonis* (Jones et al. 2006). All of these factors may increase the mobility of sea lice among different habitats, beyond their dependence on planktonic larval dispersal alone.

2.3.6 Temporal variation and population structure

A comparison of *L. salmonis* collected from 2007 and 2009 was included in this study to assess the temporal stability of Pacific *L. salmonis* population structure. The frequency of founder effects and strength of genetic drift may influence the long-term retention of particular phenotypes, including those involved in drug resistance. Pairwise F_{ST} values from temporally separated farm samples (Nodales vs. Nodales 2007, Quatsino vs. Quatsino 2007) were very low and not significant (Table 4). In addition, the AMOVA results from samples grouped by year show that F_{CT} was not significant and that 100% of the variance that does occur is due to within group variation (Table 5c).

In comparison to our results in the Pacific Ocean, the occurrence of temporal variation in *L. salmonis* population structure in the Atlantic Ocean is not clear. In one study of an

Irish farm, significant temporal differences were detected in lice collected throughout one year, an effect attributed to site-specific factors (Nolan and Powell, 2009). However, a Norwegian study of lice structure before and after a chemical delousing treatment was unable to distinguish more than one weakly significant pairwise comparison in a site where treatment failed (Kevin Glover, personal communication). Our study differs as we collected lice in the Pacific and examined temporal variation using 2 time points that are more distant than those of other studies. Our results do not suggest that bottlenecks or genetic drift frequently influence Pacific sea lice population structure. It may be possible that the time of year that collections were made has influenced our results. Sampling occurred in November in 2007 and through July and August in 2009. Our samples were taken during or shortly after the time of year when wild salmon migrate past salmon farms toward fresh water. During this migration, lice may be transferred from wild to farmed hosts. Because both of our Pacific Ocean 2007 samples are from farms, it may be possible that wild salmon migration has influenced our results. However, this is not likely because similar allele frequencies were observed in the Bering Sea sample, which was also collected in 2007 from wild salmon. More rigorous sampling at shorter time intervals may reveal genotypic shifts in farms during periods when adult Pacific salmon are not near shore. If however, seasonal changes do not occur on farms, sampling over longer intervals may provide detection of genetic drift or bottleneck events. Our results indicate that either no significant temporal variation develops over a span of two years in the louse populations of coastal BC, or a sufficiently large influx of lice from returning wild fish can lead to infection of farms at a rate that effectively masks any previous founder effects on farms.

2.3.7 Geographic distance and population structure

Identifying the scale and degree to which isolation by distance occurs in Pacific *L. salmonis* is important to consider when estimating the ability of particular phenotypes to become established in new regions. Isolation by distance can occur when boundaries between distinct subpopulations are weak. As the geographic range increases in a continuous population, the likelihood of the even distribution of allele frequencies decreases over the population range. The strength of this isolation may indicate how quickly new alleles, such as those that confer drug resistance, may become established over large areas. Correlative analysis of pairwise F_{ST} values with geographic distance did not suggest significant isolation by distance (Figure 3), which indicates that *L. salmonis* are capable of long-range dispersal.

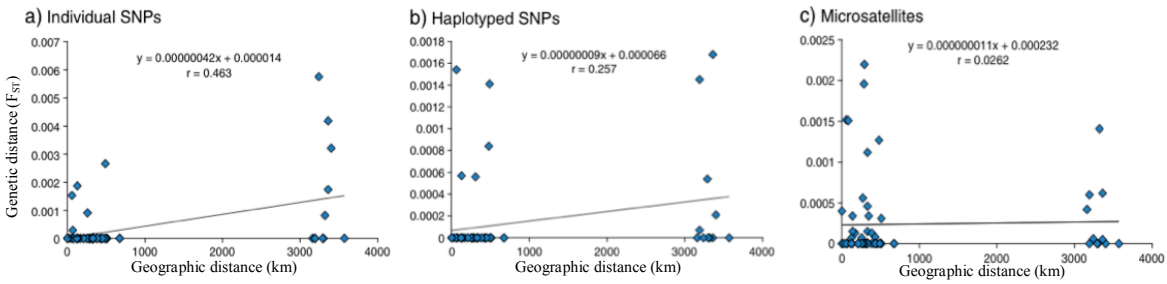

Figure 3. Plots of pairwise F_{ST} values against the geographic distances between the sample collection sites. Least-squares regression is used to plot a line of best fit, and a Mantel test used to determine the correlation between the statistical and geographical distances using Arlequin 3.11. a) Analysis using the Individual SNP data set; b) analysis using the Haplotyped SNP data set; and c) analysis using the Microsatellite data set. Geographic distance plotted on the x-axes and pairwise F_{ST} plotted on the y-axes.

The hierarchical AMOVA that partitioned variance between Pacific Ocean and Bering Sea samples detected some structure from the 2 SNP data sets. Low but significant F_{CT} values were observed between the Pacific Ocean and Bering Sea groups, with $F_{CT} = 0.004$ ($P = 0.001$) and $F_{CT} = 0.003$ ($P = 0.026$) for the individual and haplotyped SNPs

respectively (Table 5d). The corresponding variance components were still quite small, accounting for 0.37% and 0.26% of the variation within each SNP data set respectively, while most of the variance was still attributed to within group variation. In addition, the only significant (but small) pairwise F_{ST} value was observed between the Bering Sea and Donegal samples (F_{ST} = 0.006, P = 0.018; Table 4a), while the microsatellite data set showed no variation (F_{CT} = 0.0, P = 0.449; Table 5d).

The Aleutian Islands may be a barrier to dispersal between the Bering Sea and Pacific Ocean, although this was only very weakly suggested by our analysis. If structure exists between the Bering Sea and Pacific Ocean, we have found only minimal evidence of it, with one significant pairwise F_{ST} value between the two wild collection sites of Bering Sea and Donegal when individual SNP loci were compared. Because the Bering Sea and Donegal samples were collected in different years we cannot rule out the possibility that the observed structure was due to temporal rather than geographic variation.

While Todd et al. (2004) found no evidence of population structure across the Atlantic Ocean and Tjensvoll et al. (2006) found little structure between European and Atlantic Canadian lice, a re-analysis of the COI data of Tjensvoll et al. (2006) in Boulding et al. (2009) and a study of microsatellite data by Kevin Glover (personal communication) have detected isolation by distance in the Atlantic Ocean. Wild Canadian Atlantic salmon have been found in the Norwegian Sea; likewise wild European and North American Atlantic salmon have been found near Greenland (Hansen and Jacobsen 2003). These long-range salmon migrations may provide dispersal opportunities for *L. salmonis*; however, the number of wild salmonids in the Atlantic Ocean is much smaller than the number of wild salmonids in the Pacific Ocean. It therefore follows that the frequency of

long distance *L. salmonis* dispersal, facilitated by host migration, should be more frequent in the Pacific than Atlantic Ocean (Heuch et al. 2005). Pacific salmon are likely to act as a mechanism of sea louse dispersal; each summer, large numbers of louse-infected wild salmon return towards shore, destined for their natal freshwater spawning grounds (Beamish et al. 2005). When salmon become concentrated in coastal waters, louse densities also increase and the likelihood of reproduction between lice from previously distant locations should be greater than the period previous to this host migration.

Future studies should include louse samples from the northwestern Pacific, for example from Japan and Russia, to determine whether isolation by distance can be detected over a broader Pacific *L. salmonis* range. A more confident evaluation of isolation by distance could be made if all included samples were collected during the same year; this would reduce the possibility of temporal variability confounding the results.

Because 22 of 27 microsatellite loci included in this study were developed from EST sequences, it is possible that these 22 markers are linked to genes under selective pressure. Stabilizing selection on these gene regions may result in an underestimation of population divergence; however, employing a large number of loci, presumably from different locations in the genome, should help to reduce any effects that selection may have on particular loci (Ellis and Burke 2007). Previous studies have evaluated the ability of EST derived microsatellite markers to accurately estimate population structure and have found evidence that these markers give similar results to anonymous genomic microsatellites (e.g. (Woodhead et al. 2005, Kim et al. 2008). In our study, the global and pairwise F_{ST} values for the EST-microsatellites vs. the anonymous microsatellites produced very similar, low values (data not shown). These results suggest that the EST-

microsatellite loci that we have developed for *L. salmonis* are not strongly influenced by selection and therefore provide an accurate estimation of population structure.

Table 6. AMOVA. a) Analysis of each of the three data sets as a single group of samples. b) A separation into groups of wild vs. each farm separately ([BS + Dg + At + Kw + Gd + Uc + Pb vs. Qs07] vs. Qs vs. Kt vs. Nd07 vs. Nd). c) A separation by year, 2007 vs. 2009 ([BS + Qs07 + Nd07] vs. [Dg + At + Kw + Gd + Uc + Pb + Qs + Kt + Nd]). d) A separation of samples from BC waters vs. the Bering Sea (BS vs. Remainder). D.F. = degrees of freedom, S.S. = sum of squares, V.c. = variance component, % V. = percent of total variance, S.D. = standard deviation; see Table 1 for sample abbreviations.

	Individual SNPs						SNP Haplotypes						Microsatellites					
Source of Variation	**D.F.**	**S.S.**	**V.C.**	**% V.**	***F*-statistic**	***P* ± S.D**	**D.F.**	**S.S.**	**V.C.**	**% V.**	***F*-statistic**	***P* ± S.D**	**D.F.**	**S.S.**	**V.C.**	**% V.**	***F*-statistic**	***P* ± S.D**
a) Individual samples																		
Among populations	11	87.29	-0.024	-0.23	F_{IT}=-0.002	1.000 ± 0.000	11	48.08	-0.010	-0.18	F_{ST}=-0.002	1.000 ± 0.000	11	81.16	-0.004	-0.05	F_{ST}= 0.000	1.000 ± 0.000
Among individuals within populations	549	5575.47	-0.071	-0.70	F_{ST}=-0.007	0.752 ± 0.014	550	2904.99	-0.083	-1.55	F_{IS}=-0.015	0.980 ± 0.004	551	4274.73	-0.014	-0.18	F_{IS}=-0.002	0.641 ± 0.017
Within individuals	561	5777.00	10.298	100.93	F_{IS}=-0.009	0.807 ± 0.012	562	3061.50	5.448	101.73	F_{IT}=-0.017	0.981 ± 0.005	563	4383.50	7.786	100.23	F_{IT}=-0.002	0.685 ± 0.017
Total	1121	11439.76	10.203				1123	6014.58	5.355				1125	8739.40	7.768			
b) Wild vs Farm (each farm separate)																		
Among groups	5	35.04	-0.013	-0.12	F_{CT}=-0.001	0.822 ± 0.010	5	21.36	-0.002	-0.03	F_{CT}= 0.000	0.650 ± 0.016	5	35.92	-0.003	-0.03	F_{CT}= 0.000	0.731 ± 0.016
Among populations within groups	6	52.24	-0.016	-0.16	F_{SC}=-0.002	0.882 ± 0.012	6	26.72	-0.010	-0.18	F_{SC}=-0.002	0.941 ± 0.007	6	45.24	-0.002	-0.03	F_{SC}= 0.000	0.671 ± 0.015
Within populations	1110	11352.47	10.227	100.28	F_{ST}=-0.003	0.996 ± 0.002	1112	5966.49	5.366	100.21	F_{ST}=-0.002	0.994 ± 0.003	1114	8658.23	7.772	100.07	F_{ST}= 0.000	0.867 ± 0.009
Total	1121	11439.76	10.199				1123	6014.58	5.354				1125	8739.40	7.767			
c) 2007 vs 2009																		
Between groups	1	6.84	-0.003	-0.03	F_{CT}= 0.000	0.636 ± 0.015	1	2.75	-0.004	-0.08	F_{CT}=-0.001	0.930 ± 0.009	1	5.92	-0.004	-0.05	F_{CT}= 0.000	0.933 ± 0.008
Among populations within groups	10	80.45	-0.023	-0.23	F_{SC}=-0.002	0.993 ± 0.002	10	45.34	-0.009	-0.17	F_{SC}=-0.002	0.977 ± 0.005	10	75.24	-0.003	-0.03	F_{SC}= 0.000	0.750 ± 0.012
Within populations	1110	11352.47	10.227	100.26	F_{ST}=-0.003	0.997 ± 0.002	1112	5966.49	5.366	100.24	F_{ST}=-0.002	0.996 ± 0.002	1114	8658.23	7.772	100.08	F_{ST}= 0.000	0.862 ± 0.011
Total	1121	11439.76	10.201				1123	6014.58	5.352				1125	8739.40	7.766			
d) All sites vs outgroup																		
Between groups	1	14.92	0.041	0.40	F_{CT}= 0.004	0.085 ± 0.010	1	6.23	0.011	0.21	F_{CT}= 0.002	0.059 ± 0.008	1	7.85	0.003	0.04	F_{CT}= 0.000	0.324 ± 0.017
Among populations within groups	10	72.37	-0.033	-0.32	F_{SC}=-0.003	1.000 ± 0.000	10	41.86	-0.013	-0.24	F_{SC}=-0.002	0.999 ± 0.001	10	73.32	-0.005	-0.06	F_{SC}=-0.001	0.873 ± 0.009
Within populations	1110	11352.47	10.227	99.92	F_{ST}= 0.001	0.995 ± 0.002	1112	5966.49	5.366	100.03	F_{ST}= 0.000	0.995 ± 0.002	1114	8658.23	7.772	100.02	F_{ST}= 0.000	0.841 ± 0.009
Total	1121	11439.76	10.236				1123	6014.58	5.364				1125	8739.40	7.770			

2.4 Conclusions

Analysis of the microsatellite and SNP data as a whole fails to detect population structure in Pacific *L. salmonis*. Our analyses suggest that dispersal is likely sufficient to maintain a largely homogeneous population of *L. salmonis* in the Northeast Pacific Ocean and that shifts in allele frequencies are unlikely to change over short time periods (i.e. the 2year interval in this study). The lack of population structure observed between sea lice from wild and farmed hosts suggests that these parasites likely enter farms in numbers sufficient to maintain homogenous population structure between farm and wild environments. The potential absence of barriers to gene flow throughout the northeastern Pacific is important to consider when evaluating the capacity for widespread establishment of particular phenotypes, such as those that convey drug resistance. Although our data suggest that *L. salmonis* are capable of long-range dispersal and high migration rates, the differing selective pressures in wild and farm environments will likely impede the widespread establishment of phenotypes that are beneficial in only small, localized areas such as those which exist on Atlantic salmon farms. The observed pattern of genetic structure is likely to belong to a more generalist species that is capable of surviving in most environments rather than one with several subpopulations that become specialized to survive in differing local environments. Our data serve as an initial reference point of *L. salmonis* genetic structure in the Pacific Ocean. Expansion of genetic monitoring with time and geographic range could reveal large scale population structuring in this species.

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquaculture.2010.09.033.

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Chapter 3:
Research summary and future objectives

3.1 Summary of research project presented in Chapter 2

3.1.1 Sampling and technical design

In this study we examined the genetic population structure of *L. salmonis* from 12 Pacific Ocean sampling locations that ranged from the Bering Sea to southern Vancouver Island. We compared collections of *L. salmonis* from 7 populations of wild pink (*Oncorhynchus gorbuscha*) or chinook (*O. tshawytcha*) salmon and 5 populations of farmed Atlantic salmon (*Salmo salar*). All of the samples were collected in the late summer and fall of 2009, with the exception of two farm locations, which were sampled in November 2007 (Nodales & Quatsino populations), and the Bering Sea population which was collected in 2007. We extracted DNA from a total of 562 motile stage *L. salmonis*, with 38 to 56 lice from each collection site.

We developed a large set of genetic markers for this study from an *L. salmonis* expressed sequence tag (EST) library (Yazawa et al. 2008). We initially assessed 55 (primer design by S. G. Jantzen and B. F. Koop), 39 (primer design by K. P. Lubieniecki and W. S. Davidson), and 6 (Todd et al. 2004) candidate SSR loci for PCR specificity, polymorphism level, and genotyping accuracy using fluorescence-based capillary electrophoresis. We screened 118 candidate SNP loci (primer design by E. B. Rondeau) for PCR and sequencing success as well as the abundance and frequency of polymorphic sites within the targeted DNA sequences. We ultimately used 27 SSR loci and 25 SNP loci with 87 polymorphic sites to generate genotypes for the *L. salmonis* compared in our

population study. Five of the 27 SSR loci used were previously developed by Todd et al. (2004). Three data sets were generated for analysis of population structure: SSR loci, individual SNP loci, and multi-SNP haplotypes.

Data were tested for deviation from Hardy-Weinberg equilibrium (HWE), allelic and genetic diversity indices (H_e, H_o, F_{IS}, F_{ST}, Nm), isolation by distance, analysis of molecular variance (AMOVA)**,** linkage disequilibrium**,** and Bayesian inference of population structure. We compared results from SNP versus SSR data and anonymous (Todd et al. 2004) versus EST-derived SSR loci to evaluate the consistency of these different marker types. We tested for population structure among lice from farm and wild hosts, temporal change in population structure within farms, and spatial difference in population structure over the sampling range.

3.1.2 Comparisons made using genetic data

It is unlikely that many of the individual SNPs, SNP haplotypes or SSR loci deviated from HWE in our samples, which means that genetic variants had an equal chance of representation independent of the geographic location of the parental contribution. Among all three data sets, genotype frequencies deviated from HWE expectations for only one SNP haplotype sample ($p < 0.001$). Five to 39 alleles per locus were detected for the SSR loci, 2 to 16 haplotypes per locus were detected for the SNP loci, and 9 of 87 individual SNPs were detected with 3 alleles. Private allele frequencies for all three data sets were low ($\bar{p}(1) = 0.01$) and indicated that 17-19 migrant individuals may be present with sample sizes of 45-46 individuals per population (Barton and Slatkin 1986). The frequency of homozygosity over all loci within each sampled population (F_{IS}) did not indicate assortative mating, inbreeding, null alleles, or Walhund effect within particular

populations. For the SSR data set, F_{IS} ranged from −0.025 (Qs07) to 0.030 (Nd07). For the SNP haplotype data set, F_{IS} ranged from −0.068 (Gd) to 0.052 (Uc). For the individual SNP data set, F_{IS} ranged from −0.048 (Kt) to 0.022 (Uc). Estimates of allelic fixation among all samples and loci in each of the three data sets failed to indicate any genetic structure among populations (global $F_{ST} = 0.000$, $p > 0.8$). Allelic fixation among pairs of populations over all loci (pairwise F_{ST}) suggested that gene flow is not restricted among any sample pairs. One significant difference was found using the individual SNP data set to compare the allele frequencies of Bering Sea and Donegal (pairwise $F_{ST} = 0.006$, $p = 0.018$), however, this F_{ST} value is very low and may be significant due to chance alone. No correlation was detected between geographic distance and genetic differentiation (F_{ST}) between pairs of sample sites (Slatkin 1993). No variance in allele frequencies among populations or groups of populations was detected for any of the three data sets; 100% of variance was attributed to variation within individual *L. salmonis.* Strong allelic similarity was detected in all comparisons that involved hierarchical population groupings: no among-population variation was detected from comparing wild *L. salmonis* to individual farm sites, 2007 samples to 2009 samples, or Bering Sea to Pacific samples. Bias in our results is not likely due to linkage among loci, as potential linkage disequilibrium was detected among very few pairs of loci in the individual SNPs that were not known to be physically linked (12 of 44,892 tests; $p < 0.001$), SNP haplotypes (4 of 3,600 tests; $p < 0.001$), or SSR loci (27 of 4,212 tests; $p < 0.001$). It is unlikely that cryptic population structure exists among our samples (Jousson et al. 2000, Roman 2006, Locke et al. 2010); Bayesian inference of population assignment could not detect divergent populations for either the SNP haplotype or SSR data sets.

The SSR and SNP data sets produced similar results. Comparable results were obtained from both the EST-derived and anonymous SSR loci, although the anonymous SSR loci were more polymorphic on average than the EST-derived SSR loci (anon-SSRs $\bar{a}$ = 28.2, EST-SSRs $\bar{a}$ = 13).

3.1.3 Context and importance

No samples compared in this study indicated strong evidence of population structure among Pacific Ocean *L. salmonis* samples. Our results suggest that within this sampling range, *L. salmonis* gene flow has been high enough to counteract the effects of genetic drift and localized selection pressure (i.e., on farms). It is likely that dispersal has been unrestricted over the evolutionary history of Pacific *L. salmonis* and that a large panmictic population exists within the Northeast Pacific Ocean.

3.2 How do our results compare with other *Lepeophtheirus salmonis* population genetics research?

Some disagreements exist among the various assessments of *L. salmonis* population structure (e.g., Tjensvoll et al. 2006, Yazawa et al. 2008, Boulding et al. 2009). However, the general indication from *most* of these studies is that gene flow is high and genetic divergence is not likely to develop through neutral genetic drift among even geographically distant subpopulations of *L. salmonis* within the Atlantic or Pacific Oceans. Genetic comparisons of Atlantic and Pacific *L. salmonis* consistently demonstrate that significant genetic divergence exists between these two distinct populations (Todd et al. 2004, Tjensvoll et al. 2006, Yazawa et al. 2008, Yasuike et al. 2012). High resolution time series sampling that reflects important changes in *L. salmonis*

population density (i.e., rapid changes in the abundance of *L. salmonis* on individual farms) may allow us to better understand parasite population dynamics related to anti-parasitic treatment on farms, parasite transmission among host species, and variation in selection pressures between wild and farm hosts. The population structure of *L. salmonis* on farms likely does not reflect the patterns expected from natural populations of *L. salmonis* because farm hosts and environments provide optimal locations for rapid *L. salmonis* population growth, punctuated by frequent periods of high mortality (illustrated by Figure 4). Frequent changes in the population structure of *L. salmonis* on farms may have contributed to the conflicting results of some *L. salmonis* population studies, particularly considering the fact that sampling in Atlantic *L. salmonis* studies has often been biased toward farms (Isdal et al. 1997, Nolan and Powell 2009, Glover et al. 2011). Samples collected over time, when farm *L. salmonis* abundance both low and high, might reveal important differences in the genetic diversity and infection dynamics of farm *L. salmonis* (Figure 4).

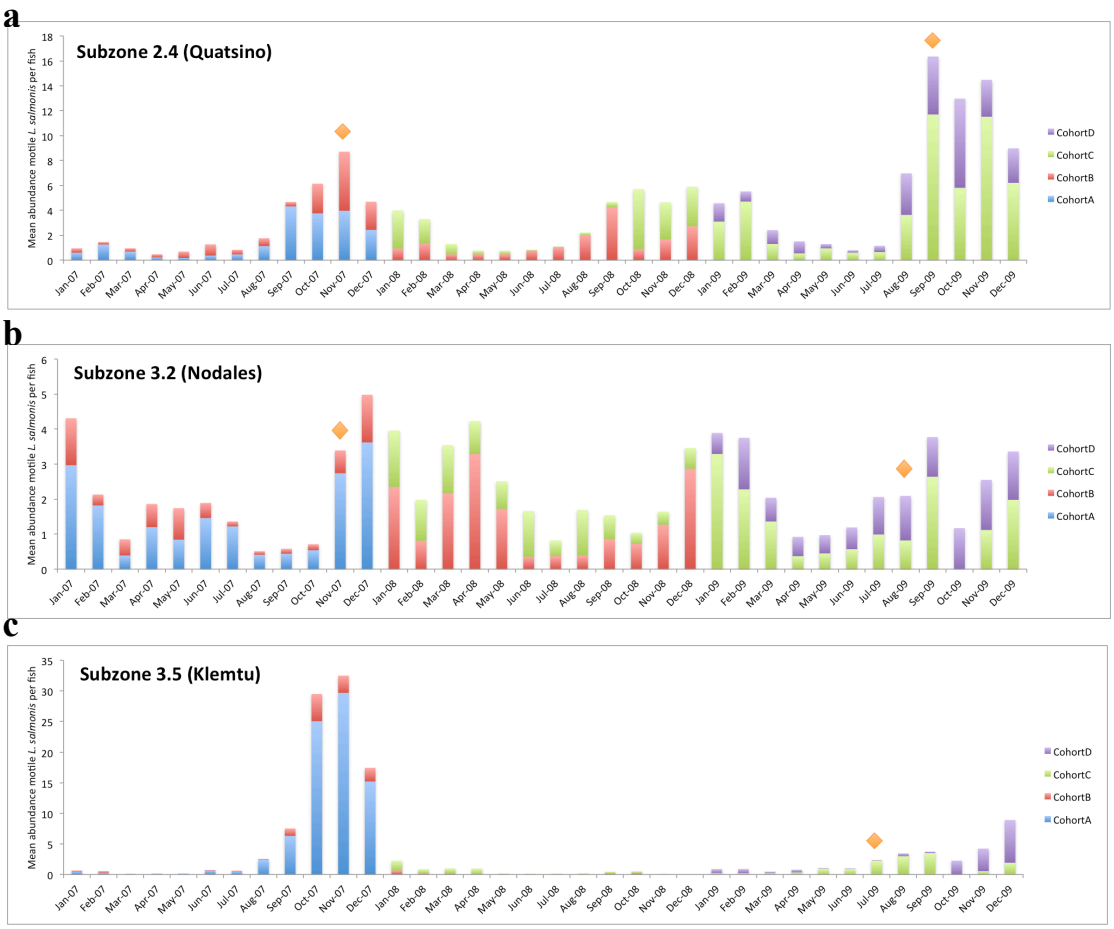

Figure 4. Average number of motile *Lepeophtheirus salmonis* per fish sampled during sea lice infection monitoring conducted by BC Salmon Farmers Association (BCSFA 2007, 2008, 2009). Data are shown for estimates taken within the management subzones where farm *L. salmonis* samples were included in our study. Orange diamonds indicate the months when our samples were collected from specific farms within each subzone: a) Qs07 and Qs; b) Nd07 and Nd; and c) Kt. The average abundance of *L. salmonis* is shown for first and second sea-year age classes of Atlantic salmon at each time point (indicated by Cohort). Cohort-specific *L. salmonis* abundance is illustrated over the full lifespan of Cohorts B and C.

Some of the SSR loci developed for our study of Pacific *L. salmonis* population structure were used in a comparable study of Atlantic *L. salmonis* population structure (Glover et al. 2011). Glover et al. (2011) compared *L. salmonis* sample locations from Atlantic salmon farms in Norway (n=19), Ireland (n=2), Shetland (n=2), the Faroe Islands

(n=2), and Atlantic Canada (n=2) using 12 SSR loci, including five loci designed by Todd et al. (2004; "Todd-SSRs"), and seven loci developed by Messmer et al. (2011; "Messmer-SSRs"). Five of the Messmer-SSRs and five of the Todd-SSRs used by Glover et al. (2011) were also used for our study of the Pacific *L. salmonis* population (Table 7).

Table 7. Number of alleles identified at loci common to Pacific and Atlantic *Lepeophtheirus salmonis* population genetics studies. I) Messmer et al. (2011), II) Glover et al. (2011), III) Todd et al. (2004). *n* = total number of individual *L. salmonis* included in study (average number of individuals per sample). The average number of alleles per sample is indicated in parentheses following the total number of alleles for each locus.

SSR locus	I) Pacific *n*=562 (47)	II) Atlantic *n*=2514 (92)	III) Atlantic *n*=1007 (55)	III) Pacific *n*=34 ***
LsalSTA1	39 (19.6)	30 (16.8)	27 (14.5)	12
LsalSTA2	25 (15.1)	37 (16.4)	33 (13.1)	8
LsalSTA3	28 (15.0)	30 (21.7)	38 (20.9)	14
LsalSTA4	17 (8.8)	15 (5.8)	14 (5.6)	6
LsalSTA5	32 (16.0)	35 (22.8)	43 (22.2)	12
Lsal101/103EUVC *	10 (4.2)	12 (4.9)	-	-
Lsal105EUVC	10 (5.2)	12 (4.6)	-	-
Lsal106EUVC	8 (5.1)	10 (4.3)	-	-
Lsal108EUVC	14 (6.3)	17 (9.3)	-	-
Lsal109EUVC	n.a.**	11 (7.2)	-	-
Lsal110EUVC	6 (3.7)	6 (4.3)	-	-
Allele total (Todd-SSRs)	141	147	155	-
Allele total (Messmer-SSRs)	48	57	-	-

* Both names refer to the same locus, reported as Lsal101EUVC by Messmer et al. (2011) and as Lsal103EUVC by Glover et al. (2011).
** This locus was not included in the published results of Messmer et al. (2011).
*** One sample of Pacific *L. salmonis* collected from a Broughton Archipelago farm was included by Todd et al. (2004). This sample was included in the III) Atlantic column.

The Pacific *L. salmonis* SSR allele frequencies published by Todd et al. (2004) are similar to the frequencies found in our study using the Todd-SSR loci. We identified a larger number of Todd-SSR alleles than was previously reported for Pacific *L. salmonis*

(Todd et al. 2004), most likely because of the difference in the number of Pacific Ocean lice sampled in each of these studies (Table 7). The level of heterozygosity at each of the Todd-SSRs is similar for Pacific and Atlantic *L. salmonis*, however, the heterozygosity of LsalSTA3 is generally higher in the Atlantic Ocean than in the Pacific Ocean (Figure 5; Todd et al. 2004, Messmer et al. 2011). Selection pressure has been associated with LsalSTA3 in Atlantic *L. salmonis* by Glover et al. (2011), but locus-specific heterozygosities were not reported by Glover et al. (2011), so average heterozygosities cannot be compared.

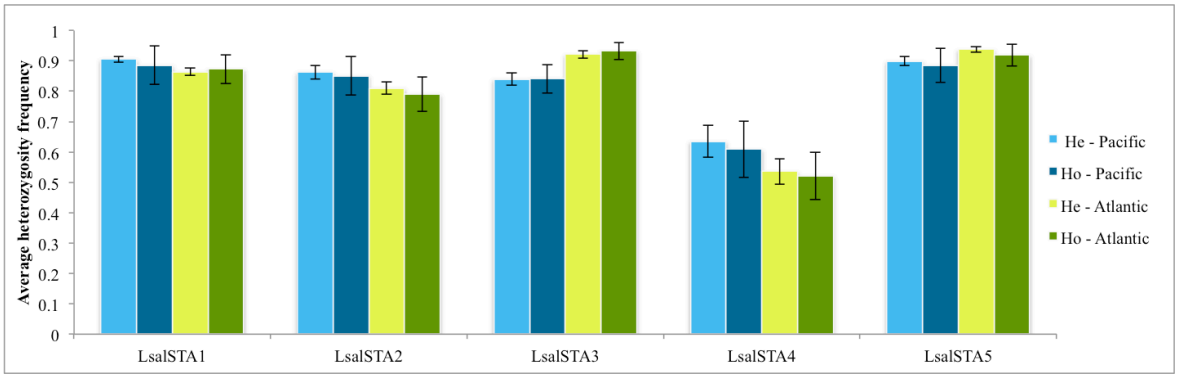

Figure 5. Comparison of locus specific expected (He) and observed (Ho) heterozygosities averaged among samples from Pacific (Messmer et al. 2011) and Atlantic (Todd et al. 2004) *Lepeophtheirus salmonis* populations. Error bars indicate standard deviations among samples.

Global F_{ST} values were reported for each locus used by Glover et al. (2011) and plotted against expected heterozygosity for each locus, in order to detect outlier loci that may be under selection pressure (using the program LOSITAN, Antao et al. 2008). Neither locus-specific global F_{ST}s, nor F_{ST} outlier analysis were reported in our study. This type of analysis would have improved our publication and made it more comparable to other *L. salmonis* population studies. The overall conclusions of our Pacific *L. salmonis*

population genetics study are similar to the conclusions of SSR-based Atlantic *L. salmonis* population genetics studies; little evidence of population structure among samples within either ocean was detected (Todd et al. 2004, Glover et al. 2011).

Several methods of control can be integrated and standardized for SSR markers, including the use of a standard control template, calculation of genotyping error rates, and reproduction of homozygotes if null alleles are suspected (Selkoe and Toonen 2006). Efforts to standardize SSR genotyping and statistical reporting among laboratories may help to make more comprehensive comparisons among SSR data sets in the future.

3.3 Expansion of *Lepeophtheirus salmonis* population genetics research

The progression of research toward understanding the genetics and genomics of *L. salmonis* has included sequencing the genome of Pacific and Atlantic *L. salmonis* as a progression from the foundational research of the Genomics of Lice and Salmon (GiLS) project (http://web.uvic.ca/grasp/gils/). One of the immediate applications of *L. salmonis* genomic libraries has been to identify approximately 40,000 SNP loci that vary among phenotypically different groups of lice. We hope to identify genetic mechanisms responsible for various phenotypic traits in *L. salmonis,* including EMB resistance, and to reassess the population structure of *L salmonis* by comparing patterns of genetic variation throughout the entire genome. These population comparisons will include Pacific Ocean *L. salmonis* samples from Japan, the Bering Sea, Alaska, BC salmon farms, and BC wild salmon as part of a larger study that includes both Pacific and Atlantic *L. salmonis*. The use of this high-resolution technology may help us to identify population structure that could not be detected with the SSRs and SNPs previously used. Genome-wide SNP

analysis may reveal loci that have undergone divergent selection among wild hosts or between farm and wild environments and provide information on the genetic mechanisms responsible for evolution of drug resistance in parasites (Helyar et al. 2011, Bourret et al. 2013).

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Appendix A
Contributions to related publications

1. Rondeau, E.B., Minkley, D.R., Leong, J.S., **Messmer, A.M.,** Jantzen, J.R., von Schalburg, K.R., Lemon, C., Bird, N.H., and Koop, B.F. 2014. The genome and linkage map for the Northern pike (*Esox lucius*): significant conserved synteny revealed between the salmonid sister group Esociformes and the Neoteleostei. PLoS ONE **9**(7): e102089. Doi:10.1371/journal.pone.0102089.
    - *Designed, conducted and evaluated the screening of 1611 putative microsatellite loci for use in linkage mapping. Managed laboratory work, collected and analyzed microsatellite data used to produce linkage map. Contributed to linkage mapping methods and results sections of manuscript.*

2. **Messmer A.M.,** Nelson R.J., Koop B.F. (In prep). Panmictic genetic structure in *Anoplopoma fimbria* (Pallas, 1814) from the northeastern Pacific Ocean inferred from mitochondrial DNA haplotypes and EST derived microsatellite markers.
    - *Conducted DNA extractions, microsatellite PCRs, mtDNA primer design, PCRs and sequencing, data collection and analysis, and wrote manuscript.*

3. Von Schalburg, K.R., Gowen, B.E., **Messmer, A.M**., Davidson, W.S., and Koop, B.F. 2014. Sex-specific expression and localization of aromatase and its regulators during embryonic and larval development of Atlantic salmon. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. **168**: 33–44. Elsevier Inc. doi: 10.1016/j.cbpb.2013.11.004.
    - *Evaluated candidate sex-linked microsatellite loci, extracted genomic DNA from salmon embryos, determined sex of salmon based on microsatellite allele inheritance, contributed to methods and results sections of manuscript.*

4. Rondeau, E.B., **Messmer, A.M.**, Sanderson, D.S., Jantzen, S.G., von Schalburg, K.R., Minkley, D.R., Leong, J.S., Macdonald, G.M., Davidsen, A.E., Parker, W. a, Mazzola, R.S. a, Campbell, B., and Koop, B.F. 2013. Genomics of sablefish (*Anoplopoma fimbria*): expressed genes, mitochondrial phylogeny, linkage map and identification of a putative sex gene. BMC Genomics **14**: 452. BMC Genomics. doi: 10.1186/1471-2164-14-452.
    - *Evaluated candidate microsatellite loci for use in linkage map, extracted DNA, ran PCRs and data collection.*

5. Nelson, R.J., Bouchard, C., Madsen, M., Praebel, K., Rondeau, E., Schalburg, K., Leong, J.S., Jantzen, S., Sandwith, Z., Puckett, S., **Messmer, A.**, Fevolden, S.E., and Koop, B.F. 2012. Microsatellite loci for genetic analysis of the arctic gadids *Boreogadus saida* and *Arctogadus glacialis*. Conserv. Genet. Resour. **5**: 445–448. doi: 10.1007/s12686-012-9824-1.
    - *Screened 150 candidate microsatellite loci to identify loci useful for population genetics research.*

6. **Messmer AM**, Rondeau EB, Jantzen SG, Lunieniecki KP, Davidson WS, Koop BF. 2011. Assessment of population structure in Pacific *Lepeophtheirus salmonis* (Krøyer) using single nucleotide polymorphism and microsatellite genetic markers. Aquaculture 320(3-4SI): 183-192.
    - *Contributed to experimental design, assessed microsatellite quality, extracted sample DNA, conducted microsatellite PCRs, collected and analyzed microsatellite data, and wrote manuscript.*

7. Glover KA, Stolen AB, **Messmer A**, Koop BF et al 2011. Population genetic structure of the parasitic copepod *Lepeophtheirus salmonis* throughout the Atlantic. Marine Ecology Progress Series 427:161-172.
    - *Contributed microsatellite loci, edited manuscript.*

8. Mol Ecology Resources Primer Dev C., Aggarwal RK, Allainguillaume J, Bajay MM, Barthwal S, Bertolino P, Chauhan P, Consuegra S, Croxford A, Dalton DL, den Belder E, Diaz-Ferguson E, Douglas MR, Drees M, Elderson J, Esselink GD, Fernandez-Manjarres JF, Frascaria-Lacoste N, Gabler-Schwarz S, de Leaniz CG, Ginwal HS, Goodisman MAD, Guo BL, Hamilton MB, Hayes PK, Hong Y, Kajita T, Kalinowski ST, Keller L, Koop BF, Kotze A, Lalremruata A, Leese F, Li CH, Liew WY, Martinelli S, Matthews EA, Medlin LK, **Messmer AM,** Meyer EI, Monteiro M, Moyer GR, Nelson RJ, Nguyen TTT, Omoto C, Ono JY, Pavinato VAC, Pearcy M, Pinheiro JB, Power LD, Rawat A, Reusch TBH, Sanderson D, Sannier J, Sathe S, Sheridan CK, Smulders MJM, Sukganah A, Takayama K, Tamura M, Tateishi Y, Vanhaecke D, Vu NV, Wickneswari R, Williams AS, Wimp GM, Witte V, Zucchi MI, and Mol Ecology Resources Primer Dev C. 2011. Permanent Genetic Resources added to Molecular Ecology Resources Database 1 August 2010-30 September 2010 Molecular Ecology Resources 11(1): 219-222.
    - *Contributed to experimental design, evaluated candidate microsatellite loci, conducted DNA extractions, PCRs and data collection, analyzed data, wrote manuscript.*

9. Yasuike M, de Boer J, von Schalburg KR, Cooper GA, McKinnel L, **Messmer A**, So S, Davidson WS, Koop BF. 2010. Evolution of duplicated IgH loci in Atlantic salmon, *Salmo salar*. BMC Genomics 11:486.
    - *Conducted and managed plasmid preparations and DNA sequencing of BAC DNA.*

10. Leong JS, Jantzen SG, von Schalburg KR, Cooper GA**, Messmer AM**, Liao NY, Munro S, Moore R, Holt RA, Jones SJM, Davidson WS, Koop BF. 2010. *Salmo salar* and *Esox lucius* full-length cDNA sequences reveal changes in evolutionary pressures on a post-tetraploidization genome. BMC Genomics**,** 11**:** 279.
    - *Conducted and managed plasmid preparations and DNA sequencing of EST DNA.*

Appendix B
Scientific presentations

1. **Messmer AM***, Rondeau E, Sanderson D, Jantzen S, Macdonald G, Davidson WS, Koop BF. Assessment of population structure in Pacific *Lepeophtheirus salmonis* using single nucleotide polymorphism and microsatellite genetic markers. Oral presentation at Pacific Ecology and Evolution Conference. Held in Bamfield BC, March 4-6, 2011.
   - *Contributed to experimental design, assessed microsatellite quality, extracted sample DNA, conducted microsatellite PCRs, collected and analyzed microsatellite data, and created presentation.*

2. **Messmer AM***, Rondeau E, Sanderson D, Jantzen S, Macdonald G, Davidson WS, Koop BF. Assessment of population structure in Pacific *Lepeophtheirus salmonis* using single nucleotide polymorphism and microsatellite genetic markers. UBC/SFU/UVic Fall Ecology and Evolution Retreat. Held in Brackendale BC, October 29 - Nov 1, 2010.
   - *Contributed to experimental design, assessed microsatellite quality, extracted sample DNA, conducted microsatellite PCRs, collected and analyzed microsatellite data, and created presentation.*

3. **Messmer AM***, Rondeau E*, Sanderson D, Jantzen S, Macdonald G, Davidson WS, Koop BF. Assessment of population structure in Pacific *Lepeophtheirus salmonis* using fine scale genetic markers. Poster presentation at Sea Lice 2010, the 8th International Sea Lice Conference. Held in Victoria BC, May 9-12, 2010.
   - *Contributed to experimental design, assessed microsatellite quality, extracted sample DNA, conducted microsatellite PCRs, collected and analyzed microsatellite data, and created presentation with ER.*

4. Nelson RJ*, Carmack EC, McLaughlin FA, Cooper GA, **Messmer AM**, Vagelatos N. (2008) Use of molecular genetics to trace Zooplankton transport through Bering Strait into the Arctic Ocean.
   - *Conducted lab work and analyzed DNA sequence data.*

* Presenter

Appendix C
Supplementary tables for Chapter 2

Table 8. Characterization of microsatellite loci used in this study. A.S.R. = allele size range, Ta = annealing temperature, * loci from Todd et al. 2004. Referred to as Table S1 in published version.

Locus	Accession Numbers	Left/Forward Primer (5'-3')	Right/Reverse Primer (5'-3')	Repeat Motif	T_a (°C)	A.S.R.
Lsal006ESFU	GW626096	GGGAACGTTAGGATGTACTG	CAGTTACGTGCAGCAGAAT	$(TTG)_{23}$	52	276-309
Lsal025ESFU	EX475550 EX475549	TTTATCATGACTTGGAGCCT	GAACATCCATTTGTTGCTTT	$(TA)_{18}$	52	218-224
Lsal050ESFU	EX480464	TCTTTTTCATCCATTTGCTT	CAGAAGATCGAAGATTGGAG	$(TTC)_9$	52	313-350
Lsal060ESFU	GW632561	GAGTAATGGAAACCAACGTG	CTATTTCCTCTACGCTCGAA	$(TGG)_9$	52	235-288
Lsal065ESFU	EY509523	TAATGGAACCATCTTCAACC	AGCACTAGGAGCACCATATC	$(CTG)_9$	52	200-220
Lsal070ESFU	GW637473	ACCTACTTCGCCAATTCATA	GATAACCGAAGAGATGGTGA	$(CATCT)_5$	52	223-264
Lsal078ESFU	GW645831	TAGTGGAGCATTTCCATTTT	AACACCTGACAAAACACTCC	$(ATG)_8$	52	204-225
Lsal079ESFU	FK932914	GTTCGGACATATTGGAGGTA	AGGTCAATTTCAACAACCAC	$(TTG)_8$	52	297-354
Lsal083ESFU	EX478504	AGCCTTCCAGTGTCTACAAA	GAGGGATGAAAAAGGAGATT	$(CAA)_8$	52	215-224
Lsal096ESFU	GW633334	TTCTCTGCTTGGTTCGTACT	AATGGAAAGGTGGAGTACCT	$(CTG)_7$	52	243-259
Lsal101EUVC	GW660598	CAGATGCTCCAGCAATTGAA	TGCGCCGACAATATTTTACA	$(GAA)_{32}$	52	222-234
Lsal105EUVC	EX482162	ACGGGTGTGGTTGTCGTATT	TTACGCCTAAGGCAACGACT	$(TGT)_{36}$	52	135-158
Lsal106EUVC	EX475929	TGTTGCTGTTGCAAATGATG	AGGTGCAGCAGCTGTGTCTA	$(CAA)_{13}$	52	129-142
Lsal108EUVC	EX485676	TGGAAAAACAATAAATGCTTCG	TTGGATCATTCCTCATTTCTCT	$(AGT)_{15}$	52	197-215
Lsal110EUVC	FK927649	TGGCACAAATAATAACGGGTTT	AAGCTCTGCCAAAGAGAGAAGA	$(TATG)_{12}$	52	117-125
Lsal201EUVC	GW620448	GGAATAAAAACAGTCGTGCG	CGTCATTACTGCTCCAACG	$(ACA)_{36}$	52	158-176
Lsal210EUVC	FK909878 FK909877	TGCTGTTTTTGTCTTTGTCG	TGTTAAGATAAGCATTTTGACCG	$(AG)_{16}$	52	199-239
Lsal225EUVC	HO701983	TTGAACAGACGGGTGTGG	ACCCAGCAACCACTACTACG	$(ACA)_{36}$	52	203-267
Lsal229EUVC	HO675729	GATTTTCTTGGGGATTGAGG	CCCTCCCATGTTCATTCC	$(CAG)_8$	52	169-193
Lsal235EUVC	EY506730	AAGTAAGAATAGGAAATTGTGA GACC	AATGTACTCGTTGTGTAAACGC	$(TAC)_{12}$	52	135-161
Lsal238EUVC	HO669906	TTCAAGTATTATCCTTCCGAGC	GGATTGAATACACGTAATGGG	$(TA)_{17}$	52	144-163
Lsal242EUVC	HO676734	GAAAAATAAGTTCGATCCATGC	TTGTATTGCTCCTTCCTTGC	$(TCT)_{11}$	52	190-204
LsalSTA1*	AY509254	CGTCGAAATTCTCATCCAA	GGGAAAGATTGGGAGTGAG	TC_{20}	60	132-219
LsalSTA2*	AY509255	TCGTGGTGGTTGACTCTACT	AGGAAATCAGGAGCAAGTG	TC_{13}	58	180-213
LsalSTA3*	AY509256	TTATCCGAATCCGTCTTATG	AGCCTGAAGTAGGTTAGTTGG	TC_{18}	57	139-197
LsalSTA4*	AY509257	AAGGCGTGCGTTGTTAAGT	CAATGCGATCCTGGAGTCT	$(GA)_2A$ $(GA)_2GT$ $(GA)_8$	58	170-205
LsalSTA5*	AY509258	GGGATAAGTGGCGAGCTACC	GTCTCAGCGGCAGAAGTCTC	GA_{15}	60	186-257

Table 9. SNP marker names and locations for polymorphisms used in the study. Referred to as Table S3 in published version. Published version includes additional column with the full Contig sequence for each marker.

SNP Marker Name	Alternate	Contig	SNP Sequence
LsaSP0507UVic	A06_1	Contig1174	ACACCCATTC [A/C] TCG
LsaSP0508UVic	A06_2	Contig1174	CGAATTTGCT [A/G] GCC
LsaSP0509UVic	A06_3	Contig1174	GGCCATCGG [A/G] AAT
LsaSP0510UVic	A06_4	Contig1174	TGGCCGCAT [A/C] TTT
LsaSP0520UVic	A09_1	Contig6222	TAACATTTCACT [C/T] CCT
LsaSP0522UVic	A09_2	Contig6222	AGTTAGTAAAT [G/T] ATA
LsaSP0523UVic	A09_3	Contig6222	CCAAAGTCAT [A/G] AAT
LsaSP0524UVic	A09_4	Contig6222	TTAAAGACATC [C/T] TTC
LsaSP0525UVic	A09_5	Contig6222	ACTATATACTT [A/G] GCC
LsaSP0526UVic	A09_6	Contig6222	GCTCGCCATAA [A/G] CAT
LsaSP1001UVic	A09_7	Contig6222	ATTTCTACTTAATCC [C/T] ATG
LsaSP0529UVic	A09_8	Contig6222	CGATTTTTG [A/T] AGG
LsaSP0533UVic	A10_2	Contig8997	TTTTTGCTC [C/G] GGC
LsaSP0534UVic	A10_3	Contig8997	GGCCAATTCAT [A/G] CCT
LsaSP0535UVic	A10_4	Contig8997	CAATTAAAATA [C/T] ATT
LsaSP1002UVic	A10_1	Contig8997	CACAGTCAAGTTG [C/T] CTG
LsaSP1003UVic	A10_5	Contig8997	TGTGTTGAATCCG [C/T] CCT
LsaSP1004UVic	A10_6	Contig8997	TGGGTAGACA [G/T] GAG
LsaSP0542UVic	B01_1	Contig9854	CCTCGAAGA [A/G] CCA
LsaSP0543UVic	B01_2	Contig9854	TTGTGCTTT [C/G] AAA
LsaSP0544UVic	B01_3	Contig9854	ATAAATGT [A/G] GAG
LsaSP0569UVic	B05_1	Contig5757	CCAGAAGAAA [C/T] GTT
LsaSP1005Uvic	B05_2	Contig5757	AAAGTCTGTT [A/T] TAGCTT
LsaSP0570UVic	B06_1	Contig8588	GAATATCTTTC [A/T] GAG
LsaSP0571UVic	B06_2	Contig8588	TGGAAAAGAA [A/G] AGG
LsaSP0573UVic	B06_3	Contig8588	GCCTTAGAGGG [C/G] TTA
LsaSP1006UVic	B12_1	Contig9860	AAAATTTTCAAA [C/T] AGTT
LsaSP0598UVic	C02_1	Contig168	GGTATGAGACGG [A/G] ATT
LsaSP0600UVic	C02_2	Contig168	ATTATTTTACT [G/T] TTT
LsaSP0604UVic	C03_2	Contig7006	TCACAATCAT [G/T] TAG
LsaSP0603UVic	C03_1	Contig7006	AAGTCATGTT [A/G] TTC
LsaSP0605UVic	C03_3	Contig7006	TCGGGAATTT [C/T] TAT
LsaSP0607UVic	C04_1	Contig3147	CTCACATATGT [A/C] TTT
LsaSP1007UVic	C04_2	Contig3147	TTTTATTGGATAATTTGT [G/T] TTCG
LsaSP1008UVic	C06_3	Contig80	GGAGTCAAA [A/G] CTAG
LsaSP0613UVic	C06_4	Contig80	CAAAGCTAGA [C/T] GCT
LsaSP1009UVic	C06_5	Contig80	GCTGCTAGAAGAAT [A/T] GTTAAG
LsaSP0614UVic	C06_7	Contig80	AAAATCTC [A/C] TCA
LsaSP1010UVic	C06_1	Contig80	TAATAATCCTCT [C/T] ACA
LsaSP1011UVic	C06_2	Contig80	GAGTCAAA [A/G] CTAG
LsaSP1012UVic	C06_6	Contig80	ATGAAATTATGC [A/T] GAA
LsaSP0615UVic	C06_8	Contig80	GTAATACTCT [C/T] CCT
LsaSP0618UVic	C07_1	Contig8283	ATAATAGTAGAT [A/G] TAA
LsaSP0619UVic	C07_2	Contig8283	GTAGATGTAAGG [A/C] TCA
LsaSP0620UVic	C07_3	Contig8283	ACGGATTTGAG [A/T] TCT
LsaSP1013UVic	C07_4	Contig8283	AAAATCTCTTTTT [A/T] GAC
LsaSP0635UVic	C11_1	Contig8590	TCAACATAATGG [C/T] TAT
LsaSP0636UVic	C11_2	Contig8590	AAAATCATTA [C/T] TTT
LsaSP1014UVic	C12_1	Contig930	CTTAAATCTGCGGC [C/G] TCAT
LsaSP0640UVic	C12_2	Contig930	CTCATCCAC [A/T] GAG

SNP Marker Name	Alternate	Contig	SNP Sequence
LsaSP0653UVic	D04_1	Contig8241	AAGGTCATATC [C/T] GGA
LsaSP0654UVic	D04_2	Contig8241	TGAGGTTCCCG [A/G] AAT
LsaSP0655UVic	D05_1	Contig10458	GCAGCAATTC [A/G] AAG
LsaSP0656UVic	D05_2	Contig10458	GGCCTTATCT [A/G] TAG
LsaSP0657UVic	D05_3	Contig10458	GTAGCGCCAC [A/C] ATCATTAT
LsaSP0662UVic	D06_1	Contig7499	GCCAGCTCTTCC [C/T] GGA
LsaSP0664UVic	D06_2	Contig7499	CGGAGTAGAG [G/T] GCA
LsaSP1015UVic	D08_1	Contig8731	CAGTTGGTAGTGAGC [A/C] CGA
LsaSP0673UVic	D08_2	Contig8731	AGTGAGCACGA [A/G] GTTA
LsaSP0674UVic	D08_3	Contig8731	AGTGAGCACGAA [A/G] TTA
LsaSP0680UVic	D09_2	Contig234	ACGGAACTAA [G/T] CTA
LsaSP0683Uvic	D09_3	Contig234	TGTCTACAGG [C/T] CAG
LsaSP0678UVic	D09_1	Contig234	AAAGAGAGTT [C/T] TTA
LsaSP0686UVic	D10_2	Contig10103	GATGATTCA [A/G] TTA
LsaSP0687UVic	D10_3	Contig10103	TCTCTCAATA [A/G] CAT
LsaSP0685UVic	D10_1	Contig10103	CAACTCTGA [G/T] GAT
LsaSP0700UVic	D12_1	Contig2055	ATCTGCTTATGT [G/T] TCC
LsaSP0701UVic	D12_2	Contig2055	CTGCTCCTC [C/T] GTC
LsaSP0703UVic	D12_3	Contig2055	CGCAGTATC [A/C] CCG
LsaSP0704UVic	E01_5	Contig396	ATGTAAGATT [A/C] ATT
LsaSP0707UVic	E01_1	Contig396	GCATTTTTTT [A/T] AAAA
LsaSP0708UVic	E01_2	Contig396	GCATTTTTTTT [A/T] AAA
LsaSP0709UVic	E01_3	Contig396	AAATCTCTGC [C/G] ATG
LsaSP0710UVic	E01_4	Contig396	AGATATATTTAC [A/T] ATA
LsaSP0717UVic	E04_2	Contig7844	TAGATGTTCAT [A/T] AAG
LsaSP0718UVic	E04_3	Contig7844	GTATTAGTTTT [C/G] TTT
LsaSP0720UVic	E04_4	Contig7844	AATACAAATA [A/T] TTAT
LsaSP0721UVic	E04_5	Contig7844	AATACAAATAT [G/T] TAT
LsaSP0722UVic	E04_6	Contig7844	TCATCTCAGAG [A/C/G] TGT
LsaSP0715UVic	E04_1	Contig7844	GATGCAGGTGG [G/T] TCT
LsaSP0740Uvic	E08_1	Contig5803	ACTTCGTTATTC [A/C/T] CAC
LsaSP0741UVic	E08_2	Contig5803	TTCGTTATTCTCA [C/T] ATT
LsaSP0747UVic	E10_1	Contig10295	TAATTATAATT [A/G] TCA
LsaSP0748UVic	E10_2	Contig10295	CTTGAGTTTT [A/G] CGA
LsaSP0749UVic	E10_3	Contig10295	AAATAAGGTTT [A/T] AAA
LsaSP0750UVic	E10_4	Contig10295	TATGCTGAACT [A/G] AGTCT
LsaSP0751UVic	E10_5	Contig10295	TATGCTGAACTGA [G/T] TCT

Full versions of Table S2 (Amplified sequences from SNP analysis, concatenated into one, alphabetically, from A06 to E10, separated by the short linker sequence WMDDMW. See Table 1 for sample abbreviations) and Table 9 (SNP marker names and locations for polymorphisms used in the study) are available from <http://www.sciencedirect.com./science/article/pii/S0044848610006630>. These tables are too large to include here due to the large amount of DNA sequence data they contain.

Table 10. Summarized statistics for each locus in each data set. a) Individual SNP data set b) Haplotyped SNP data set and c) Microsatellite data set. H_O = observed heterozygosity, H_E = expected heterozygosity, P = P-value from test of Hardy–Weinberg Equilibrium, A = observed number of alleles, n = number of individuals included in the analysis. Referred to as Table S4, S5, and S6 in published version.

a) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
LsaSP0507UVic (2)	H_o	0.21	0.26	0.17	0.21	0.26	0.23	0.34	0.28	0.21	0.23	0.15	0.13
	H_e	0.19	0.26	0.20	0.19	0.26	0.29	0.29	0.24	0.23	0.20	0.14	0.16
	P	1.000	1.000	0.419	1.000	1.000	0.143	0.566	0.572	0.529	1.000	1.000	0.279
	F_{IS}	-0.111	0.004	0.113	-0.106	0.000	0.224	-0.194	-0.150	0.071	-0.117	-0.071	0.191
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	46	48	38	47	42	44	46	47
LsaSP0508UVic (2)	H_o	0.41	0.43	0.41	0.33	0.37	0.48	0.63	0.43	0.36	0.45	0.41	0.26
	H_e	0.42	0.44	0.44	0.42	0.42	0.47	0.47	0.42	0.47	0.42	0.45	0.36
	P	1.000	1.000	0.743	0.175	0.484	1.000	0.042	1.000	0.179	0.724	0.740	0.093
	F_{IS}	0.021	0.032	0.054	0.203	0.120	-0.024	-0.345	-0.007	0.244	-0.080	0.087	0.298
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	46	48	38	47	42	44	46	47
LsaSP0509UVic (2)	H_o	0.41	0.43	0.41	0.33	0.37	0.48	0.63	0.43	0.36	0.45	0.41	0.26
	H_e	0.42	0.44	0.44	0.42	0.42	0.47	0.47	0.42	0.47	0.42	0.45	0.36
	P	1.000	1.000	0.744	0.178	0.479	1.000	0.041	1.000	0.186	0.724	0.744	0.089
	F_{IS}	0.021	0.032	0.054	0.203	0.120	-0.024	-0.345	-0.007	0.244	-0.080	0.087	0.298
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	46	48	38	47	42	44	46	47
LsaSP0510UVic (2)	H_o	0.21	0.17	0.28	0.17	0.35	0.23	0.21	0.26	0.31	0.27	0.28	0.26
	H_e	0.22	0.19	0.25	0.25	0.29	0.24	0.19	0.26	0.30	0.24	0.25	0.26
	P	1.000	0.412	0.573	0.043	0.319	1.000	1.000	1.000	1.000	1.000	0.570	1.000
	F_{IS}	0.029	0.115	-0.154	0.340	-0.200	0.032	-0.104	0.004	-0.043	-0.147	-0.154	0.004
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	46	48	38	47	42	44	46	47
LsaSP0520UVic (2)	H_o	0.15	0.17	0.13	0.15	0.20	0.32	0.24	0.24	0.11	0.26	0.17	0.16
	H_e	0.14	0.16	0.16	0.17	0.18	0.27	0.25	0.25	0.10	0.23	0.16	0.15
	P	1.000	1.000	0.284	0.336	1.000	0.576	0.571	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.074	-0.084	0.189	0.152	-0.098	-0.179	0.057	0.026	-0.042	-0.138	-0.084	-0.075
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	52	46	46	48	46	47	38	46	38	38	46	44
LsaSP0522UVic (2)	H_o	0.57	0.39	0.50	0.40	0.49	0.57	0.50	0.59	0.47	0.53	0.41	0.49
	H_e	0.50	0.50	0.50	0.50	0.51	0.49	0.48	0.49	0.50	0.47	0.50	0.49
	P	0.422	0.145	1.000	0.154	1.000	0.363	1.000	0.239	0.759	0.508	0.250	1.000
	F_{IS}	-0.140	0.226	0.007	0.215	0.032	-0.173	-0.046	-0.191	0.077	-0.149	0.181	-0.007
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	46	46	48	47	47	38	46	43	43	46	45
LsaSP0523UVic (3)	H_o	0.59	0.41	0.52	0.40	0.49	0.55	0.50	0.61	0.47	0.53	0.39	0.49
	H_e	0.50	0.51	0.50	0.50	0.51	0.49	0.48	0.50	0.50	0.47	0.50	0.49
	P	0.274	0.244	1.000	0.157	1.000	0.378	1.000	0.166	0.759	0.506	0.149	1.000
	F_{IS}	-0.173	0.184	-0.040	0.215	0.032	-0.138	-0.046	-0.213	0.077	-0.149	0.226	-0.007
	A	2	2	2	2	2	2	2	3	2	2	2	2
	n	56	46	46	48	47	47	38	46	43	43	46	45
LsaSP0524UVic (2)	H_o	0.11	0.15	0.07	0.10	0.02	0.06	0.13	0.04	0.09	0.05	0.11	0.04
	H_e	0.10	0.14	0.06	0.10	0.02	0.06	0.12	0.04	0.09	0.05	0.14	0.04
	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.219	1.000
	F_{IS}	-0.048	-0.071	-0.023	-0.044	0.000	-0.022	-0.057	-0.011	-0.037	-0.012	0.237	-0.011
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	46	46	48	47	47	38	46	43	43	46	45
LsaSP0525UVic (2)	H_o	0.59	0.43	0.50	0.42	0.49	0.55	0.55	0.57	0.44	0.53	0.35	0.49
	H_e	0.50	0.51	0.50	0.50	0.51	0.49	0.49	0.49	0.50	0.47	0.50	0.49
	P	0.284	0.380	1.000	0.259	1.000	0.370	0.505	0.371	0.536	0.503	0.040	1.000
	F_{IS}	-0.173	0.141	-0.001	0.175	0.032	-0.138	-0.131	-0.155	0.126	-0.149	0.313	-0.007
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	46	46	48	47	47	38	46	43	43	46	45
LsaSP0526UVic (2)	H_o	0.57	0.43	0.50	0.46	0.49	0.55	0.50	0.61	0.47	0.53	0.35	0.51
	H_e	0.50	0.51	0.50	0.50	0.51	0.49	0.50	0.50	0.50	0.47	0.50	0.50
	P	0.287	0.385	1.000	0.565	1.000	0.548	1.000	0.150	0.760	0.506	0.042	1.000
	F_{IS}	-0.147	0.141	-0.001	0.092	0.032	-0.121	-0.004	-0.228	0.077	-0.149	0.313	-0.030

a) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	46	46	48	47	47	38	46	43	43	46	45
LsaSP1001UVic (2)	H_o	0.18	0.11	0.13	0.17	0.21	0.04	0.13	0.30	0.05	0.07	0.15	0.13
	H_e	0.16	0.14	0.12	0.19	0.19	0.04	0.12	0.26	0.05	0.11	0.21	0.13
	P	1.000	0.217	1.000	0.406	1.000	1.000	1.000	0.571	1.000	0.117	0.102	1.000
	F_{IS}	-0.089	0.237	-0.059	0.117	-0.108	-0.011	-0.057	-0.169	-0.012	0.373	0.287	-0.060
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	46	46	48	47	47	38	46	43	43	46	45
LsaSP0529UVic (2)	H_o	0.59	0.39	0.50	0.42	0.48	0.57	0.55	0.54	0.45	0.56	0.33	0.48
	H_e	0.50	0.51	0.50	0.50	0.51	0.49	0.49	0.49	0.50	0.46	0.51	0.48
	P	0.285	0.135	1.000	0.251	0.773	0.365	0.509	0.543	0.552	0.192	0.019	1.000
	F_{IS}	-0.173	0.238	-0.001	0.171	0.054	-0.173	-0.131	-0.119	0.103	-0.217	0.357	0.015
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	44	46	48	46	47	38	46	42	43	46	44
LsaSP1002UVic (2)	H_o	0.04	0.09	0.06	0.04	0.04	0.08	0.05	0.09	0.07	0.07	0.11	0.11
	H_e	0.07	0.08	0.06	0.04	0.04	0.08	0.05	0.08	0.07	0.07	0.10	0.10
	P	0.058	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	0.488	-0.034	-0.022	-0.011	-0.011	-0.033	-0.014	-0.034	-0.023	-0.025	-0.047	-0.047
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	47	48	47	48	38	47	45	42	46	46
LsaSP0533UVic (3)	H_o	0.26	0.28	0.38	0.35	0.26	0.40	0.37	0.32	0.27	0.52	0.33	0.48
	H_e	0.33	0.30	0.40	0.39	0.31	0.37	0.37	0.35	0.30	0.43	0.38	0.46
	P	0.197	0.625	0.725	0.709	0.334	1.000	1.000	0.670	0.603	0.274	0.432	1.000
	F_{IS}	0.210	0.077	0.054	0.091	0.186	-0.062	-0.006	0.091	0.099	-0.214	0.141	-0.043
	A	2	2	2	2	2	3	2	2	2	2	2	2
	n	54	47	47	48	47	48	38	47	45	42	46	46
LsaSP0534UVic (2)	H_o	0.22	0.28	0.26	0.15	0.17	0.25	0.13	0.21	0.20	0.10	0.28	0.09
	H_e	0.20	0.24	0.23	0.17	0.16	0.22	0.12	0.19	0.18	0.09	0.25	0.08
	P	1.000	0.574	1.000	0.334	1.000	1.000	1.000	1.000	1.000	1.000	0.574	1.000
	F_{IS}	-0.116	-0.150	-0.136	0.152	-0.082	-0.133	-0.057	-0.108	-0.100	-0.038	-0.154	-0.034
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	47	48	47	48	38	47	45	42	46	46
LsaSP0535UVic (2)	H_o	0.15	0.23	0.19	0.23	0.23	0.21	0.29	0.15	0.20	0.21	0.17	0.30
	H_e	0.20	0.21	0.18	0.21	0.21	0.22	0.25	0.27	0.22	0.23	0.16	0.29
	P	0.107	1.000	1.000	1.000	1.000	0.543	1.000	0.008	0.502	0.531	1.000	1.000
	F_{IS}	0.259	-0.122	-0.095	-0.119	-0.122	0.058	-0.156	0.453	0.079	0.071	-0.084	-0.048
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	47	48	47	48	38	47	45	42	46	46
LsaSP1003UVic (2)	H_o	0.06	0.04	0.09	0.06	0.13	0.06	-	0.04	0.07	0.02	0.04	0.04
	H_e	0.09	0.04	0.08	0.06	0.12	0.06	-	0.04	0.07	0.07	0.04	0.04
	P	0.095	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	0.036	1.000	1.000
	F_{IS}	0.379	-0.011	-0.034	-0.022	-0.057	-0.022	-	-0.011	-0.023	0.661	-0.011	-0.011
	A	2	2	2	2	2	2	1	2	2	2	2	2
	n	54	47	47	48	47	48	38	47	45	42	46	46
LsaSP1004UVic (3)	H_o	-	0.04	0.02	0.10	0.09	0.10	0.03	-	0.02	0.02	0.07	0.04
	H_e	-	0.04	0.02	0.10	0.08	0.10	0.03	-	0.02	0.02	0.06	0.04
	P	-	1.000	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000
	F_{IS}	-	-0.011	0.000	-0.044	-0.034	-0.044	0.000	-	0.000	0.000	-0.015	-0.011
	A	1	2	2	2	2	2	2	1	2	2	3	2
	n	54	47	47	48	47	48	38	47	45	42	46	46
LsaSP0542UVic (2)	H_o	0.48	0.48	0.43	0.57	0.42	0.50	0.42	0.56	0.55	0.45	0.48	0.45
	H_e	0.48	0.50	0.51	0.50	0.50	0.47	0.51	0.50	0.51	0.48	0.50	0.50
	P	1.000	1.000	0.386	0.388	0.343	0.748	0.348	0.541	0.758	0.757	0.773	0.553
	F_{IS}	-0.010	0.038	0.141	-0.141	0.161	-0.069	0.171	-0.112	-0.084	0.053	0.047	0.111
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	46	46	47	38	44	38	41	42	44	46	47
LsaSP0543UVic (2)	H_o	0.27	0.24	0.28	0.36	0.26	0.36	0.26	0.37	0.31	0.39	0.35	0.30
	H_e	0.26	0.28	0.30	0.39	0.23	0.33	0.27	0.36	0.35	0.37	0.37	0.31
	P	1.000	0.325	0.633	0.706	1.000	0.662	1.000	1.000	0.404	1.000	0.696	0.661
	F_{IS}	-0.031	0.135	0.073	0.084	-0.138	-0.106	0.024	-0.015	0.128	-0.052	0.055	0.049
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	46	46	47	38	44	38	41	42	44	46	47
LsaSP0544UVic (2)	H_o	0.02	0.13	0.11	0.17	0.05	-	0.13	0.07	0.02	0.05	0.09	0.04
	H_e	0.02	0.12	0.10	0.16	0.05	-	0.12	0.07	0.02	0.04	0.08	0.04
	P	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	0.000	-0.059	-0.047	-0.082	-0.014	-	-0.057	-0.026	0.000	-0.012	-0.034	-0.011
	A	2	2	2	2	2	1	2	2	2	2	2	2
	n	56	46	46	47	38	44	38	41	42	44	46	47
LsaSP0569UVic (2)	H_o	0.41	0.43	0.54	0.48	0.38	0.54	0.42	0.44	0.53	0.55	0.61	0.45

a) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	H_e	0.50	0.46	0.47	0.48	0.45	0.49	0.49	0.50	0.45	0.49	0.50	0.48
	P	0.186	0.751	0.338	1.000	0.335	0.746	0.506	0.538	0.313	0.535	0.143	0.758
	F_{IS}	0.185	0.053	-0.171	0.007	0.158	-0.093	0.149	0.110	-0.189	-0.117	-0.228	0.075
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	46	46	46	47	41	38	43	45	44	46	47
LsaSP1005Uvic (2)	H_o	0.04	0.04	0.07	-	0.02	-	0.03	-	0.02	0.09	0.11	0.02
	H_e	0.04	0.04	0.06	-	0.02	-	0.03	-	0.02	0.09	0.10	0.02
	P	1.000	1.000	1.000	-	1.000	-	1.000	-	1.000	1.000	1.000	1.000
	F_{IS}	-0.009	-0.011	-0.023	-	0.000	-	0.000	-	0.000	-0.036	-0.047	0.000
	A	2	2	2	1	2	1	2	1	2	2	2	2
	n	56	46	46	46	47	41	38	43	45	44	46	47
LsaSP0570UVic (2)	H_o	0.55	0.51	0.46	0.48	0.36	0.52	0.53	0.55	0.55	0.42	0.45	0.36
	H_e	0.50	0.49	0.51	0.49	0.49	0.50	0.50	0.50	0.50	0.48	0.50	0.50
	P	0.433	1.000	0.753	1.000	0.120	1.000	1.000	0.549	0.550	0.522	0.559	0.077
	F_{IS}	-0.116	-0.047	0.089	0.031	0.259	-0.039	-0.051	-0.100	-0.099	0.136	0.095	0.275
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	39	39	48	44	44	38	44	42	43	44	47
LsaSP0571UVic (2)	H_o	0.63	0.56	0.43	0.44	0.33	0.43	0.61	0.45	0.57	0.39	0.52	0.26
	H_e	0.49	0.46	0.48	0.43	0.43	0.47	0.50	0.47	0.48	0.46	0.49	0.45
	P	0.049	0.305	0.530	1.000	0.150	0.747	0.325	1.000	0.322	0.330	0.765	0.004
	F_{IS}	-0.292	-0.215	0.103	-0.027	0.239	0.090	-0.205	0.029	-0.200	0.165	-0.065	0.440
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	41	42	48	43	44	38	44	42	44	46	47
LsaSP0573UVic (2)	H_o	0.63	0.56	0.43	0.44	0.33	0.43	0.61	0.45	0.57	0.39	0.52	0.24
	H_e	0.49	0.46	0.48	0.43	0.43	0.47	0.50	0.47	0.48	0.46	0.49	0.47
	P	0.050	0.300	0.528	1.000	0.157	0.746	0.330	1.000	0.324	0.331	0.766	0.001
	F_{IS}	-0.292	-0.215	0.103	-0.027	0.239	0.090	-0.205	0.029	-0.200	0.165	-0.065	0.489
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	41	42	48	43	44	38	44	42	44	46	46
LsaSP1006UVic (2)	H_o	0.17	0.13	0.13	0.08	0.13	0.14	0.08	0.15	0.20	0.14	0.13	0.13
	H_e	0.16	0.12	0.13	0.12	0.12	0.17	0.08	0.14	0.19	0.13	0.16	0.16
	P	1.000	1.000	1.000	0.153	1.000	0.294	1.000	1.000	1.000	1.000	0.288	0.287
	F_{IS}	-0.083	-0.059	-0.060	0.299	-0.057	0.186	-0.028	-0.070	-0.103	-0.062	0.188	0.188
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	53	46	45	48	47	44	38	47	44	44	45	45
LsaSP0598UVic (2)	H_o	0.46	0.45	0.51	0.49	0.49	0.36	0.34	0.52	0.54	0.51	0.43	0.53
	H_e	0.49	0.50	0.48	0.49	0.45	0.48	0.50	0.48	0.50	0.50	0.47	0.50
	P	0.760	0.753	0.742	1.000	0.736	0.124	0.138	0.754	0.737	1.000	0.746	0.757
	F_{IS}	0.076	0.094	-0.064	-0.004	-0.081	0.252	0.310	-0.080	-0.088	-0.030	0.090	-0.079
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	46	42	37	39	43	47	32	44	37	39	44	43
LsaSP0600UVic (2)	H_o	-	0.02	0.08	0.10	0.07	0.06	-	0.09	0.08	0.08	0.09	0.05
	H_e	-	0.02	0.08	0.10	0.11	0.06	-	0.09	0.08	0.07	0.09	0.05
	P	-	1.000	1.000	1.000	0.116	1.000	-	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-	0.000	-0.029	-0.041	0.373	-0.022	-	-0.036	-0.029	-0.027	-0.036	-0.012
	A	1	2	2	2	2	2	1	2	2	2	2	2
	n	46	42	37	39	43	47	32	44	37	39	44	43
LsaSP0603UVic (2)	H_o	0.07	0.11	-	0.06	0.07	0.07	0.03	0.13	0.11	0.14	0.05	0.09
	H_e	0.07	0.11	-	0.06	0.07	0.06	0.08	0.12	0.11	0.13	0.05	0.09
	P	1.000	1.000	-	1.000	1.000	1.000	0.041	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.029	-0.049	-	-0.022	-0.024	-0.023	0.660	-0.059	-0.049	-0.065	-0.012	-0.036
	A	2	2	1	2	2	2	2	2	2	2	2	2
	n	54	44	37	48	43	46	37	46	44	42	43	44
LsaSP0604UVic(2)	H_o	0.46	0.43	0.35	0.40	0.40	0.48	0.30	0.33	0.39	0.38	0.30	0.41
	H_e	0.45	0.37	0.43	0.37	0.44	0.39	0.39	0.40	0.41	0.43	0.37	0.40
	P	1.000	0.407	0.294	0.712	0.723	0.243	0.199	0.272	0.717	0.479	0.231	1.000
	F_{IS}	-0.018	-0.177	0.189	-0.076	0.094	-0.230	0.234	0.187	0.062	0.121	0.192	-0.020
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	44	46	48	43	46	37	46	44	42	43	44
LsaSP0605UVic (2)	H_o	0.07	0.11	-	0.06	0.07	0.07	0.03	0.13	0.11	0.14	0.05	0.09
	H_e	0.07	0.11	-	0.06	0.07	0.06	0.08	0.12	0.11	0.13	0.05	0.09
	P	1.000	1.000	-	1.000	1.000	1.000	0.042	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.029	-0.049	-	-0.022	-0.024	-0.023	0.660	-0.059	-0.049	-0.065	-0.012	-0.036
	A	2	2	1	2	2	2	2	2	2	2	2	2
	n	54	44	46	48	43	46	37	46	44	42	43	44
LsaSP0607UVic (2)	H_o	0.40	0.20	0.26	0.38	0.33	0.29	0.26	0.40	0.26	0.29	0.33	0.31
	H_e	0.38	0.21	0.31	0.34	0.33	0.25	0.30	0.38	0.26	0.34	0.30	0.35
	P	1.000	0.490	0.333	0.661	1.000	0.569	0.581	1.000	1.000	0.354	1.000	0.666
	F_{IS}	-0.045	0.082	0.186	-0.133	0.016	-0.155	0.138	-0.054	0.015	0.163	-0.071	0.111

a) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	46	47	47	46	42	38	40	43	42	46	45
LsaSP1007UVic (2)	H_o	0.07	0.13	0.09	0.13	0.13	0.12	0.08	0.03	0.09	0.07	0.11	0.18
	H_e	0.07	0.12	0.12	0.12	0.12	0.11	0.08	0.03	0.09	0.07	0.10	0.16
	P	1.000	1.000	0.155	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.029	-0.059	0.298	-0.057	-0.059	-0.051	-0.028	0.000	-0.037	-0.025	-0.047	-0.086
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	46	47	47	46	42	38	40	43	42	46	45
LsaSP1010UVic (3)	H_o	0.09	0.06	0.09	0.02	0.09	0.08	0.05	0.13	0.09	0.09	0.13	0.02
	H_e	0.09	0.06	0.08	0.02	0.09	0.08	0.10	0.12	0.09	0.09	0.12	0.02
	P	1.000	1.000	1.000	1.000	1.000	1.000	0.081	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.039	-0.022	-0.034	0.000	-0.035	-0.033	0.483	-0.057	-0.026	-0.037	-0.059	0.000
	A	2	2	2	2	2	2	2	2	3	2	2	2
	n	54	47	47	45	45	48	38	47	45	43	46	47
LsaSP1011UVic (2)	H_o	0.15	0.11	0.09	0.07	0.09	0.10	0.05	0.06	0.11	0.07	0.07	0.09
	H_e	0.14	0.10	0.08	0.07	0.09	0.10	0.05	0.06	0.11	0.07	0.06	0.08
	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.071	-0.045	-0.034	-0.023	-0.035	-0.044	-0.014	-0.022	-0.048	-0.024	-0.023	-0.034
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	47	45	45	48	38	47	45	43	46	47
LsaSP1008UVic (2)	H_o	0.17	0.17	0.11	0.22	0.16	0.17	0.24	0.11	0.07	0.23	0.17	0.09
	H_e	0.15	0.16	0.10	0.23	0.15	0.15	0.21	0.10	0.07	0.24	0.16	0.12
	P	1.000	1.000	1.000	0.570	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.153
	F_{IS}	-0.082	-0.082	-0.045	0.050	-0.073	-0.080	-0.121	-0.045	-0.023	0.043	-0.084	0.298
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	47	45	45	48	38	47	45	43	46	47
LsaSP0613UVic (2)	H_o	0.19	0.13	0.28	0.22	0.24	0.23	0.16	0.19	0.24	0.19	0.13	0.30
	H_e	0.17	0.16	0.27	0.20	0.22	0.21	0.19	0.18	0.25	0.17	0.12	0.26
	P	1.000	0.281	1.000	1.000	1.000	1.000	0.333	1.000	1.000	1.000	1.000	0.572
	F_{IS}	-0.093	0.191	-0.020	-0.114	-0.128	-0.119	0.175	-0.095	0.022	-0.091	-0.059	-0.165
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	47	45	45	48	38	47	45	43	46	47
LsaSP1009UVic (2)	H_o	0.17	0.17	0.11	0.22	0.13	0.17	0.24	0.11	0.07	0.23	0.17	0.09
	H_e	0.15	0.16	0.10	0.23	0.13	0.15	0.21	0.10	0.07	0.24	0.16	0.12
	P	1.000	1.000	1.000	0.572	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.158
	F_{IS}	-0.082	-0.082	-0.045	0.050	-0.060	-0.080	-0.121	-0.045	-0.023	0.043	-0.084	0.298
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	47	45	45	48	38	47	45	43	46	47
LsaSP1012UVic (2)	H_o	0.09	0.06	0.11	0.02	0.09	0.10	0.05	0.15	0.11	0.12	0.15	0.02
	H_e	0.12	0.06	0.10	0.02	0.09	0.10	0.10	0.14	0.11	0.11	0.14	0.02
	P	0.185	1.000	1.000	1.000	1.000	1.000	0.081	1.000	1.000	1.000	1.000	1.000
	F_{IS}	0.245	-0.022	-0.045	0.000	-0.035	-0.044	0.483	-0.070	-0.048	-0.050	-0.071	0.000
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	47	45	45	48	38	47	45	43	46	47
LsaSP0614UVic (3)	H_o	0.22	0.28	0.15	0.20	0.16	0.15	0.05	0.11	0.29	0.23	0.24	0.09
	H_e	0.25	0.24	0.14	0.18	0.15	0.17	0.05	0.24	0.31	0.24	0.21	0.16
	P	0.314	0.574	1.000	1.000	1.000	0.337	1.000	0.002	0.636	1.000	1.000	0.023
	F_{IS}	0.129	-0.150	-0.070	-0.100	-0.062	0.152	-0.014	0.561	0.068	0.043	-0.125	0.462
	A	2	2	2	2	3	2	2	2	2	2	2	2
	n	54	47	47	45	45	48	38	47	45	43	46	47
LsaSP0615UVic (2)	H_o	0.02	0.02	0.04	0.11	0.07	0.02	0.03	-	0.07	0.05	0.07	0.09
	H_e	0.02	0.02	0.04	0.11	0.07	0.02	0.03	-	0.07	0.05	0.10	0.08
	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	0.107	1.000
	F_{IS}	0.000	0.000	-0.011	-0.048	-0.023	0.000	0.000	-	-0.023	-0.012	0.375	-0.034
	A	2	2	2	2	2	2	2	1	2	2	2	2
	n	54	47	47	45	45	48	38	47	45	43	46	47
LsaSP0618UVic (2)	H_o	0.43	0.50	0.45	0.43	0.39	0.32	0.44	0.48	0.48	0.39	0.42	0.38
	H_e	0.50	0.50	0.48	0.49	0.51	0.45	0.51	0.50	0.50	0.46	0.48	0.47
	P	0.284	1.000	0.760	0.377	0.146	0.085	0.518	0.769	0.764	0.489	0.528	0.207
	F_{IS}	0.157	-0.001	0.075	0.140	0.228	0.302	0.125	0.047	0.057	0.144	0.123	0.197
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	46	47	47	46	44	36	46	42	41	45	45
LsaSP0619UVic (2)	H_o	0.37	0.46	0.40	0.47	0.39	0.34	0.42	0.46	0.33	0.34	0.38	0.29
	H_e	0.45	0.42	0.43	0.44	0.48	0.39	0.46	0.44	0.45	0.42	0.38	0.44
	P	0.226	0.725	0.737	0.744	0.227	0.439	0.714	1.000	0.159	0.267	1.000	0.037
	F_{IS}	0.176	-0.090	0.063	-0.066	0.189	0.128	0.095	-0.047	0.261	0.187	0.018	0.349
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	46	47	47	46	44	36	46	42	41	45	45
LsaSP0620UVic (2)	H_o	0.46	0.48	0.47	0.38	0.39	0.34	0.42	0.48	0.45	0.41	0.44	0.42

a) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	H_e	0.50	0.50	0.50	0.50	0.51	0.47	0.51	0.51	0.51	0.48	0.49	0.48
	P	0.595	0.773	0.770	0.144	0.149	0.108	0.327	0.773	0.550	0.504	0.755	0.529
	F_{IS}	0.083	0.053	0.064	0.235	0.228	0.283	0.180	0.054	0.107	0.130	0.085	0.123
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	46	47	47	46	44	36	46	42	41	45	45
LsaSP1013UVic (2)	H_o	0.02	0.07	0.06	0.04	-	0.02	0.03	0.09	0.02	0.02	0.04	0.07
	H_e	0.02	0.06	0.06	0.04	-	0.02	0.03	0.08	0.02	0.02	0.04	0.07
	P	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	0.000	-0.023	-0.022	-0.011	-	0.000	0.000	-0.034	0.000	0.000	-0.011	-0.023
	A	2	2	2	2	1	2	2	2	2	2	2	2
	n	54	46	47	47	46	44	36	46	42	41	45	45
LsaSP0635UVic (2)	H_o	0.39	0.50	0.44	0.43	0.50	0.36	0.42	0.55	0.39	0.64	0.41	0.57
	H_e	0.50	0.50	0.49	0.49	0.49	0.50	0.47	0.49	0.50	0.50	0.49	0.50
	P	0.158	1.000	0.758	0.550	1.000	0.075	0.722	0.549	0.137	0.071	0.361	0.557
	F_{IS}	0.218	0.007	0.085	0.127	-0.013	0.275	0.108	-0.121	0.235	-0.286	0.165	-0.128
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	51	46	45	47	46	47	38	47	44	44	46	46
LsaSP0636UVic (2)	H_o	0.39	0.50	0.44	0.45	0.50	0.36	0.42	0.55	0.39	0.64	0.41	0.57
	H_e	0.50	0.50	0.49	0.49	0.49	0.50	0.47	0.49	0.50	0.50	0.49	0.50
	P	0.157	1.000	0.754	0.567	1.000	0.073	0.724	0.547	0.136	0.070	0.361	0.550
	F_{IS}	0.218	0.007	0.085	0.090	-0.013	0.275	0.108	-0.121	0.235	-0.286	0.165	-0.128
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	51	46	45	47	46	47	38	47	44	44	46	46
LsaSP1014UVic (2)	H_o	0.06	0.15	0.07	0.11	0.07	0.04	0.19	0.04	0.11	0.14	0.07	0.15
	H_e	0.05	0.14	0.06	0.10	0.06	0.04	0.17	0.04	0.11	0.13	0.06	0.14
	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.019	-0.070	-0.023	-0.045	-0.023	-0.011	-0.091	-0.011	-0.049	-0.063	-0.023	-0.070
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	46	47	46	47	37	47	44	43	46	47
LsaSP0640UVic (2)	H_o	0.61	0.55	0.57	0.49	0.48	0.47	0.35	0.47	0.43	0.56	0.43	0.55
	H_e	0.50	0.49	0.50	0.48	0.49	0.49	0.45	0.47	0.50	0.51	0.50	0.49
	P	0.172	0.548	0.548	1.000	1.000	1.000	0.264	1.000	0.541	0.553	0.549	0.378
	F_{IS}	-0.217	-0.121	-0.122	-0.014	0.025	0.039	0.228	-0.003	0.136	-0.105	0.126	-0.138
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	46	47	46	47	37	47	44	43	46	47
LsaSP0653UVic (2)	H_o	0.35	0.31	0.24	0.40	0.30	0.27	0.26	0.34	0.18	0.24	0.24	0.28
	H_e	0.33	0.30	0.28	0.32	0.26	0.35	0.27	0.29	0.27	0.25	0.28	0.37
	P	1.000	1.000	0.326	0.171	0.572	0.197	1.000	0.320	0.051	1.000	0.579	0.107
	F_{IS}	-0.035	-0.053	0.135	-0.237	-0.165	0.218	0.024	-0.195	0.333	0.040	0.131	0.262
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	45	46	48	47	48	38	47	45	42	45	47
LsaSP0654UVic (2)	H_o	0.04	0.11	0.07	0.08	0.02	0.06	0.03	0.04	0.04	-	0.02	0.04
	H_e	0.04	0.11	0.06	0.08	0.02	0.06	0.03	0.04	0.04	-	0.02	0.04
	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000
	F_{IS}	-0.009	-0.048	-0.023	-0.033	0.000	-0.022	0.000	-0.011	-0.011	-	0.000	-0.011
	A	2	2	2	2	2	2	2	2	2	1	2	2
	n	55	45	46	48	47	48	38	47	45	42	45	47
LsaSP0655UVic (3)	H_o	0.37	0.37	0.34	0.42	0.32	0.30	0.32	0.19	0.31	0.35	0.35	0.22
	H_e	0.35	0.36	0.34	0.36	0.35	0.26	0.31	0.24	0.32	0.32	0.39	0.23
	P	0.208	1.000	1.000	0.412	0.672	0.571	1.000	0.192	1.000	1.000	0.467	0.559
	F_{IS}	-0.078	-0.038	-0.005	-0.169	0.091	-0.165	-0.043	0.207	0.039	-0.088	0.109	0.053
	A	3	2	2	2	2	2	2	2	2	2	2	2
	n	51	46	47	48	47	47	37	47	45	43	46	46
LsaSP0656UVic (2)	H_o	0.24	0.22	0.19	0.10	0.17	0.23	0.22	0.13	0.16	0.23	0.13	0.13
	H_e	0.24	0.23	0.18	0.10	0.16	0.24	0.24	0.16	0.15	0.21	0.12	0.12
	P	1.000	0.558	1.000	1.000	1.000	1.000	0.505	0.276	1.000	1.000	1.000	1.000
	F_{IS}	0.016	0.053	-0.095	-0.044	-0.082	0.029	0.089	0.191	-0.073	-0.120	-0.059	-0.059
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	51	46	47	48	47	47	37	47	45	43	46	46
LsaSP0657UVic (3)	H_o	0.33	0.26	0.34	0.44	0.32	0.36	0.30	0.38	0.42	0.33	0.28	0.43
	H_e	0.31	0.29	0.31	0.35	0.39	0.39	0.29	0.36	0.34	0.33	0.33	0.44
	P	1.000	0.599	1.000	0.092	0.095	0.707	1.000	1.000	0.170	1.000	0.370	1.000
	F_{IS}	-0.090	0.103	-0.089	-0.270	0.182	0.084	-0.013	-0.057	-0.257	0.028	0.148	0.022
	A	2	2	2	2	3	2	2	2	2	2	2	2
	n	51	46	47	48	47	47	37	47	45	43	46	46
LsaSP0662UVic (2)	H_o	0.32	0.32	0.19	0.32	0.26	0.31	0.32	0.34	0.32	0.36	0.35	0.32
	H_e	0.30	0.35	0.27	0.37	0.29	0.29	0.37	0.31	0.33	0.33	0.32	0.27
	P	1.000	0.672	0.073	0.420	0.604	1.000	0.410	1.000	1.000	0.664	1.000	0.578
	F_{IS}	-0.074	0.091	0.296	0.147	0.103	-0.062	0.133	-0.089	0.034	-0.106	-0.094	-0.179

a) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	50	47	47	47	46	48	37	47	44	44	46	47
LsaSP0664UVic (2)	H_o	0.36	0.40	0.36	0.47	0.41	0.44	0.43	0.53	0.43	0.55	0.54	0.40
	H_e	0.41	0.46	0.45	0.45	0.40	0.46	0.48	0.41	0.46	0.45	0.44	0.41
	P	0.485	0.521	0.208	1.000	1.000	1.000	0.731	0.072	0.742	0.308	0.166	1.000
	F_{IS}	0.117	0.123	0.192	-0.032	-0.033	0.041	0.094	-0.289	0.065	-0.203	-0.249	0.023
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	50	47	47	47	46	48	37	47	44	44	46	47
LsaSP1015UVic (2)	H_o	0.10	0.13	0.07	0.13	0.09	0.08	0.11	0.11	0.14	0.13	0.05	0.18
	H_e	0.09	0.16	0.11	0.16	0.08	0.08	0.11	0.10	0.13	0.12	0.05	0.16
	P	1.000	0.279	0.116	0.276	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.042	0.189	0.372	0.191	-0.034	-0.033	-0.045	-0.047	-0.063	-0.054	-0.014	-0.086
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	51	46	42	47	46	48	36	46	43	40	38	45
LsaSP0673UVic (2)	H_o	0.10	0.09	0.10	0.06	0.22	0.06	0.14	0.15	0.14	0.15	0.11	0.18
	H_e	0.09	0.12	0.09	0.06	0.23	0.10	0.13	0.18	0.17	0.14	0.10	0.16
	P	1.000	0.160	1.000	1.000	0.558	0.104	1.000	0.355	0.300	1.000	1.000	1.000
	F_{IS}	-0.042	0.297	-0.038	-0.022	0.053	0.376	-0.061	0.149	0.184	-0.068	-0.042	-0.086
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	51	46	42	47	46	48	36	46	43	40	38	45
LsaSP0674UVic (2)	H_o	0.16	0.22	0.19	0.21	0.22	0.21	0.17	0.17	0.16	0.25	0.18	0.24
	H_e	0.15	0.23	0.21	0.19	0.20	0.19	0.20	0.16	0.15	0.22	0.21	0.22
	P	1.000	0.561	0.456	1.000	1.000	1.000	0.350	1.000	1.000	1.000	0.415	1.000
	F_{IS}	-0.075	0.053	0.104	-0.108	-0.111	-0.106	0.170	-0.084	-0.077	-0.130	0.131	-0.128
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	51	46	42	47	46	48	36	46	43	40	38	45
LsaSP0678UVic (2)	H_o	0.05	0.04	0.02	0.10	0.04	0.06	0.13	0.04	0.02	0.09	0.11	0.04
	H_e	0.05	0.04	0.02	0.10	0.04	0.06	0.12	0.04	0.02	0.09	0.11	0.04
	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.019	-0.011	0.000	-0.044	-0.011	-0.022	-0.057	-0.011	0.000	-0.036	-0.048	-0.011
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	46	42	48	46	48	38	47	45	44	45	47
LsaSP0680UVic (2)	H_o	0.24	0.22	0.21	0.31	0.28	0.33	0.34	0.21	0.22	0.36	0.27	0.17
	H_e	0.21	0.20	0.23	0.29	0.30	0.31	0.29	0.19	0.20	0.33	0.23	0.16
	P	1.000	1.000	0.528	1.000	0.631	1.000	0.564	1.000	1.000	0.662	1.000	1.000
	F_{IS}	-0.125	-0.111	0.071	-0.062	0.073	-0.084	-0.194	-0.108	-0.114	-0.106	-0.143	-0.082
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	46	42	48	46	48	38	47	45	44	45	47
LsaSP0683Uvic (2)	H_o	0.13	0.17	0.19	0.15	0.26	0.21	0.13	0.13	0.11	0.16	0.11	0.09
	H_e	0.15	0.16	0.17	0.14	0.23	0.19	0.12	0.12	0.11	0.15	0.11	0.08
	P	0.295	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	0.162	-0.084	-0.093	-0.068	-0.139	-0.106	-0.057	-0.057	-0.048	-0.075	-0.048	-0.034
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	46	42	48	46	48	38	47	45	44	45	47
LsaSP0685UVic (2)	H_o	-	0.04	0.04	0.06	0.02	0.02	0.05	0.11	0.04	0.02	0.09	0.06
	H_e	-	0.04	0.04	0.06	0.02	0.06	0.05	0.10	0.04	0.02	0.08	0.06
	P	-	1.000	1.000	1.000	1.000	0.032	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-	-0.011	-0.011	-0.022	0.000	0.662	-0.014	-0.047	-0.011	0.000	-0.034	-0.022
	A	1	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	47	48	38	46	45	44	46	47
LsaSP0686UVic (2)	H_o	0.29	0.26	0.30	0.31	0.32	0.31	0.16	0.28	0.38	0.36	0.37	0.38
	H_e	0.27	0.31	0.29	0.35	0.27	0.37	0.23	0.36	0.38	0.36	0.30	0.34
	P	1.000	0.333	1.000	0.668	0.579	0.420	0.098	0.204	1.000	1.000	0.315	0.662
	F_{IS}	-0.050	0.186	-0.048	0.096	-0.179	0.153	0.321	0.208	0.018	-0.024	-0.216	-0.133
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	47	48	38	46	45	44	46	47
LsaSP0687UVic (2)	H_o	0.54	0.40	0.59	0.46	0.49	0.40	0.37	0.46	0.44	0.57	0.43	0.53
	H_e	0.44	0.45	0.48	0.46	0.45	0.49	0.42	0.49	0.49	0.50	0.49	0.46
	P	0.130	0.528	0.137	1.000	0.742	0.244	0.690	0.762	0.755	0.542	0.546	0.349
	F_{IS}	-0.220	0.096	-0.235	0.009	-0.096	0.190	0.118	0.062	0.085	-0.130	0.114	-0.157
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	47	48	38	46	45	44	46	47
LsaSP0700UVic (2)	H_o	0.46	0.45	0.39	0.46	0.43	0.40	0.50	0.59	0.49	0.53	0.35	0.34
	H_e	0.50	0.47	0.50	0.50	0.48	0.49	0.51	0.49	0.50	0.50	0.50	0.49
	P	0.601	0.759	0.229	0.572	0.549	0.234	1.000	0.236	1.000	0.766	0.043	0.039
	F_{IS}	0.076	0.055	0.214	0.087	0.098	0.190	0.013	-0.191	0.021	-0.060	0.313	0.313
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	46	48	38	46	45	43	46	47
LsaSP0701UVic (2)	H_o	0.14	0.09	0.09	0.15	0.04	0.08	0.11	0.17	0.16	0.12	0.09	0.13

a) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	H_e	0.13	0.12	0.12	0.17	0.04	0.08	0.10	0.16	0.15	0.11	0.08	0.12
	P	1.000	0.156	0.162	0.341	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	F_{IS}	-0.068	0.298	0.297	0.152	-0.011	-0.033	-0.042	-0.084	-0.073	-0.050	-0.034	-0.057
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	46	48	38	46	45	43	46	47
LsaSP0703UVic (2)	H_o	0.20	0.13	0.13	0.17	0.17	0.08	0.11	0.13	0.18	0.14	0.20	0.09
	H_e	0.18	0.12	0.12	0.15	0.16	0.08	0.10	0.16	0.16	0.17	0.18	0.12
	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.284	1.000	0.298	1.000	0.155
	F_{IS}	-0.100	-0.057	-0.059	-0.080	-0.084	-0.033	-0.042	0.189	-0.086	0.184	-0.098	0.298
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	47	46	48	46	48	38	46	45	43	46	47
LsaSP0707UVic (2)	H_o	0.28	0.21	0.11	0.19	0.20	0.09	0.27	0.07	0.21	0.12	0.07	0.16
	H_e	0.27	0.26	0.19	0.24	0.18	0.16	0.24	0.07	0.19	0.11	0.11	0.18
	P	1.000	0.248	0.045	0.185	1.000	0.025	1.000	1.000	1.000	1.000	0.109	0.356
	F_{IS}	-0.038	0.171	0.391	0.209	-0.096	0.461	-0.143	-0.025	-0.105	-0.050	0.374	0.147
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	44	48	41	46	37	42	43	43	45	45
LsaSP0708UVic (2)	H_o	0.48	0.34	0.52	0.48	0.51	0.48	0.43	0.50	0.40	0.40	0.42	0.49
	H_e	0.47	0.51	0.50	0.50	0.50	0.50	0.49	0.50	0.50	0.50	0.50	0.50
	P	1.000	0.041	0.769	0.778	1.000	1.000	0.513	1.000	0.224	0.218	0.365	1.000
	F_{IS}	-0.023	0.328	-0.048	0.048	-0.014	0.038	0.117	-0.002	0.205	0.219	0.151	0.029
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	44	48	41	46	37	42	43	43	45	45
LsaSP0709UVic (2)	H_o	0.26	0.13	0.09	0.10	0.12	0.11	0.22	0.12	0.21	0.07	0.09	0.16
	H_e	0.23	0.16	0.17	0.17	0.12	0.18	0.20	0.11	0.19	0.11	0.13	0.15
	P	0.577	0.275	0.026	0.037	1.000	0.042	1.000	1.000	1.000	0.115	0.160	1.000
	F_{IS}	-0.140	0.191	0.459	0.396	-0.053	0.394	-0.108	-0.051	-0.105	0.373	0.296	-0.073
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	44	48	41	46	37	42	43	43	45	45
LsaSP0710UVic (2)	H_o	0.20	0.13	0.11	0.04	0.12	0.09	0.27	0.07	0.09	0.14	0.04	0.13
	H_e	0.18	0.16	0.15	0.12	0.12	0.12	0.24	0.07	0.09	0.13	0.09	0.13
	P	1.000	0.277	0.231	0.005	1.000	0.159	1.000	1.000	1.000	1.000	0.067	1.000
	F_{IS}	-0.104	0.191	0.235	0.651	-0.053	0.297	-0.143	-0.025	-0.037	-0.063	0.485	-0.060
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	44	48	41	46	37	42	43	43	45	45
LsaSP0704UVic (2)	H_o	0.20	0.28	0.39	0.29	0.32	0.22	0.38	0.21	0.23	0.30	0.33	0.27
	H_e	0.18	0.24	0.34	0.28	0.30	0.26	0.34	0.23	0.24	0.35	0.30	0.23
	P	1.000	0.574	0.658	1.000	1.000	0.259	1.000	0.525	1.000	0.390	1.000	1.000
	F_{IS}	-0.104	-0.150	-0.130	-0.039	-0.048	0.168	-0.103	0.071	0.043	0.133	-0.071	-0.143
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	54	47	44	48	41	46	37	42	43	43	46	45
LsaSP0715UVic (2)	H_o	0.09	0.18	0.13	0.11	0.04	0.07	0.18	0.09	0.07	0.07	0.09	0.09
	H_e	0.09	0.17	0.16	0.14	0.04	0.07	0.17	0.13	0.07	0.07	0.09	0.09
	P	1.000	1.000	0.282	0.216	1.000	1.000	1.000	0.164	1.000	1.000	1.000	1.000
	F_{IS}	-0.038	-0.089	0.189	0.238	-0.011	-0.025	-0.088	0.295	-0.023	-0.026	-0.037	-0.035
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	44	46	47	46	42	38	44	45	41	43	45
LsaSP0717UVic (2)	H_o	0.45	0.50	0.48	0.49	0.46	0.43	0.45	0.59	0.56	0.56	0.44	0.38
	H_e	0.46	0.49	0.48	0.46	0.49	0.48	0.48	0.47	0.50	0.46	0.44	0.48
	P	1.000	1.000	1.000	0.750	0.760	0.525	0.738	0.109	0.554	0.300	1.000	0.210
	F_{IS}	0.016	-0.023	0.007	-0.063	0.062	0.103	0.065	-0.266	-0.109	-0.215	-0.014	0.216
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	44	46	47	46	42	38	44	45	41	43	45
LsaSP0718UVic (2)	H_o	0.24	0.18	0.20	0.19	0.13	0.12	0.26	0.14	0.22	0.24	0.19	0.20
	H_e	0.21	0.20	0.18	0.21	0.16	0.11	0.23	0.13	0.20	0.25	0.17	0.22
	P	1.000	0.440	1.000	0.483	0.282	1.000	1.000	1.000	1.000	1.000	1.000	0.503
	F_{IS}	-0.125	0.109	-0.098	0.084	0.189	-0.051	-0.138	-0.062	-0.114	0.036	-0.091	0.079
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	44	46	47	46	42	38	44	45	41	43	45
LsaSP0720UVic (2)	H_o	0.13	0.23	0.13	0.11	0.04	0.07	0.21	0.09	0.07	0.07	0.14	0.11
	H_e	0.12	0.20	0.16	0.14	0.04	0.07	0.19	0.17	0.07	0.07	0.13	0.11
	P	1.000	1.000	0.287	0.213	1.000	1.000	1.000	0.027	1.000	1.000	1.000	1.000
	F_{IS}	-0.059	-0.117	0.189	0.238	-0.011	-0.025	-0.104	0.459	-0.023	-0.026	-0.063	-0.048
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	44	46	47	46	42	38	44	45	41	43	45
LsaSP0721UVic (2)	H_o	0.13	0.23	0.13	0.11	0.07	0.07	0.21	0.09	0.09	0.10	0.14	0.11
	H_e	0.12	0.20	0.16	0.14	0.06	0.07	0.19	0.17	0.09	0.09	0.13	0.11
	P	1.000	1.000	0.283	0.212	1.000	1.000	1.000	0.027	1.000	1.000	1.000	1.000
	F_{IS}	-0.059	-0.117	0.189	0.238	-0.023	-0.025	-0.104	0.459	-0.035	-0.039	-0.063	-0.048

a) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	44	46	47	46	42	38	44	45	41	43	45
LsaSP0722UVic (3)	H_o	0.44	0.50	0.50	0.47	0.59	0.40	0.47	0.55	0.60	0.46	0.60	0.40
	H_e	0.50	0.52	0.51	0.50	0.52	0.52	0.49	0.52	0.50	0.50	0.53	0.50
	P	0.416	1.000	1.000	0.770	0.431	0.164	1.000	0.764	0.224	0.752	0.206	0.229
	F_{IS}	0.136	0.033	0.011	0.071	-0.124	0.220	0.042	-0.056	-0.211	0.083	-0.148	0.207
	A	2	3	2	2	3	3	2	3	2	2	3	2
	n	55	44	46	47	46	42	38	44	45	41	43	45
LsaSP0740Uvic (3)	H_o	0.47	0.67	0.50	0.57	0.59	0.59	0.68	0.70	0.49	0.50	0.46	0.60
	H_e	0.61	0.60	0.60	0.65	0.59	0.62	0.57	0.63	0.58	0.60	0.60	0.61
	P	0.066	0.094	0.253	0.169	0.053	0.143	0.455	0.220	0.345	0.363	0.118	0.229
	F_{IS}	0.225	-0.117	0.165	0.113	0.002	0.054	-0.194	-0.118	0.162	0.173	0.243	0.026
	A	3	3	3	3	3	3	3	3	3	3	3	3
	n	55	46	44	47	46	46	37	44	45	40	46	47
LsaSP0741UVic (2)	H_o	0.13	0.30	0.27	0.23	0.17	0.17	0.24	0.18	0.27	0.20	0.24	0.21
	H_e	0.12	0.29	0.27	0.24	0.20	0.20	0.26	0.17	0.23	0.18	0.21	0.23
	P	1.000	1.000	1.000	1.000	0.424	0.421	1.000	1.000	1.000	1.000	1.000	0.555
	F_{IS}	-0.059	-0.048	-0.008	0.029	0.113	0.113	0.053	-0.089	-0.143	-0.099	-0.125	0.055
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	55	46	44	47	46	46	37	44	45	40	46	47
LsaSP0747UVic (2)	H_o	0.36	0.31	0.33	0.44	0.40	0.38	0.42	0.37	0.34	0.33	0.30	0.38
	H_e	0.32	0.33	0.30	0.37	0.32	0.31	0.37	0.33	0.37	0.31	0.35	0.34
	P	0.667	0.658	1.000	0.248	0.170	0.183	0.649	0.658	0.682	1.000	0.393	0.661
	F_{IS}	-0.122	0.053	-0.071	-0.191	-0.239	-0.221	-0.152	-0.117	0.073	-0.054	0.133	-0.133
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	42	46	48	45	48	38	46	44	40	43	47
LsaSP0748UVic (2)	H_o	0.36	0.31	0.33	0.40	0.40	0.38	0.42	0.37	0.34	0.35	0.30	0.38
	H_e	0.32	0.33	0.30	0.35	0.32	0.31	0.37	0.33	0.37	0.32	0.35	0.34
	P	0.669	0.659	1.000	0.420	0.170	0.176	0.651	0.658	0.685	1.000	0.392	0.660
	F_{IS}	-0.122	0.053	-0.071	-0.148	-0.239	-0.221	-0.152	-0.117	0.073	-0.081	0.133	-0.133
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	42	46	48	45	48	38	46	44	40	43	47
LsaSP0749UVic (2)	H_o	0.27	0.17	0.15	0.17	0.16	0.25	0.18	0.20	0.18	0.25	0.21	0.23
	H_e	0.28	0.15	0.21	0.19	0.15	0.33	0.21	0.18	0.17	0.22	0.23	0.21
	P	0.642	1.000	0.100	0.406	1.000	0.096	0.414	1.000	1.000	1.000	0.521	1.000
	F_{IS}	0.058	-0.079	0.287	0.117	-0.073	0.252	0.131	-0.098	-0.089	-0.130	0.074	-0.122
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	42	46	48	45	48	38	46	44	40	43	47
LsaSP0750UVic (2)	H_o	0.14	0.29	0.20	0.13	0.16	0.02	0.26	0.13	0.20	0.20	0.09	0.11
	H_e	0.13	0.25	0.18	0.12	0.15	0.02	0.23	0.16	0.19	0.18	0.13	0.10
	P	1.000	0.572	1.000	1.000	1.000	1.000	1.000	0.282	1.000	1.000	0.168	1.000
	F_{IS}	-0.068	-0.155	-0.098	-0.056	-0.073	0.000	-0.138	0.189	-0.103	-0.099	0.294	-0.045
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	42	46	48	45	48	38	46	44	40	43	47
LsaSP0751UVic (2)	H_o	0.38	0.31	0.24	0.31	0.42	0.33	0.24	0.48	0.27	0.30	0.35	0.43
	H_e	0.33	0.30	0.28	0.29	0.34	0.33	0.32	0.41	0.33	0.38	0.37	0.38
	P	0.428	1.000	0.320	1.000	0.170	1.000	0.129	0.303	0.347	0.212	0.684	0.705
	F_{IS}	-0.140	-0.043	0.135	-0.062	-0.257	0.000	0.265	-0.169	0.173	0.212	0.067	-0.108
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	56	42	46	48	45	48	38	46	44	40	43	47
Over all loci	*FIS*	-0.029	-0.048	0.008	-0.009	-0.034	-0.006	0.005	-0.048	-0.048	-0.035	0.022	0.004

b) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
A06 (4)	*Ho*	0.64	0.60	0.61	0.50	0.72	0.69	0.76	0.66	0.69	0.61	0.65	0.53
	He	0.61	0.61	0.64	0.62	0.65	0.67	0.65	0.63	0.70	0.62	0.64	0.57
	P	0.490	0.919	0.247	0.140	0.544	0.409	0.575	0.991	0.297	0.335	0.740	0.300
	FIS	-0.061	0.030	0.045	0.199	-0.105	-0.023	-0.173	-0.041	0.011	0.010	-0.018	0.074
	A	4	4	4	4	4	4	4	4	4	4	4	4
	n	56	47	46	48	46	48	38	47	42	44	46	47
A09 (19)	*Ho*	0.75	0.73	0.63	0.71	0.71	0.81	0.71	0.78	0.57	0.74	0.65	0.70
	He	0.73	0.72	0.67	0.74	0.68	0.72	0.73	0.76	0.64	0.67	0.74	0.69
	P	0.324	0.245	0.583	0.504	0.627	0.606	0.687	0.784	0.890	0.398	0.271	0.757
	FIS	-0.028	-0.005	0.066	0.040	-0.053	-0.129	0.032	-0.033	0.109	-0.106	0.116	-0.016
	A	9	9	7	8	10	10	7	5	6	9	10	8
	n	52	44	46	48	45	47	38	46	37	38	46	43
A10 (11)	*Ho*	0.61	0.70	0.77	0.69	0.66	0.81	0.79	0.64	0.59	0.76	0.72	0.76
	He	0.70	0.71	0.73	0.73	0.71	0.77	0.66	0.72	0.69	0.71	0.74	0.74

b) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	P	0.014	0.438	0.397	0.500	0.553	0.566	0.558	0.051	0.168	0.217	0.914	0.882
	FIS	0.123	0.005	-0.049	0.057	0.078	-0.059	-0.194	0.113	0.146	-0.079	0.030	-0.032
	A	8	7	8	7	9	6	7	7	7	8	6	6
	n	54	47	47	48	47	48	38	47	46	42	46	46
B01 (5)	*Ho*	0.64	0.63	0.59	0.77	0.53	0.68	0.58	0.66	0.57	0.64	0.61	0.66
	He	0.62	0.64	0.65	0.67	0.59	0.65	0.63	0.63	0.63	0.67	0.67	0.64
	P	0.895	0.570	0.919	0.458	0.578	0.914	0.598	0.975	0.127	0.786	0.791	0.361
	FIS	-0.034	0.019	0.093	-0.140	0.111	-0.055	0.080	-0.039	0.095	0.052	0.087	-0.023
	A	4	5	4	4	3	4	4	4	4	4	4	4
	n	56	46	46	47	38	44	38	41	42	44	46	47
B05 (3)	*Ho*	0.43	0.46	0.57	0.48	0.38	0.54	0.42	0.44	0.53	0.57	0.65	0.45
	He	0.52	0.47	0.49	0.48	0.46	0.49	0.50	0.50	0.46	0.52	0.54	0.49
	P	0.393	1.000	0.569	1.000	0.457	0.747	0.406	0.542	0.541	0.751	0.330	0.723
	FIS	0.176	0.035	-0.164	0.007	0.171	-0.093	0.168	0.110	-0.170	-0.089	-0.213	0.090
	A	3	3	3	3	2	2	3	3	3	2	3	3
	n	56	46	46	46	47	41	38	43	45	44	46	47
B06 (5)	*Ho*	0.63	0.62	0.54	0.58	0.47	0.57	0.63	0.57	0.62	0.44	0.52	0.41
	He	0.53	0.54	0.58	0.57	0.56	0.57	0.59	0.55	0.58	0.52	0.54	0.57
	P	0.022	0.338	0.884	0.910	0.212	0.763	0.142	0.692	0.009	0.547	0.484	0.013
	FIS	-0.191	-0.136	0.070	-0.022	0.176	0.007	-0.077	-0.040	-0.059	0.146	0.031	0.278
	A	4	3	3	4	3	3	4	3	3	3	3	3
	n	56	39	39	48	43	44	38	44	42	43	44	46
B12 (2)	*Ho*	0.17	0.13	0.13	0.08	0.13	0.14	0.08	0.15	0.20	0.14	0.13	0.13
	He	0.16	0.12	0.13	0.12	0.12	0.17	0.08	0.14	0.19	0.13	0.16	0.16
	P	1.000	1.000	1.000	0.154	1.000	0.295	1.000	1.000	1.000	1.000	0.286	0.288
	FIS	-0.083	-0.059	-0.060	0.299	-0.057	0.186	-0.028	-0.070	-0.103	-0.062	0.188	0.188
	A	2	2	2	2	2	2	2	2	2	2	2	2
	n	53	46	45	48	47	44	38	47	44	44	45	45
C02 (3)	*Ho*	0.46	0.48	0.57	0.56	0.53	0.43	0.34	0.61	0.59	0.54	0.50	0.56
	He	0.49	0.51	0.53	0.54	0.52	0.52	0.50	0.54	0.54	0.54	0.53	0.52
	P	0.763	0.866	0.887	0.909	0.199	0.192	0.141	0.212	0.891	0.805	0.833	0.875
	FIS	0.076	0.071	-0.072	-0.040	-0.022	0.183	0.310	-0.147	-0.101	0.001	0.053	-0.071
	A	3	3	3	3	3	3	3	3	3	3	2	2
	n	46	42	37	39	43	47	32	44	37	39	44	43
C03 (3)	*Ho*	0.46	0.43	0.35	0.40	0.42	0.50	0.32	0.39	0.43	0.45	0.33	0.41
	He	0.48	0.39	0.43	0.38	0.46	0.40	0.40	0.43	0.44	0.47	0.38	0.42
	P	0.508	0.738	0.291	1.000	0.870	0.136	0.020	0.299	0.782	0.541	0.282	0.573
	FIS	0.031	-0.113	0.189	-0.038	0.082	-0.238	0.201	0.085	0.013	0.032	0.153	0.031
	A	3	2	3	3	3	3	3	3	3	3	3	3
	n	54	44	46	48	43	46	37	46	44	42	43	44
C04 (3)	*Ho*	0.45	0.33	0.34	0.49	0.43	0.40	0.34	0.43	0.35	0.36	0.39	0.47
	He	0.44	0.32	0.41	0.43	0.43	0.34	0.37	0.40	0.34	0.40	0.39	0.47
	P	1.000	0.652	0.107	0.804	1.000	0.530	0.636	1.000	1.000	0.462	0.553	0.747
	FIS	-0.045	-0.018	0.169	-0.135	-0.018	-0.179	0.068	-0.068	-0.041	0.097	-0.008	0.014
	A	3	3	3	3	3	3	3	3	3	3	3	3
	n	55	46	47	47	46	42	38	40	43	42	46	45
C06 (16)	*Ho*	0.63	0.55	0.60	0.69	0.64	0.60	0.50	0.49	0.62	0.67	0.63	0.53
	He	0.59	0.56	0.56	0.62	0.57	0.55	0.52	0.56	0.66	0.67	0.61	0.56
	P	0.857	0.670	0.898	0.656	0.989	0.933	0.714	0.046	0.121	0.967	0.933	0.379
	FIS	-0.060	0.011	-0.065	-0.106	-0.134	-0.093	0.030	0.122	0.054	-0.013	-0.035	0.046
	A	7	10	9	9	10	9	11	11	9	8	11	8
	n	54	47	47	45	45	48	38	47	45	43	46	47
C07 (8)	*Ho*	0.57	0.74	0.64	0.60	0.50	0.45	0.61	0.72	0.57	0.46	0.58	0.49
	He	0.63	0.67	0.63	0.61	0.59	0.55	0.66	0.68	0.63	0.55	0.59	0.57
	P	0.854	0.599	0.958	0.231	0.340	0.094	0.985	0.510	0.301	0.157	0.993	0.283
	FIS	0.097	-0.100	-0.015	0.023	0.156	0.181	0.072	-0.062	0.088	0.152	0.023	0.136
	A	6	5	5	5	5	5	5	3	5	5	6	6
	n	54	46	47	47	46	44	36	46	42	41	45	45
C11 (3)	*Ho*	0.39	0.50	0.44	0.45	0.50	0.36	0.42	0.55	0.39	0.64	0.41	0.57
	He	0.50	0.50	0.49	0.50	0.49	0.50	0.47	0.49	0.50	0.50	0.49	0.50
	P	0.165	1.000	0.758	0.734	1.000	0.079	0.724	0.548	0.140	0.071	0.362	0.557
	FIS	0.218	0.007	0.085	0.106	-0.013	0.275	0.108	-0.121	0.235	-0.286	0.165	-0.128
	A	2	2	2	2	2	2	2	2	2	3	2	2
	n	51	46	45	47	46	47	38	47	44	44	46	46
C12 (3)	*Ho*	0.61	0.60	0.61	0.57	0.54	0.51	0.46	0.51	0.50	0.58	0.50	0.66
	He	0.53	0.57	0.54	0.54	0.53	0.51	0.56	0.49	0.56	0.56	0.53	0.57
	P	0.117	0.534	0.518	0.690	0.440	0.772	0.319	0.785	0.735	0.497	0.358	0.540
	FIS	-0.152	-0.047	-0.136	-0.061	-0.032	0.002	0.185	-0.036	0.103	-0.030	0.061	-0.169
	A	3	3	3	3	3	3	3	3	3	3	3	3

b) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	n	54	47	46	47	46	47	37	47	44	43	46	47
D04 (3)	*Ho*	0.35	0.31	0.24	0.40	0.30	0.27	0.26	0.34	0.18	0.24	0.24	0.28
	He	0.34	0.31	0.28	0.33	0.26	0.36	0.27	0.29	0.27	0.25	0.28	0.38
	P	1.000	0.811	0.211	0.530	0.640	0.097	1.000	0.689	0.043	1.000	0.400	0.087
	FIS	-0.014	-0.006	0.161	-0.188	-0.151	0.244	0.038	-0.168	0.348	0.040	0.142	0.280
	A	3	3	3	3	3	3	3	3	2	3	3	3
	n	55	45	46	48	47	48	38	47	45	42	45	47
D05 (6)	*Ho*	0.76	0.67	0.66	0.77	0.70	0.70	0.70	0.60	0.73	0.65	0.61	0.61
	He	0.70	0.69	0.67	0.65	0.70	0.70	0.67	0.63	0.66	0.68	0.67	0.65
	P	0.739	0.919	0.660	0.134	0.239	0.540	0.553	0.562	0.520	0.559	0.413	0.530
	FIS	-0.096	0.023	0.010	-0.181	-0.010	-0.009	-0.043	0.057	-0.120	0.049	0.098	0.065
	A	4	4	4	4	4	4	4	5	4	4	5	4
	n	51	46	47	48	47	47	37	47	45	43	46	46
D06 (4)	*Ho*	0.46	0.57	0.47	0.60	0.48	0.58	0.59	0.60	0.55	0.61	0.59	0.55
	He	0.47	0.61	0.58	0.60	0.48	0.55	0.61	0.50	0.59	0.56	0.53	0.52
	P	0.820	0.684	0.030	0.643	0.267	0.527	0.625	0.308	0.399	0.215	0.065	0.888
	FIS	0.031	0.056	0.193	0.008	0.010	-0.054	0.028	-0.196	0.077	-0.104	-0.110	-0.057
	A	4	4	4	4	4	4	4	4	4	4	4	4
	n	50	47	47	47	46	48	37	47	44	44	46	47
D08 (4)	*Ho*	0.33	0.41	0.29	0.38	0.43	0.31	0.33	0.37	0.40	0.43	0.32	0.49
	He	0.31	0.46	0.39	0.38	0.46	0.34	0.40	0.41	0.41	0.44	0.34	0.48
	P	0.563	0.290	0.008	0.674	0.486	0.131	0.027	0.377	0.286	0.081	0.556	0.408
	FIS	-0.063	0.106	0.262	-0.004	0.055	0.095	0.172	0.089	0.044	0.026	0.080	-0.009
	A	4	4	4	4	4	4	4	4	4	4	4	4
	n	51	46	42	47	46	48	36	46	43	40	38	45
D09 (4)	*Ho*	0.35	0.39	0.38	0.44	0.54	0.46	0.45	0.32	0.31	0.43	0.36	0.26
	He	0.35	0.34	0.38	0.42	0.49	0.47	0.40	0.30	0.30	0.46	0.33	0.24
	P	0.263	0.857	0.884	0.751	0.303	0.206	0.695	0.792	0.767	0.255	0.664	1.000
	FIS	0.006	-0.149	0.004	-0.047	-0.105	0.019	-0.111	-0.054	-0.053	0.060	-0.067	-0.087
	A	4	4	4	4	4	4	4	4	4	4	4	4
	n	55	46	42	48	46	48	38	47	45	44	45	47
D10 (4)	*Ho*	0.55	0.51	0.65	0.56	0.57	0.52	0.47	0.59	0.56	0.66	0.61	0.64
	He	0.49	0.53	0.58	0.56	0.52	0.60	0.50	0.63	0.59	0.63	0.62	0.56
	P	0.517	0.621	0.427	0.869	0.855	0.123	0.266	0.588	0.924	0.729	0.462	0.679
	FIS	-0.126	0.034	-0.135	-0.003	-0.115	0.139	0.044	0.063	0.053	-0.055	0.024	-0.142
	A	4	4	4	4	4	4	4	4	4	4	3	4
	n	56	47	46	48	47	48	38	46	45	44	46	47
D12 (6)	*Ho*	0.59	0.53	0.57	0.56	0.50	0.50	0.63	0.61	0.64	0.58	0.57	0.47
	He	0.63	0.54	0.62	0.63	0.60	0.55	0.59	0.61	0.61	0.62	0.61	0.59
	P	0.858	0.038	0.225	0.419	0.190	0.709	0.691	0.138	0.838	0.349	0.001	0.229
	FIS	0.062	0.022	0.091	0.110	0.162	0.094	-0.065	-0.005	-0.049	0.056	0.080	0.203
	A	4	5	5	5	5	5	5	4	4	5	6	4
	n	56	47	46	48	46	48	38	46	45	43	46	47
E01 (10)	*Ho*	0.44	0.47	0.48	0.42	0.51	0.30	0.57	0.31	0.44	0.40	0.42	0.44
	He	0.42	0.47	0.49	0.50	0.45	0.43	0.54	0.33	0.43	0.47	0.41	0.43
	P	0.317	0.295	0.050	0.052	0.931	0.004	0.421	0.377	0.964	0.058	0.301	0.378
	FIS	-0.052	0.002	0.021	0.164	-0.137	0.297	-0.060	0.064	-0.035	0.166	-0.024	-0.038
	A	6	5	5	7	7	6	6	6	8	6	6	7
	n	54	47	44	48	41	46	37	42	43	43	45	45
E04 (8)	*Ho*	0.69	0.73	0.72	0.64	0.72	0.60	0.63	0.77	0.80	0.68	0.77	0.64
	He	0.72	0.74	0.72	0.71	0.71	0.68	0.69	0.73	0.71	0.69	0.73	0.73
	P	0.404	0.638	0.599	0.467	0.643	0.671	0.444	0.209	0.917	0.344	0.285	0.730
	FIS	0.037	0.022	-0.003	0.103	-0.017	0.127	0.092	-0.062	-0.135	0.014	-0.052	0.118
	A	7	5	7	6	6	7	6	7	6	5	6	6
	n	55	44	46	47	46	42	38	44	45	41	43	45
E08 (4)	*Ho*	0.53	0.78	0.57	0.68	0.63	0.63	0.76	0.77	0.67	0.63	0.57	0.70
	He	0.65	0.70	0.66	0.72	0.66	0.70	0.67	0.68	0.66	0.65	0.67	0.68
	P	0.208	0.413	0.188	0.399	0.079	0.213	0.764	0.573	0.124	0.196	0.165	0.242
	FIS	0.190	-0.114	0.145	0.049	0.042	0.105	-0.133	-0.133	-0.019	0.045	0.158	-0.037
	A	4	4	4	4	4	4	4	4	4	4	4	4
	n	55	46	44	47	46	46	37	44	46	40	46	47
E10 (8)	*Ho*	0.79	0.83	0.70	0.77	0.82	0.71	0.71	0.76	0.73	0.78	0.67	0.81
	He	0.76	0.75	0.73	0.73	0.72	0.74	0.78	0.76	0.76	0.78	0.77	0.75
	P	0.712	0.012	0.733	0.620	0.069	0.202	0.436	0.330	0.009	0.855	0.246	0.920
	FIS	-0.028	-0.106	0.053	-0.050	-0.138	0.039	0.095	0.002	0.042	0.007	0.120	-0.079
	A	5	5	5	5	5	5	5	5	6	7	5	5
	n	56	42	46	48	45	48	38	46	44	40	43	47
Over all loci	*FIS*	-0.029	-0.048	0.008	-0.009	-0.034	-0.006	0.005	-0.048	-0.048	-0.035	0.022	0.004

c) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
Lsal101EUVC (10)	*Ho*	0.07	0.11	0.02	0.10	0.09	0.09	-	0.06	0.10	0.14	0.02	0.15
	He	0.07	0.10	0.02	0.10	0.08	0.09	-	0.06	0.10	0.13	0.02	0.14
	P	1.000	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000	1.000	1.000
	FIS	-0.016	-0.022	0.000	-0.024	-0.019	-0.020	-	-0.011	-0.024	-0.032	0.000	-0.035
	A	6	2	2	7	4	4	5	4	6	5	4	1
	n	56	47	47	48	47	46	-	47	48	44	46	47
Lsal108 EUVC (14)	*Ho*	0.36	0.49	0.38	0.34	0.43	0.36	0.43	0.28	0.50	0.34	0.51	0.34
	He	0.39	0.45	0.38	0.34	0.46	0.37	0.41	0.33	0.43	0.38	0.48	0.36
	P	0.381	0.677	0.582	0.416	0.573	0.872	1.000	0.265	0.634	0.181	0.747	0.812
	FIS	0.078	-0.089	-0.016	0.001	0.085	0.026	-0.044	0.156	-0.171	0.105	-0.070	0.058
	A	7	6	5	7	7	6	8	7	6	5	8	4
	n	56	47	47	47	47	47	37	47	48	44	47	47
Lsal083ESFU (10)	*Ho*	0.55	0.57	0.68	0.79	0.72	0.72	0.55	0.70	0.71	0.64	0.81	0.68
	He	0.67	0.70	0.65	0.72	0.71	0.71	0.72	0.71	0.70	0.69	0.72	0.69
	P	0.151	0.148	0.552	0.112	0.150	0.402	0.005	0.270	0.741	0.527	0.556	0.933
	FIS	0.171	0.185	-0.056	-0.097	-0.009	-0.025	0.234	0.007	-0.010	0.073	-0.132	0.013
	A	5	4	5	5	6	6	4	5	4	5	4	5
	n	56	47	47	47	46	47	38	47	48	44	47	47
Lsal242EUVC (9)	*Ho*	0.70	0.74	0.68	0.51	0.62	0.66	0.59	0.68	0.67	0.68	0.79	0.60
	He	0.65	0.67	0.74	0.58	0.66	0.64	0.65	0.69	0.66	0.68	0.70	0.67
	P	0.563	0.647	0.386	0.093	0.237	0.904	0.588	0.460	0.738	0.698	0.898	0.084
	FIS	-0.070	-0.105	0.079	0.124	0.067	-0.023	0.090	0.013	-0.018	-0.002	-0.126	0.114
	A	6	5	7	6	5	6	5	6	6	5	5	7
	n	56	47	47	39	47	47	37	47	48	44	47	47
Lsal060ESFU (18)	*Ho*	0.43	0.40	0.30	0.38	0.48	0.38	0.45	0.43	0.36	0.36	0.35	0.30
	He	0.40	0.40	0.33	0.40	0.49	0.35	0.45	0.46	0.34	0.34	0.36	0.36
	P	0.446	0.527	0.276	0.327	0.896	0.741	0.498	0.359	0.546	0.766	0.270	0.222
	FIS	-0.067	-0.015	0.100	0.055	0.022	-0.097	-0.003	0.046	-0.076	-0.069	0.034	0.163
	A	7	4	5	7	7	8	6	7	7	8	9	9
	n	54	47	47	48	46	47	38	46	47	44	46	47
Lsal210EUVC (21)	*Ho*	0.87	0.83	0.76	0.74	0.75	0.80	0.71	0.70	0.79	0.80	0.74	0.76
	He	0.84	0.82	0.82	0.80	0.75	0.75	0.81	0.78	0.82	0.78	0.80	0.83
	P	0.962	0.667	0.110	0.203	0.419	0.855	0.013	0.134	0.740	0.461	0.788	0.176
	FIS	-0.042	-0.008	0.079	0.065	-0.006	-0.073	0.124	0.105	0.036	-0.017	0.080	0.090
	A	13	14	15	13	13	14	13	11	14	15	16	13
	n	55	47	45	47	44	46	38	46	48	44	42	41
Lsal229EUVC (13)	*Ho*	0.84	0.91	0.81	0.81	0.85	0.81	0.84	0.89	0.87	0.86	0.78	0.89
	He	0.83	0.86	0.81	0.86	0.86	0.83	0.83	0.84	0.80	0.83	0.85	0.81
	P	0.999	0.865	0.197	0.912	0.110	0.746	0.937	0.928	0.042	0.851	0.225	0.383
	FIS	-0.007	-0.070	0.003	0.055	0.015	0.022	-0.011	-0.068	-0.083	-0.047	0.075	-0.105
	A	11	8	10	8	9	9	7	10	9	10	8	8
	n	56	47	47	47	47	47	37	47	46	44	46	47
Lsal079ESFU (17)	*Ho*	0.73	0.79	0.85	0.91	0.79	0.89	0.84	0.85	0.90	0.84	0.81	0.81
	He	0.80	0.86	0.85	0.87	0.85	0.84	0.83	0.85	0.86	0.80	0.85	0.81
	P	0.387	0.396	0.860	0.809	0.048	0.568	0.606	0.961	0.893	0.556	0.456	0.127
	FIS	0.089	0.088	-0.005	-0.048	0.080	-0.060	-0.019	0.004	-0.040	-0.048	0.045	-0.003
	A	11	11	11	11	12	11	11	13	10	12	12	11
	n	56	47	47	47	47	47	38	47	48	44	47	47
Lsal105EUVC (10)	*Ho*	0.29	0.40	0.45	0.46	0.30	0.40	0.21	0.28	0.40	0.39	0.30	0.38
	He	0.25	0.41	0.46	0.49	0.30	0.42	0.22	0.30	0.40	0.49	0.30	0.38
	P	1.000	0.872	0.009	0.641	0.323	0.881	0.216	0.512	0.424	0.081	1.000	0.908
	FIS	-0.127	0.010	0.034	0.073	0.012	0.036	0.045	0.086	0.002	0.215	-0.002	-0.017
	A	5	5	4	6	7	4	6	4	6	6	4	5
	n	56	47	47	48	47	47	38	47	48	44	47	47
Lsal025ESFU (5)	*Ho*	0.13	0.15	0.15	0.08	0.09	0.04	0.05	0.13	0.17	0.05	0.07	0.06
	He	0.12	0.14	0.14	0.08	0.08	0.04	0.10	0.12	0.16	0.05	0.06	0.06
	P	1.000	1.000	1.000	1.000	1.000	1.000	0.080	1.000	1.000	1.000	1.000	1.000
	FIS	-0.043	-0.040	-0.045	-0.019	-0.025	-0.005	0.488	-0.042	-0.062	-0.006	-0.023	-0.015
	A	5	4	2	3	3	3	3	3	3	4	3	3
	n	56	47	47	48	47	47	38	47	48	44	46	47
Lsal006ESFU (19)	*Ho*	0.75	0.83	0.79	0.81	0.74	0.87	0.81	0.85	0.81	0.74	0.80	0.81
	He	0.74	0.80	0.79	0.78	0.75	0.80	0.78	0.78	0.79	0.76	0.78	0.78
	P	0.649	0.533	0.572	0.483	0.265	0.221	0.609	0.800	0.334	0.404	0.326	0.006
	FIS	-0.007	-0.037	0.008	-0.039	0.010	-0.087	-0.034	-0.092	-0.020	0.026	-0.029	-0.044
	A	10	9	11	12	11	11	10	9	10	10	11	8
	n	55	47	47	47	47	47	37	47	47	43	46	47
Lsal201EUVC (15)	*Ho*	0.20	0.43	0.34	0.29	0.34	0.38	0.45	0.34	0.38	0.39	0.23	0.32

c) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	He	0.19	0.44	0.32	0.29	0.37	0.36	0.39	0.39	0.33	0.39	0.22	0.31
	P	1.000	0.085	0.840	0.649	0.291	0.809	1.000	0.152	1.000	0.792	1.000	0.653
	FIS	-0.060	0.023	-0.052	-0.023	0.091	-0.073	-0.150	0.126	-0.127	0.015	-0.075	-0.039
	A	7	7	5	8	6	6	6	8	7	6	5	7
	n	56	47	47	48	47	47	38	47	48	44	47	47
Lsal106EUVC (8)	*Ho*	0.62	0.60	0.59	0.65	0.60	0.46	0.61	0.47	0.57	0.57	0.54	0.55
	He	0.56	0.60	0.58	0.58	0.59	0.50	0.60	0.50	0.54	0.63	0.56	0.53
	P	0.855	0.916	0.662	0.431	0.933	0.749	0.289	0.393	0.313	0.148	0.604	0.606
	FIS	-0.097	0.007	-0.014	-0.118	-0.008	0.094	-0.009	0.061	-0.072	0.105	0.022	-0.040
	A	5	6	5	6	4	5	5	5	5	6	5	4
	n	55	47	46	48	47	46	38	47	47	44	46	47
Lsal070ESFU (8)	*Ho*	0.164	0.045	0.159	0.208	0.085	0.128	0.184	0.109	0.111	0.182	0.128	0.217
	He	0.155	0.045	0.151	0.195	0.082	0.121	0.173	0.106	0.107	0.172	0.124	0.202
	P	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	FIS	-0.057	-0.012	-0.054	-0.067	-0.034	-0.057	-0.066	-0.030	-0.038	-0.060	-0.034	-0.075
	A	2	4	4	4	2	4	3	2	4	4	3	3
	n	55	44	44	48	47	47	38	46	45	44	47	46
Lsal238EUVC (16)	*Ho*	0.63	0.74	0.72	0.58	0.60	0.60	0.76	0.64	0.65	0.67	0.60	0.70
	He	0.66	0.69	0.68	0.64	0.63	0.65	0.72	0.74	0.61	0.70	0.63	0.70
	P	0.293	0.925	0.913	0.125	0.055	0.179	0.738	0.130	0.318	0.466	0.224	0.260
	FIS	0.049	-0.082	-0.069	0.085	0.060	0.087	-0.063	0.134	-0.055	0.042	0.050	0.000
	A	10	10	9	10	10	8	11	10	9	9	9	12
	n	56	47	47	48	47	47	38	47	48	43	47	47
Lsal225EUVC (29)	*Ho*	0.88	0.80	0.72	0.65	0.81	0.89	0.79	0.94	0.85	0.91	0.80	0.89
	He	0.89	0.89	0.89	0.86	0.89	0.89	0.88	0.90	0.89	0.87	0.88	0.90
	P	0.639	0.114	0.009	0.016	0.266	0.957	0.209	0.827	0.759	0.349	0.376	0.092
	FIS	0.014	0.106	0.194	0.241	0.089	0.003	0.099	-0.040	0.048	-0.047	0.089	0.013
	A	21	14	17	20	17	18	18	18	14	14	18	15
	n	56	40	39	43	47	46	38	47	47	44	45	46
Lsal050ESFU (14)	*Ho*	0.82	0.81	0.83	0.87	0.66	0.85	0.82	0.85	0.81	0.84	0.83	0.89
	He	0.80	0.80	0.79	0.80	0.79	0.82	0.85	0.81	0.84	0.83	0.83	0.83
	P	0.757	0.940	0.479	0.587	0.048	0.768	0.312	0.561	0.252	0.632	0.844	0.227
	FIS	-0.033	-0.010	-0.052	-0.091	0.170	-0.043	0.044	-0.052	0.033	-0.012	-0.002	-0.078
	A	10	11	12	9	12	12	11	12	11	12	11	12
	n	56	47	47	47	47	47	38	47	48	44	47	47
Lsal065ESFU (13)	*Ho*	0.71	0.77	0.51	0.64	0.61	0.62	0.61	0.62	0.63	0.59	0.62	0.57
	He	0.58	0.65	0.58	0.60	0.63	0.65	0.58	0.61	0.64	0.64	0.60	0.58
	P	0.125	0.557	0.412	0.035	0.081	0.017	0.571	0.538	0.719	0.369	0.614	0.867
	FIS	-0.234	-0.173	0.114	-0.062	0.028	0.052	-0.038	-0.013	0.018	0.081	-0.024	0.009
	A	8	5	7	5	5	6	6	6	6	5	5	5
	n	56	47	47	47	46	47	38	47	48	44	47	47
Lsal078ESFU (12)	*Ho*	0.48	0.57	0.60	0.67	0.57	0.39	0.45	0.57	0.46	0.52	0.50	0.54
	He	0.53	0.49	0.57	0.58	0.54	0.51	0.50	0.58	0.44	0.50	0.55	0.50
	P	0.245	0.235	0.868	0.358	0.537	0.072	0.312	0.961	0.223	0.869	0.143	0.787
	FIS	0.088	-0.179	-0.043	-0.150	-0.067	0.229	0.112	0.005	-0.053	-0.044	0.087	-0.064
	A	5	7	7	6	5	5	5	5	6	6	6	6
	n	56	47	47	48	47	46	38	47	48	44	42	41
Lsal110EUVC (6)	*Ho*	0.30	0.34	0.38	0.40	0.34	0.40	0.34	0.23	0.27	0.39	0.26	0.28
	He	0.31	0.39	0.36	0.42	0.33	0.35	0.30	0.21	0.25	0.35	0.34	0.28
	P	0.843	0.283	0.530	0.718	0.419	0.454	1.000	1.000	1.000	1.000	0.065	1.000
	FIS	0.019	0.120	-0.060	0.051	-0.030	-0.146	-0.124	-0.099	-0.101	-0.092	0.241	0.010
	A	4	3	4	3	3	4	4	4	5	3	3	4
	n	56	47	47	48	47	47	38	47	48	44	47	47
Lsal096ESFU (8)	*Ho*	0.57	0.57	0.55	0.63	0.67	0.62	0.50	0.65	0.69	0.45	0.55	0.57
	He	0.64	0.60	0.56	0.56	0.66	0.57	0.51	0.65	0.60	0.56	0.58	0.53
	P	0.451	0.453	0.217	0.323	0.248	0.969	0.052	0.894	0.201	0.380	0.898	0.395
	FIS	0.112	0.044	0.012	-0.114	-0.022	-0.078	0.016	-0.003	-0.150	0.187	0.045	-0.078
	A	6	6	5	5	8	6	6	6	5	6	6	5
	n	56	47	47	48	46	47	38	46	48	44	47	47
Lsal235EUVC (11)	*Ho*	0.27	0.15	0.47	0.40	0.45	0.32	0.34	0.35	0.35	0.34	0.28	0.32
	He	0.34	0.18	0.40	0.40	0.41	0.32	0.32	0.34	0.44	0.39	0.31	0.31
	P	0.020	0.351	0.479	0.017	0.649	1.000	0.358	0.198	0.141	0.285	0.575	0.366
	FIS	0.204	0.167	-0.173	-0.010	-0.087	-0.012	-0.069	-0.013	0.201	0.127	0.108	-0.043
	A	4	5	5	8	6	8	7	6	8	7	8	5
	n	56	47	47	47	47	47	38	46	48	44	47	47
LsalSTA4 (17)	*Ho*	0.57	0.77	0.72	0.73	0.60	0.55	0.58	0.45	0.60	0.59	0.64	0.51
	He	0.58	0.71	0.70	0.69	0.70	0.61	0.60	0.58	0.57	0.65	0.64	0.59
	P	0.103	0.849	0.472	0.594	0.180	0.311	0.363	0.093	0.406	0.330	0.631	0.706

c) Locus (No. of alleles)		Bs	Kt	Qs	Qs07	At	Nd	Nd07	Gd	Kw	Dg	Uc	Pb
	FIS	0.019	-0.086	-0.022	-0.065	0.145	0.101	0.032	0.235	-0.059	0.098	0.003	0.129
	A	8	12	8	7	9	7	8	9	8	12	9	8
	n	56	47	46	48	47	47	38	47	48	44	47	47
LsalSTA3													
(28)	*Ho*	0.80	0.94	0.83	0.80	0.87	0.81	0.84	0.87	0.81	0.80	0.80	0.91
	He	0.86	0.85	0.85	0.84	0.80	0.82	0.86	0.84	0.84	0.82	0.82	0.86
	P	0.010	0.926	0.839	0.068	0.716	0.834	0.912	0.525	0.310	0.188	0.516	0.912
	FIS	0.071	-0.108	0.029	0.046	-0.089	0.018	0.024	-0.039	0.037	0.026	0.025	-0.061
	A	14	16	17	16	17	14	14	15	13	13	16	15
	n	56	47	47	46	47	47	38	47	48	44	46	47
LsalSTA2													
(25)	*Ho*	0.89	0.79	0.89	0.78	0.93	0.79	0.76	0.91	0.83	0.88	0.80	0.93
	He	0.85	0.84	0.90	0.85	0.85	0.87	0.84	0.90	0.86	0.87	0.84	0.89
	P	0.577	0.280	0.323	0.249	0.721	0.086	0.076	0.996	0.529	0.390	0.044	0.649
	FIS	-0.049	0.059	0.011	0.084	-0.106	0.092	0.094	-0.018	0.028	-0.015	0.044	-0.049
	A	13	17	16	16	15	17	14	15	14	15	17	12
	n	56	47	46	46	46	47	38	46	48	43	46	46
LsalSTA5													
(32)	*Ho*	0.88	0.89	0.74	0.91	0.96	0.85	0.89	0.87	0.90	0.95	0.87	0.91
	He	0.91	0.90	0.86	0.91	0.92	0.90	0.90	0.88	0.89	0.90	0.90	0.91
	P	0.568	0.813	0.126	0.883	0.999	0.467	0.721	0.444	0.845	0.756	0.958	0.779
	FIS	0.037	0.005	0.146	0.003	-0.043	0.058	0.010	0.004	-0.002	-0.060	0.035	-0.002
	A	17	14	15	18	15	15	14	18	15	18	18	15
	n	56	47	46	44	47	47	38	47	48	44	46	45
LsalSTA1													
(39)	*Ho*	0.86	0.89	0.87	0.82	1.00	0.85	0.79	0.95	0.96	0.93	0.87	0.83
	He	0.91	0.89	0.90	0.90	0.92	0.89	0.90	0.90	0.92	0.90	0.91	0.91
	P	0.313	0.550	0.742	0.218	0.615	0.311	0.282	0.367	0.692	0.588	0.147	0.214
	FIS	0.064	0.001	0.027	0.091	-0.088	0.050	0.122	-0.057	-0.037	-0.032	0.039	0.088
	A	16	20	18	20	18	21	23	23	19	17	21	19
	n	56	47	47	45	47	47	38	43	48	44	47	47
Over all loci	*FIS*	0.008	-0.018	0.002	-0.025	0.005	0.005	0.030	0.002	-0.026	0.014	0.003	-0.017