

Population Structure of River Herring (Alewife, *Alosa pseudoharengus*, and Blueback Herring, *Alosa aestivalis*) Examined using Neutral Genetic Markers

by

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DALHOUSIE UNIVERSITY

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Abstract

Incorporating molecular genetic data into management decisions would provide fisheries managers with new tools to identify the magnitude of population structure, metapopulation dynamics, at risk populations, and possible genetic interactions between species, so that management strategies could be tailored to better support long-term viability of species and populations. Alewife (*Alosa pseudoharengus*) and Blueback Herring (*Alosa aestivalis*) are two closely related anadromous clupeid fishes native to the Atlantic coast of North America. Current management strategies for these two species, collectively known as river herring, take no account of stock structure or even the distinction between the two species. Using 12 neutral microsatellite loci, I examined at multiple spatial scales the patterns of population structure and genetic differentiation for river herring, with particular reference to comparisons of population structure in the U.S., where extensive stocking has occurred, and Canada, where there has been no stocking. Results suggested strong population structure within the Canadian portion of the range and weaker population structure in the U.S. portion. Highly significant genetic isolation by distance (IBD) for parts of the range unaffected by stocking provided evidence of natal homing and limited dispersal. The differing IBD results obtained in three Canadian regions may reflect differences in historical biogeographical factors and contemporary dispersal patterns among regions. Very weak differentiation and an absence of significant IBD for American stocked populations provided evidence that stocking has reduced genetic differentiation between populations in the U.S. portion of the range, except for two divergent stocked populations that appeared to have resisted homogenization. Evidence of recent population bottlenecks were identified in both U.S. and Canadian populations. The genetic structure of Blueback Herring populations appeared to be similar to that seen in Alewife. When sampled in the same river, Alewife and Blueback Herring were strongly differentiated, although hybrids of the two species were detected. Evidence of genetic impacts of stocking, regional variation in patterns of population structure and complex genetic interactions between the species suggest that current management strategies should be re-evaluated.

List of Abbreviations and Symbols Used

A_e	Allelic richness
ABI	Applied Biosystems
AMOVA	Analysis of molecular variance
ASMFC	Atlantic States Marine Fisheries Commission
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
F_{IS}	Inbreeding coefficient
H_o	Observed level of heterozygosity
H_e	Expected level of heterozygosity
HWE	Hardy-Weinberg equilibrium
IAM	Inifinite Allele Model
IBD	Isolation by distance
K	Number of clusters
MCMC	Markov chain Monte Carlo
MSA	Microsatellite Analyser
mtDNA	Mitochondrial DNA
N	Number of individual samples
N_a	Number of alleles
N_e	Effective Population Size
NJ	Neighbor-adjoining
NOAA	National Oceanic and Atmospheric Administration
PCoA	Principle coordinates analysis
PCR	Polymerase chain reaction
p-val	Probablity of departures from HWE
SDS	Sodium dodecyl sulphate
SMM	Stepwise Mutation Model
US	United States

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Chapter 1: Introduction

1.1 Population Structure

In most species, population structure is determined by a complex mix of factors, including historical demographic factors, micro-evolutionary processes, and anthropogenic influences. The long-term persistence of a species typically relies on a network of interconnected locally adapted populations that do not necessarily have equivalent levels of genetic variation or a tendency to respond to future environmental conditions in similar ways. This intraspecific diversity provides an adaptive buffer that promotes overall species abundance and survival (Hilborn *et al.* 2003, Hutchinson 2008, Roy *et al.* 2012). Peripheral populations, located near the limits of species ranges, tend to have a high adaptive significance to the species as a whole (Scudder 1989) and can express unique, polymodal, or otherwise variable genotypes, life histories, morphologies, dispersal abilities, biological tolerance or plasticity (Hardie and Hutchings 2010 and references therein). Additionally, small or less productive populations within the species range may have unique genetic compositions that are important for the future adaptation of the species (Reiss *et al.* 2009, Roy *et al.* 2012). When a species is managed as a single population, and equal fishing pressure is applied to multiple populations that differ significantly in biomass and resilience, there is significant risk of collapse for the less productive populations (Hutchinson 2008). Further, a loss of any populations would reduce gene flow among locally adapted populations that rely on immigration and emigration to maintain genetic diversity (Reiss *et al.* 2009, Schindler *et al.* 2010, Roy *et al.* 2012). Maintaining connectivity and variation among populations across the species' range will lead to enhanced resilience, reduced risk of over-exploitation, and greater ability for the species to buffer environmental change (Hilborn *et al.* 2003).

The goal for conservation and the development of management strategies should be the longevity and persistence of the fish species, but currently fisheries management often ignores the biocomplexity of stock structure, especially diversity in life history characteristics and adaptations to local spawning and nursery habitats (Hilborn *et al.* 2003). Instead fisheries managers rely heavily on political boundaries, species and fishery distributions, and patterns of fisheries participation to develop management strategies

(Reiss *et al.* 2009). However, incorporating molecular genetic data into management decisions can provide new tools to gauge the magnitude of the population structure, metapopulation dynamics and to prioritize populations for protection, thereby allowing management strategies to be tailored for long-term resource use (Roy *et al.* 2012).

1.2 River Herring

River herring is a term applied collectively to Alewife (*Alosa pseudoharengus*) and Blueback Herring (*Alosa aestivalis*), where these closely related anadromous iteroparous species co-occur along the Atlantic coast of North America (Loesch *et al.* 1982, Jessop and Anderson 1989, Bentzen *et al.* 1993). These two species have similar life history traits and differ only slightly in terms of morphometrics, growth, and time of spawning (Bigelow and Schroeder 1953, Leim and Scott 1966, Messieh 1977).

Both species spawn in headwater lakes, back eddies and still waters (DFO 2001), preferring deep and slow water with no particular preference to substrate (gravel, sand, detritus, submerged vegetation, clay and silt; Mather *et al.* 2012 and references therein). Alewife spawning runs occur as early as late February (Holland and Yelverton 1973) in North Carolina, whereas in the Gulf of St. Lawrence spawning does not begin until June (Neves 1981, Kuhn and Kornfield 2004, ASMFC 2009). Blueback Herring spawning runs occur from December in Florida (Williams *et al.* 1975, Greene *et al.* 2009) through August (Leim and Scott 1966, Greene *et al.* 2009) in the tributaries of the Gulf of St. Lawrence (Bigelow and Schroeder 1953). Blueback Herring are typically one or two orders of magnitude more abundant along the middle and southern part of their range than in the northern portion (Schmidt *et al.* 2003, Greene *et al.* 2009). Where there is considerable spatiotemporal overlap in spawning within rivers (Loesch 1987), peak spawning of the two species may differ by only 2-3 weeks (Hildebrand and Schroeder 1928), thereby increasing the potential for hybridization.

Young-of-year (YOY) will remain in freshwater for the summer and early autumn before migrating to the sea, where they remain in mixed assemblages along the coast until they reach sexual maturity at age 3-6 (DFO 2001, 2007). In response to warming water temperatures adults will migrate from the ocean and presumably return to their natal rivers to spawn. Alewives can live as long as 10 years, spawning approximately five

times (DFO 2001), while Blueback Herring are known to live up to 7-8 years (NOAA 2009).

River herring have supported commercial fisheries for more than 350 years in some areas (DFO 2001, ASMFC 2009, Schultz and Davis 2009). Recent range-wide declines in commercial landings from long-term harvest levels (DFO 2001, NOAA 2009) have been caused by human activities (overexploitation, habitat loss or degradation). This led the National Oceanic and Atmospheric Administration (NOAA) to place river herring on the Species of Concern List in 2006 (NOAA 2009), and these two species may be upgraded to 'threatened' under the Endangered Species Act (Cournane *et al.* 2012). A moratorium on directed fisheries for river herring in individual U.S. state managed waters was imposed 10 years ago, but recently (2012) it was extended coast-wide. All fisheries in which there was no approved sustainable harvest plans in place were closed (ASMFC 2009, Cournane *et al.* 2012).

Recovery efforts have focused on spawning habitat reclamation (through modification of fish passages and dam removal; Hasselman and Limburg 2012, McIntyre *et al.* 2007, DFO 2012), reduction in water pollution (DFO 2007), and restrictions on both commercial and recreational fishing (DFO 2001, ASFMC 2009). Additionally, the U.S. has implemented stock translocation programs that move stocks within (in-basin) and between (out-of-basin) watersheds for the recolonization of extirpated populations (ASFMC 1985). These translocation programs were developed and implemented without any genetic information (i.e., population structure) for the species, and stock transfers among divergent source populations may unintentionally reduce population fitness and jeopardize genetically distinct populations leading to introgression and homogenization (Hansen *et al.* 2000, Hasselman and Limburg 2012).

Comprehensive data on population structure for river herring, where population restoration has relied heavily on out-of-basin stock transfers only within the U.S. portion of the range, are largely unavailable. A recent study by Bentzen *et al.* (2009) identified weak population structure within Maine, and Palkovacs *et al.* (2008) identified weak population structure within Connecticut, but both studies were confined to small spatial scales (< 450 km) and restricted to relatively few loci and limited in sample numbers and sites. The lack of genetic information on river herring leaves the development of fisheries

management to be based on stock assessments, which rely on conventional fisheries management indices (i.e., mortality estimates, juvenile abundance indices, stock-recruitment, and yield per recruit; ASMFC 1999, 2008, Hasselman and Limburg 2012). Thus far, river herring are managed as a single stock species, instead of two separate species (ASMFC 1985, Loesch 1987). To properly manage these species, identification of the population structure is needed so that management bodies can identify and preserve unique populations.

1.3 Thesis Overview

The purpose of this thesis is to examine the pattern of genetic diversity and differentiation in river herring species within the northern portion of their range, corresponding to the upper Virginian and Acadian biogeographic provinces, using neutral genetic markers. Within this portion of the range, population restoration involving out-of-basin stock transfers has been limited to the U.S. portion of the range. Identifying and comparing river herring population structure within and between the two countries will provide valuable information about ‘natural’ patterns of population genetic structure and how they are altered by stocking which can aid in the development of proper management strategies to achieve conservation goals.

Chapter 2 focuses on the broad geographic scale (up to 2500 km). Alewife samples were collected from 44 rivers and ponds from Nemasket, Massachusetts to Miramichi, New Brunswick. Blueback Herring samples were collected from four river locations from Orland, Maine to Margaree, Nova Scotia. For Alewife, the use of 12 microsatellite markers and a variety of analytical approaches suggested substantial population structure within the Canadian portion of the range that may reflect both historical and contemporary dispersal patterns, and weaker population structure in the U.S. where long-term stocking has occurred. The magnitude and patterns of genetic differentiation also differed among regions in Canada, possibly as a result of a mix of historical and contemporary influences on dispersal. Evidence of recent genetic bottlenecks in a number of U.S. and Canadian river populations provided further evidence that human activities have influenced patterns of genetic diversity.

Blueback Herring appear to have a genetic structure that is similar to that seen in Alewives which suggests that the species are similar with regard to natal homing behaviour and dispersal. Yet, when sampled in the same river, Alewife and Blueback Herring were strongly differentiated, indicating reproductive isolation and little if any introgressive gene flow. Nonetheless, these two species do hybridize, and at some locations hybrids appear relatively abundant, thus hybridization rates between the two species deserve further study. In general, more Blueback Herring populations need to be studied in order to make stronger comparisons of the population structure of the two species.

Chapter 3 uses the same 12 microsatellite loci, but more intensive sampling to examine Alewife populations in Maine in greater detail. Temporally replicated samples were collected over a 3-4 year period for 14 of the 16 rivers or ponds to examine the pattern of genetic diversity and differentiation in a portion of the Alewife range that has been subject to long-term and extensive stocking. The temporally replicated sampling revealed inter-annual variation in allele frequencies, as well as a more detailed picture of the effects of stocking on population structure. The greater number of samples employed confirmed the effects of stocking on the genetic structure of Maine populations, but also showed that stocking has not entirely obliterated the 'natural' IBD pattern in non-stocked populations. Moreover, two stocked populations were found to be genetically divergent, indicating that they have resisted the homogenizing effects of stocking. Overall, the results from this study suggest that the long-term viability of Alewife populations may be best served by a shift in management focus from stocking to habitat restoration efforts, such as dam removal.

Chapter 2: Population Genetics of Alewife (*Alosa pseudoharengus*) and Blueback Herring (*Alosa aestivalis*) Examined using Neutral Genetic Markers

2.1 Introduction

Proper identification of population structure is essential for effective management of fish stocks (Ward 2000, Reiss *et al.* 2009, Roy *et al.* 2012) and identification of conservation units. However, population structure is often overlooked in the development of management strategies that typically rely heavily on species and fishery distributions, political boundaries, and fisheries participation (Reiss *et al.* 2009). This approach ignores the biocomplexity of the stock structure, and specifically the diversity in life history characteristics and adaptations to local spawning and nursery habitats, that may help fisheries adjust to environmental changes, enhance resilience, and resist the pressures of exploitation (Hilborn *et al.* 2003). Additionally, managing a stock as a single population, thereby applying equal fishing pressure to multiple populations that differ significantly in biomass and resilience, will potentially bring about the collapse of less productive populations (Hutchinson 2008). Although extirpation of less productive populations may seem of minor consequence, such populations may have unique genetic compositions that are important for the future adaptation of the species to environmental change (Reiss *et al.* 2009, Roy *et al.* 2012). For example, peripheral populations within a species range tend to have a high adaptive significance to the species as a whole (Scudder 1989) and can express unique, polymodal, or otherwise variable genotypes, life histories, morphologies, dispersal abilities, biological tolerance or plasticity (Hardie and Hutchings 2010) and references therein). However, a loss of any population could lead to an interruption in gene flow among locally adapted populations which may rely on immigration and emigration to maintain genetic diversity (Reiss *et al.* 2009, Schindler *et al.* 2010, Roy *et al.* 2012). The erosion of genetic diversity may result in a corresponding decreases in fitness, with affected traits including developmental stability (Gautschi *et al.* 2002), body size (Wisely *et al.* 2008), fecundity (Culver *et al.* 2008) and disease resistance (Reber *et al.* 2008). Additionally, populations that have recently suffered a severe reduction in size (i.e., bottleneck) and/or have a small effective population size

could be increasingly at risk for extinction because their adaptive potential has been greatly reduced as a result of an increased rate of inbreeding, loss of genetic variation and fixation of mildly deleterious alleles (Ryman and Laikre 1991, Cornuet and Luikart 1996).

Discontinuities in environmental conditions can form barriers to species distributions, creating distinct biogeographic provinces that can act as filters to gene flow, creating population structure within wide-ranging species that span biogeographic boundaries (Mach *et al.* 2011). Therefore, identifying and understanding population structure will provide the tools necessary to tailor a management strategy for conservation and long-term resource use (Roy *et al.* 2012).

Alewife (*Alosa pseudoharengus*) and Blueback Herring (*A. aestivalis*) (collectively termed river herring) are iteroparous anadromous fish species that exhibit overlapping distributions over much of their range along the Atlantic coast of North America, and can occur in sympatry in fresh water (Loesch *et al.* 1982, Jessop and Anderson 1989), estuarine (Stone and Daborn 1987), and marine (Neves 1981) habitats. These closely related species (Bentzen *et al.* 1993) have similar life history traits and differ only slightly in terms of morphometrics, growth parameters, and time of spawning (Bigelow and Schroeder 1953, Leim and Scott 1966, Messieh 1977). Alewives spawn in drainages from North Carolina to tributaries in the Gulf of St. Lawrence (Neves 1981, Kuhn and Kornfield 2004, ASMFC 2009) while Blueback Herring spawn in drainages from Florida (Williams *et al.* 1975) to the tributaries of the Gulf of St. Lawrence (Bigelow and Schroeder 1953). However, Blueback Herring are one or two orders of magnitude more abundant along the middle and southern part of their range than in the northern portion (Schmidt *et al.* 2003, Greene *et al.* 2009). Spawning runs occur in chronological progression from south to north (Neves 1981), from late February (Holland and Yelverton 1973) through June (ASMFC 2009, DFO 2001) for Alewives, and December (Greene *et al.* 2009) through August (Leim and Scott 1966, Greene *et al.* 2009) for Blueback Herring. There may be considerable spatiotemporal overlap in spawning within rivers (Loesch 1987), and peak periods may differ only by 2-3 weeks (Hildebrand and Schroeder 1928) between the species leading to the potential for

hybridization as seen in some other pairs of closely related anadromous species (e.g., *Oncorhynchus clarki clarki* and *Oncorhynchus mykiss*; Campton and Utter 1985).

River herring are ecologically important as prey for a variety of terrestrial, freshwater and marine animals, and as a conduit for nutrient exchanges between marine and riverine ecosystems (Durbin *et al.* 1979, Garman and Macko 1998, Post and Palkovacs 2009). Additionally, these species have supported commercial fisheries for centuries (DFO 2001, ASMFC 2009, Davis and Schultz 2009) with landings dating back over 350 years in some areas (NOAA 2009). Fishing occurs primarily during their spring migration and no distinction is made between the two species (ASMFC 1985, DFO 2001, Loesch 1987). Significant declines in commercial landings from long-term harvest levels in many river specific fisheries (DFO 2001, NOAA 2009) and in extreme cases extirpation (Limburg *et al.* 2003) in response to human impacts (i.e., overfishing and spawning habitat loss or degradation; Bilkovic *et al.* 2002) resulted in the National Oceanic and Atmospheric Administration (NOAA) placing river herring on the Species of Concern List in 2006 (NOAA 2009). Currently there is an investigation into whether to list these species as 'threatened' under the Endangered Species Act (Cournane *et al.* 2012). Recovery efforts have focused on spawning habitat reclamation (through modification of fish passages and dam removal; Hasselman and Limburg 2012, McIntyre *et al.* 2007, DFO 2012), reduction in water pollution (DFO 2007) and restrictions on both commercial and recreational fishing (DFO 2001, ASMFC 2009).

Another restoration approach implemented by the U.S. in absence of any genetic data was a stock transplantation program that moved stocks within (in-basin) and between (out-of-basin) watersheds for the recolonization of extirpated populations (ASMFC 1985). However, stock transfers among divergent source populations may unintentionally reduce population fitness and jeopardize genetically distinct populations by removing the reproductive barriers established through philopatry, thereby creating opportunities for nonnative genes or gene complexes to introgress into native populations (Nelson and Soule 1987, Hasselman and Limburg 2012). Stock transfers may inadvertently result in the loss of local adaptations and the breakdown of co-adapted gene complexes (i.e., outbreeding; Edmands 2006, McClelland and Naish 2007, Bentzen *et al.* 2009). Repeated stock transfers may result in the replacement of the native population

and in the long term could lead to homogenization and introgression (Evans and Willox 1991, Hansen *et al.* 2000, Hasselman and Limburg 2012).

Thus far, management of alosine fishes has been primarily guided by stock assessments that rely on conventional fisheries management indices (i.e., mortality estimates, juvenile abundance indices, stock-recruitment, and yield per recruit; ASMFC 1999, 2008, Hasselman and Limburg 2012). Despite recognition of the need to incorporate molecular methods (i.e., microsatellites, mtDNA, etc.) into fisheries management strategies (Okumus and Çiftci 2003), this has not yet occurred for management of alosines (Hasselman and Limburg 2012). Microsatellites provide a powerful tool for the investigation of relationships at population and familial scales because of their variability, codominant inheritance, extensive distribution throughout the genome of many species (Wright and Bentzen 1994), and relatively easy assay, and could contribute to the development of alosine management strategies (Ferguson and Danzmann 1998, Ward 2000, Chistiakov *et al.* 2006).

Previous molecular studies of alosines used microsatellites and mtDNA, and have predominantly focused on American Shad (*Alosa sapidissima*), which has mirrored the same range wide declines and restoration approaches as river herring. Currently, few data are available on population structure for river herring in the northern part of the range, corresponding to the upper Virginian and Acadian biogeographic provinces. Within this portion of the range, population restoration involving out-of-basin stock transfers has been limited to the U.S. portion of the range. Weak population structure in Maine Alewife populations was detected in studies by Bentzen *et al.* (2009) and Labbe (2012), but both these studies were somewhat limited in geographic scope, number of loci and sample sizes. The goal of this chapter is to examine the pattern of genetic diversity and differentiation in Alewife and Blueback Herring populations at a large spatial scale (up to 2500 km). The specific objectives are to use 14 microsatellite loci to identify and separate Alewife and Blueback Herring species and a) evaluate the pattern of population structure for both Alewife and Blueback Herring populations, b) determine whether isolation by distance exists among Alewife populations, c) examine the implication of management strategies for patterns of genetic differentiation and gene flow among Alewife

populations, and d) test for evidence whether Alewife populations have experienced recent population bottlenecks.

2.2 Material and Methods

2.2.1 Sample Collection

A total of 4,065 Alewife were collected as fin clips from 48 locations during the time period of 2005-2011 within the upper Virginian and Acadian Biogeographic Provinces (Southern New England to Gulf of St. Lawrence; Figure 2.1, Table 2.1). Of the 48 locations, 12 have been subject to stocking (Table 2.2). Most samples came from adults collected during the spring spawning migration, but Alewife from Soudabscook and Veazie Dam on the Penobscot River, ME, were collected as young-of-year specimens in September. The majority of samples were collected at fish ladders or at commercial dip-stands and weirs, but a few locations were sampled using dip-nets. Collections of fin clips per location were obtained through the cooperation of multiple governmental organizations in Canada and the U.S., non-profit organizations, and Unama'ki Institute of Natural Resources. All samples were stored in 95% ethanol prior to DNA extraction.

A total of 213 spawning Blueback Herring samples were collected from five locations over three years in Atlantic Canada and Maine (2009-2011; Table 2.1). Samples were collected at fish ladders, and before tissue collection species identity was verified by observing peritoneum colouration (i.e., dark/sooty for Blueback Herring, pink for Alewife; Messieh 1977). Assistance in the collection of fin clips per a sampling location was given by governmental organizations. All samples were stored in 95% ethanol.

2.2.2 Laboratory Protocol

Whole genomic DNA was extracted from 3,934 Alewife and Blueback Herring samples. A small piece of tissue was excised from each sample and dried to remove all traces of ethanol. Samples were incubated overnight in 200 μ L digestion buffer (100 mM NaCl, 50 mM Tris·HCl pH 8, 10 mM EDTA, 0.5% SDS, 40 μ g Proteinase K) at 55°C with continuous mixing at 200 rpm. DNA isolation followed the sodium iodide and glassmilk binding protocol of Elphinstone *et al.* (2003), with slight modifications to

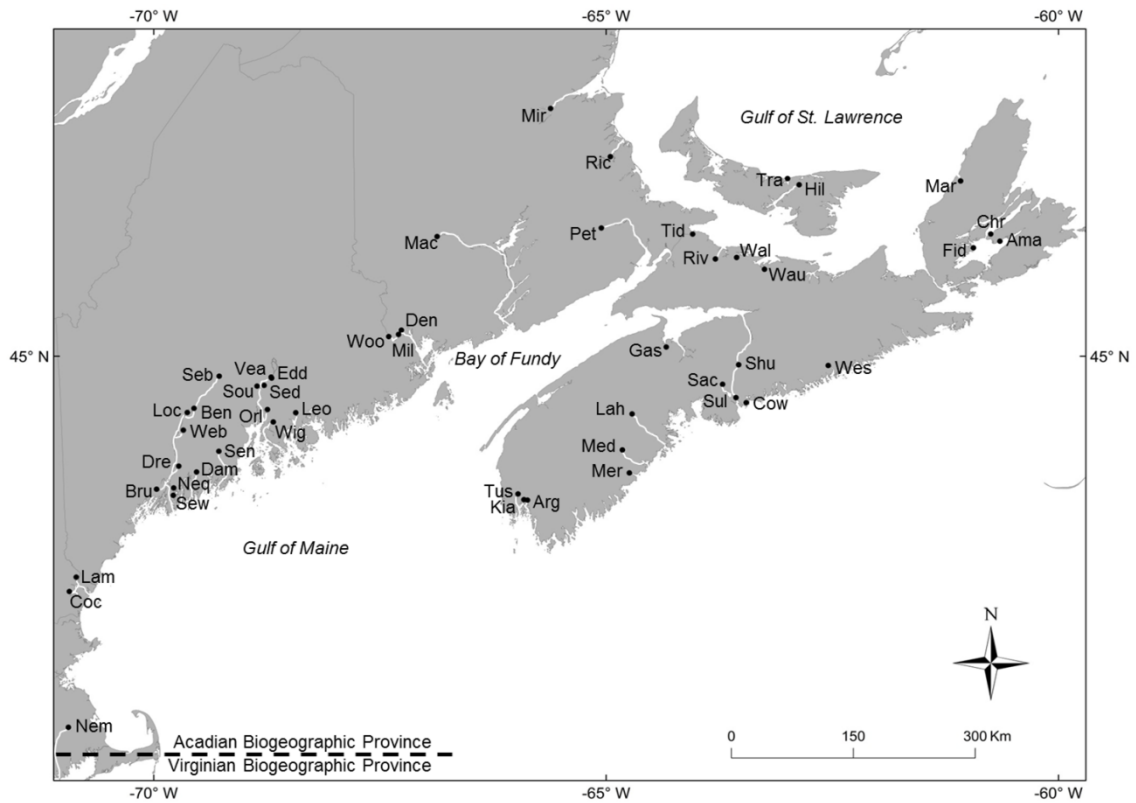


Figure 2.1: Alewife (*A. pseudoharengus*) sample locations within the upper Virginian and Acadian Biogeographic Provinces. Blueback Herring (*A. aestivalis*) were also sampled at Dre, Orl, Mac, Pet and Mar. Full names are given in Table 2.1.

Table 2.1: Sample sizes of Alewife and Blueback Herring collected from various locations in the Acadian and upper Virginian Biogeographic Provinces during 2005-2011.

Species	Code	State/Province	Sampling Location	'05	'08	'09	'10	'11	'12	Total	
Alewife	Nem	Massachusetts	Nemasket River				60			60	
	Coc	New Hampshire	Cocheco River						54	54	
	Lam		Lamprey River						21	21	
	Sew	Maine	Sewell Pond			65		51		116	
	Neq		Nequasset Lake Dam			65	60	46		171	
	Bru		Brunswick Dam			60	60	51		171	
	Dre		Dresden Mills Dam		63	34	60	57		214	
	Web		Webber Pond			37	60	52		149	
	Ben		Benton Falls Dam			65	63	60		188	
	Seb		Sebasticook Lake Dam			22				22	
	Loc		Lockwood Dam			66		51		117	
	Dam		Damariscotta Mills Dam			60	70	51		181	
	Sen		Sennebec Pond		52		63	50		165	
	Wig		Wight Pond			53		55		108	
	Orl		Orland Dam			5	75	53		133	
	Sou		Souadabscook Falls			65	60	114		239	
	Sed		Sedgeunkedunk Dam			15				15	
	Edd		Eddington Bend			16				16	
	Vea		Veazie Dam			70	53	52		175	
	Leo		Leonard Lake Dam			66		50		116	
	Den	Border	Dennis Stream		48						48
	Mil		Milltown		56						56
	Woo		Woodland		7						7
	Mac	New Brunswick	Mactaquac Dam					50			50
	Pet		Petitcodiac River					50			50
	Ric		Richibucto River					63			63
	Mir		Miramachi River					55			55
Tra	Prince Edward	Tracadie Bay					60			60	
Hil	Island	Hillsborough River					75			75	
Shu	Nova Scotia	Shubenacadie River					50			50	
Gas		Gaspereau River					58			58	
Tus		Tusket River					53			53	
Kia		Kiack Brook					50			50	
Arg		Argyle Brook					51			51	
Med		Medway River					51			51	
Mer		Mersey River					57			57	
Lah		LaHave River					56			56	
Sul		Sullivan's Pond Outlet					52			52	
Sac		Sackville River					61			61	
Cow		Cow Bay River					10			10	
Wes		West River, Sheet Harbour					52			52	
Ama		Amaguadees Pond					51			51	
Fid		Fiddle Head					52			52	
Chr		Christmas Island Pond					54			54	
Mar		Margaree River					49			49	
Wau		Waughs River					104			104	
Wal		Wallace River					102			102	
Riv		River Phillip					57			57	
Tid		Tidnish River					100			100	
Blueback Herring	Mac	New Brunswick	Mactaquac Dam				50			50	
	Pet		Petitcodiac River				53			53	
Mar	Nova Scotia	Margaree River				50			50		
Dre	Maine	Dresden Mills Dam		21						21	
Orl		Orland Dam				55				55	
Ale Total				111	115	764	684	2316	75	4065	
Bbh Total					21	55		153		229	
Overall Total				111	136	819	684	2469	75	4294	

Table 2.2: Summary of stocking history for all sample locations from 1983-2009 with some recorded events from the 1940s. Stocked locations are abbreviated ‘N’ and non-stocked locations are abbreviated ‘Y’. The rivers or watersheds in which sampling locations are located are abbreviated as follows: A, Androscoggin River; K, Kennebec River; S, Sebasticook River; O, Orland River; M, Machias River; R, Royal River.

State/Province	Sampling Location	River	Major Watershed	Stocking History
Massachusetts	Nemasket River	Nemasket River	Taunton	N
New Hampshire	Cochecho River	Cochecho River	Piscataqua	N
	Lamprey River	Lamprey River	Piscataqua	N
Maine	Sewell Pond	Sewell Creek	Kennebec	Y-A
	Nequasset Lake Dam	Nequasset Brook	Kennebec	N
	Brunswick Dam	Androscoggin River	Androscoggin	Y-K, S, R
	Dresden Mills Dam	Eastern River	Kennebec	Y-K, S
	Webber Pond	Seven-mile Stream	Kennebec	Y-A, S
	Benton Falls Dam	Sebasticook River	Kennebec	Y-K, A, R
	Sebasticook Lake Dam	Sebasticook River	Kennebec	Y-K, A, R
	Lockwood Dam	Kennebec River	Kennebec	Y-A
	Damariscotta Mills Dam	Damariscotta River	Central Coastal	N
	Sennebec Pond	St. George River	Central Coastal	Y-K, S
	Wight Pond	Bagaduce River	Penobscot	Y-O
	Orland Dam	Orland River	Penobscot	N
	Soudabscook Falls	Soudabscook Stream	Penobscot	N
	Sedgeunkedunk Dam	Sedgeunkedunk Stream	Penobscot	N
	Eddington Bend	Penobscot River	Penobscot	N
	Veazie Dam	Penobscot River	Penobscot	N
	Leonard Lake Dam	Union River	Eastern Coastal	Y-O*
Border	Dennis Stream	Dennis Stream	St. Croix	N
	Milltown	St. Croix River	St. Croix	Y-M*
	Woodland	St. Croix River	St. Croix	Y-M*
New Brunswick	Mactaquac Dam	Saint John River	Saint John	N
	Petitcodiac River	Petitcodiac River	Petitcodiac	N
	Richibucto River	Richibucto River	Richibucto	N
	Miramachi River	Miramachi River	Miramachi	N
Prince Edward Island	Tracadie Bay		Tracadie	N
	Hillsborough River	Hillsborough River	Hillsborough	N
Nova Scotia	Shubenacadie River	Shubenacadie River	Shubenacadie, Stewiacke	N
	Gaspereau River	Gaspereau River	Gaspereau	N
	Tusket River	Tusket River	Tusket	N
	Kiack Brook	Kiack Brook	Tusket	N
	Argyle Brook	Argyle Brook	Tusket	N
	Medway River	Medway River	Herring Cove, Medway	N
	Mersey River	Mersey River	Mersey	N
	LaHave River	LaHave River	LaHave	N
	Sullivan's Pond Outlet	Sullivan's Pond Outlet	Sackville	N
	Sackville River	Sackville River	Sackville	N
	Cow Bay River	Cow Bay River	Sackville	N
	West River, Sheet Harbour	West River, Sheet Harbour	East, West (Sheet Hbr)	N
	Amaguadees Pond	Bras d'Or Lake	Bras d'Or Lake	N
	Fiddle Head	Bras d'Or Lake	Bras d'Or Lake	N
	Christmas Island Pond	Bras d'Or Lake	Bras d'Or Lake	N
	Margaree River	Margaree River	Margaree	N
	Waugh's River	Waugh's River	River John	N
	Wallace River	Wallace River	River John	N
	River Phillip	River Phillip	Phillip Wallace	N
	Tidnish River	Tidnish River	Tidnish, Shinimicas	N

*word of mouth

accommodate use of a Perkin Elmer MPEII Liquid handling robot. Agarose gel electrophoresis was used to assess DNA quality and quantity from extracted subsamples.

Extracted DNA was used in each of three multiplex polymerase chain reactions (PCRs) that amplified a total of 14 microsatellite loci developed for *A. pseudoharengus* (*Aps2A*, *Aps-1*: Bentzen and Paterson 2005; *Ap058*, *Ap010*, *Ap071*: Labbe *et al.* 2012), *A. aestivalis* (*Aa082*, *Aa081*, *Aa046*, *Aa070*, *Aa039*, *Aa093*: Labbe *et al.* 2012) and *A. sapidissima* (*AsaC249*, *AsaD042*: Julian and Bartron 2007; *Asa8*: Waters *et al.* 2000; Table 2.3). Some primers (i.e., *Aa039*, *Ap071*) were redesigned using Primer3 Input v.2.0 software (Rozen and Skaletsky 2000) to facilitate their inclusion in the three multiplex mixes. PCRs were conducted in 5 μ L volumes that included 2xType-it Microsatellite PCR Master Mix (HotStarTaq plus DNA Polymerase, dNTP mix, 3 mM MgCl₂; Qiagen, Canada), RNase-free water, 10 μ M Primer mix and template DNA. Amplifications were conducted using Eppendorf 384-well thermocyclers. Amplification for mixes 1 and 2 consisted of an initial denature step at 95°C for 5 min, followed by 30 cycles of denature for 30 s at 95°C, annealing for 90 s at 60°C, extension for 30 s at 72°C, and a final extension at 60°C for 30 min. For mix 3, reaction parameters were the same except that the annealing temperature was increased to 62°C and the cycles were decreased from 30 to 28. PCR products from the three multiplexes were combined into one 384-well plate and sent to the Genome Quebec facility at McGill University where they were visualized using an Applied Biosystems (ABI) 3730XL capillary electrophoresis system. Alleles were scored using GeneMarker software (SoftGenetics) and a molecular weight standard ladder (GeneScan 500 LIZ) within each sample. Electropherograms were scored in reference to positive controls and redundant samples and negative controls were used to ensure consistency and reproducibility of genotypes.

2.2.3 Statistical Analysis

Not all genotyped specimens generated useable data, here defined as interpretable genotypes at a minimum of seven loci. In addition, since sample size can influence estimates of population genetic parameters, sampled locations with fewer than 50 specimens were excluded from further analysis (Table 2.1; Dresden (Blueback Herring), Cow Bay, Sedgeunkedunk, Eddington and Woodland), with the exception of Lamprey, which is one of two populations representing New Hampshire. A minimum sample size

Table 2.3: Summary information for 14 microsatellite markers used in this study.

Locus	Repeat motif	Primer sequence (5'-3')	T _A (°C)	Size range (bp)	Reference	Accession #
Asa-8	(TTTG) ₈	F: TCCATTCCATTACGTAGAGCACT R: CCGGCAGGGCACAGAAC	60	132-148	Waters et al. 2000	AF039660
Aa082	(ACAT) ₄	F: TGCTTCCCACACAAACACAC R: ATCGAAATCGTGACACCCC	60	168-184	Labbe et al. 2012	JN383996
AsaC249	(CATA) ₈ (TTCT) ₁₃	F: TTATTACAACGGTGAATTGAGTG R: TAAGTGCATGTTGTGTGTGATG	60	210-254	Julian & Bartron, 2007	EF014994
MGPL- Aa093*	(ATCT) ₈	F: ACAGTGTCTCCAGTATAGATGAATCG R: AGCCTATTGTGTTGGCGATATT	60	272-284	Labbe et al. 2012	JN384003
Aa081	(ATCT) ₅	F: TGTCCCATGAACTGCACAAC R: GTCATTGCAGCAGCACACC	60	152-184	Labbe et al. 2012	JN384000
Aa070	(GATT) ₅	F: TGACAGGTTGATTGATGATTGC R: TGACCGTTGTGGCCGTAG	60	210-270	Labbe et al. 2012	JN383999
Aps2A	(TCAA) ₈	F: CCAGTTACGTCAGTCACACGA R: TGGGCAGACAACAGAAGTTTT	60	94-122	Bentzen & Paterson, 2005	HM190305
AsaD042	(CTAT) ₁₂	F: ACTGGTCAATTGTAAGACACCC R: CAAGATGACCAAGGGTTAAGAC	60	167-191	Julian & Bartron, 2007	EF015000
Ap010	(AATG) ₉	F: GGGGACATCGTTGATTAATTGC R: GAACTTGCCATTTACTGGATAC	60	223-263	Labbe et al. 2012	JN383997
MGPL- Ap071	(CATT) ₈	F: TTTTCCCATATTTTGCTCATTTC R: TCGTTTCGGCATAAACACAAA	62	280-292	Labbe et al. 2012*	JN383998
Aps-1	(CTGT) ₈	F: CTGCACGTCTGACTGTCTGC R: TATGGGATGGATGGGATCAG	62	84-104	Bentzen & Paterson, 2005	HM190304
Aa046	(GGAT) ₈	F: GTCACCTCCTTCACCAGGG R: TTCCTGCCAGCATTATTGAGC	62	212-228	Labbe et al. 2012	JN383992
Ap058	(ACGC) ₅	F: TGCTTACCCCTTCACTGGC R: GGCGGAAATGGTGTGGAAC	62	254-278	Labbe et al. 2012	JN383995
MGPL- Aa039*	(CTTT) ₄	F: TCGGTACGTCTGCTCATCTG R: GCCTAGGCCTTGAGTATGAAA	62	300-320	Labbe et al. 2012	JN384006

*Both forward and reverse primers were redesigned for locus Aa093 to increase the size range. The reverse primer was redesigned for locus Ap071 and the forward primer was redesigned for locus Aa039 to increase their size ranges.

of 50 individuals has been advocated as a suitable number for conducting analyses of population structure using microsatellites (Ruzzante 1998). However, this criterion would exclude all but one Blueback Herring collection from analysis; therefore, a minimum sample size of 35 Blueback Herring was used for inclusion of this species in genetic analyses. The final dataset consisted of 48 locations (44 Alewife; 4 Blueback Herring) and 3,880 individuals. File formatting of the database to conduct analysis was done using both CREATE (v.1.35; Coombs *et al.* 2008) and PGDSpider (v.2.0.1.7; Lischer and Excoffier 2012).

STRUCTURE (v.2.2; Pritchard *et al.* 2000, Falush *et al.* 2003), a Bayesian model-based clustering method, was used on this dataset to identify and filter all Alewife, Blueback Herring and hybrid individuals (Table 2.4). A burn-in of 50,000 replicates was followed by 1,000,000 replicates of the Markov Chain Monte Carlo (MCMC) simulations, employing the admixture model and correlated allele frequencies among populations. Ten iterations of this parameter set were performed for $K = 2$ (number of clusters). Each individual was separated and categorized based on their proportion of genotypes that represent each species (q-value). An individual was categorized as a pure Alewife if it had a q-value > 0.90 or a pure Blueback Herring if it had $q < 0.10$, and individuals with q-value between 0.10 and 0.90 were considered to be hybrids. All identified individuals were placed into separate databases specifically for Alewives (3,549 individuals), Blueback Herring (180 individuals) and hybrids (151 individuals; Table 2.4).

Microsatellite genotypes were checked for scoring errors due to stuttering, null alleles and large allele dropouts using MICROCHECKER (v.2.2.3; van Oosterhout *et al.* 2004). No scoring errors or large allele dropout were detected, but evidence of null alleles was detected for *Aa082* and *Ap071* within the Alewife dataset and *Ap058* in the Blueback Herring dataset. These loci showed an excess of homozygotes at a few alleles that were consistent across populations, and were removed prior to further analyses. Markers were tested for neutrality using LOSITAN: Selection Detection Workbench (Antao *et al.* 2008) which applies an F_{ST} outlier detection algorithm (Beaumont and Nichols 1996) to identify loci that may be under selection. Analyses used the stepwise mutation model and 80,000 permutations and a sample size reflecting the smallest population size (Alewife, n

Table 2.4: Filtered dataset indicating the number of samples per location genetically identified using Bayesian clustering analyses as Alewife, Hybrid or Blueback Herring (Bbh). Full names of sampling locations are given in Table 2.1.

Population	Alewife	Hybrid	Blueback
Nem	47	13	
Coc	48	5	
Lam	16	5	
Sew	113	2	
Neq	161	3	
Bru	169		
Dre	177	12	1
Web	142	7	
Ben	189		
Loc	98	4	9
Dam	166	5	
Sen	158	6	
Wig	110	2	
Orl	111	2	
Orl (Bbh)		6	29
Sou	176	4	
Vea	173	2	
Leo	107	1	
Den	47	1	
Mil	51	5	
Mac	48	1	
Mac (Bbh)		2	48
Pet	22	28	
Pet (Bbh)		1	45
Shu	50		
Gas	56	1	
Tus	54		
Kia	50		
Arg	50	1	
Med	50	1	
Mer	56	1	
Lah	52		
Sul	51	1	
Sac	60		
Wes	51		
Ama	50		
Fid	50	1	
Chr	49	3	
Mar	47	2	
Mar (Bbh)	2	9	37
Tra	59	1	
Hil	57	2	
Wau	46	2	9
Wal	51	6	1
Riv	57		
Tid	55	3	
Ric	63		
Mir	54		1
Total	3549	151	180

= 16; Blueback Herring, $n = 29$). In the Alewife dataset, most loci showed patterns of differentiation consistent with neutrality; however, *Aa093* was outside the 95% confidence interval for neutral markers, and thus may be under selection (Antao *et al.* 2008). Based on these findings, analyses were conducted both with and without *Aa093*. Results did not differ, so all 12 loci were retained in further analyses. No evidence of non-neutrality was detected among the loci within the Blueback Herring database.

Linkage between loci within each population (using 100,000 permutations) and the adherence of loci and population to Hardy-Weinberg Equilibrium (HWE; 1,000,000 permutations and 100,000 dememorization steps) were assessed using Arlequin (v.3.5.1.3; Excoffier *et al.* 2005). Results were evaluated using sequential Bonferroni corrections (Rice 1989) with an initial α level of 0.05. The same software was used to estimate the population-specific sample size (N), number of alleles (N_a), probability of departures from HWE (p -val), observed (H_o) and expected heterozygosity (H_e). Allelic richness (A_e) was assessed with FSTAT (v.2.9.3; Goudet 1995) standardized to a minimum sample size of 39 individuals (Leberg 2008) for Alewife. Allelic richness was not calculated for Lamprey, Petitcodiac or for Blueback Herring populations due to small sample sizes. Population-specific inbreeding coefficient (F_{IS}), allele range and common allele were estimated by GENEPOP (v.4.1.4; Raymond and Rousset 1995) using default parameters for all tests.

Genetic differentiation was calculated in FSTAT (10,000 permutations; Goudet 1995) between all possible pair of populations using the F_{ST} estimate θ (Weir and Cockerham 1984). Sequential Bonferroni adjustments were used to assess significance (Rice 1989). The pairwise F_{ST} values generated by FSTAT were visualized in a Principal Coordinate Analysis (PCoA) using GenAlEx (v.6.41; Peakall and Smouse 2006). All population genetic statistics were conducted on the Alewife populations, while only the summary statistics and F_{ST} calculations were calculated for the Blueback Herring populations.

Genetic affinities were also examined among populations using a neighbor-joining (NJ) tree on Cavalli-Sforza and Edwards chord distance (D_c) and bootstrapped over all loci (5,000 replications) in POPULATIONS (v.1.2.3; Langella 1999) and visualized in TREEVIEW (Page 1996). The distance measure of D_c was chosen due to its

ability to accurately convey phylogenetic relationships with microsatellite data (Takesaki and Nei 1996).

Bayesian model-based hierarchical clustering, implemented in STRUCTURE (v.2.2; Pritchard *et al.* 2000, Falush *et al.* 2007), was used to infer the number of homogenous clusters. Structure analysis was conducted twice, first without prior information on sampling, and then with sampling location information. Both analyses employed the admixture model and correlated allele frequencies among populations with a burn in period of 50,000 steps followed by an MCMC chain of 500,000 steps. Three iterations of K (1-44) for Alewives and K (1-4) for Blueback Herring were tested. The most likely value of K was estimated using the ΔK statistic proposed by Evanno *et al.* (2005) and implemented by Structure Harvester (Earl and vonHoldt 2012). Results from all STRUCTURE iterations were amalgamated into a single population assignment using CLUMPP (v.1.1.2; Jakobsson and Rosenberg 2007). To quantify the patterns of genetic variation among clusters, populations and within populations, a hierarchical analysis of molecular variance (AMOVA) was assessed with Arlequin (10,000 permutations; Excoffier *et al.* 2005).

The significance of the relationship between genetic distance and geographic distance (i.e., isolation by distance (IBD)) was evaluated for different management strategies (U.S. stocked vs. U.S. Non-stocked vs. Canadian Non-stocked) and geographic regions (i.e., Bay of Fundy, Atlantic coast and Gulf of St. Lawrence) within the Canadian portion of the species' range using Mantel Tests implemented in GenAlEx (10,000 permutations; Peakall and Smouse 2006). Following Rousset (1997) pairwise F_{ST} values were linearized ($F_{ST}/(1-F_{ST})$). Geographic distances between sample locations were measured as the most direct route between river mouths following within 5 km of the shoreline using Google Earth v.6.2, following Bradbury *et al.* (2008). Bay of Fundy distance was measured as the most direct route and counterclockwise following Hasselman *et al.* (2010). Two distance measures were calculated for Maine; (i) 'total' and (ii) sea water, where the latter measured only the distance between river mouths at head of tide, whereas the former included freshwater distances to the mouths of tributary rivers where the river herring spawn.

Geographic discontinuities in gene flow among rivers were identified using BARRIER (v.2.2; Manni *et al.* 2004). Barriers were first computed using the geographic coordinates of sampling locations along with the multilocus F_{ST} matrix for each population. To assess the robustness of these barriers identified from the multilocus F_{ST} , the F_{ST} matrix for each of the 12 loci were then used separately to identify ‘consensus barriers’ (i.e., ≥ 7 loci).

To detect recent historical bottlenecks in populations, allele frequency data were calculated and assessed in BOTTLENECK (v.1.2.02; 10,000 permutations; Cornuet and Luikart 1996). Sign tests were used to determine if the proportion of loci with heterozygosity excess was significantly larger than expected at equilibrium (Cornuet and Luikart 1996, Luikart and Cornuet 1998). Following Luikart *et al.* (1998) the allele frequency mode shift was plotted and examined for each population to determine whether the allele frequency distribution diverted from the null hypothesis of an L-shaped distribution.

Linkage disequilibrium data were used in LDNe (Waples and Do 2008) to estimate the effective population sizes (N_e) for a population of randomly mating individuals. To reduce the possibility of parent-offspring sampling affecting the N_e estimate, samples that were collected in 2008 for Dresden and Sennebec were removed as well as samples collected in 2009 for Sewell.

2.3 Results

2.3.1 Population Genetic Statistics

No evidence of linkage disequilibrium was detected among the 12 loci examined among 44 Alewife populations or the 13 loci among the four Blueback Herring populations. After sequential Bonferroni correction, 15 locus-population combinations were out of HWE from a possible 528 comparisons overall Alewife samples and five locus-population combinations were out of HWE from a possible 52 comparisons overall Blueback Herring samples (Appendix 1). The loci that were out of HWE were not consistent among populations of either species.

Among the 44 Alewife populations the amount of polymorphism varied depending on the locus and location. The number of alleles per locus ranged from five

(*Aa082*, *Aps1*, *Aa046*) to 16 (*Aps2A*; Appendix 1), with five loci exhibiting ≤ 10 alleles. Observed heterozygosity varied between 0.020 (Kiack; *Aps1*) and 0.860 (West River; *Aps2A*), and allelic richness ranged from 1.000 (Nemasket; *Aps1*) to 9.502 (Argyle; *Ap010*; Appendix 1). Among the four Blueback Herring populations the number of alleles per locus ranged from four (*Ap010*, *Aps1*) to 10 (*Ap071*, *Ap058*, *AsaD042*) and the observed heterozygosity varied between 0.042 (Mactaquac; *Aa039*) and 0.838 (Margaree; *AsaD042*).

Pairwise genetic differentiation (F_{ST}) between Alewife populations revealed 905/946 (95.7%) significant ($p < 0.05$) pairwise comparisons and a highly significant ($p < 0.001$) global differentiation of 0.036 (Appendix 2). Genetic differentiation ranged from an insignificant ($p > 0.05$) value of -0.002 (Mersey vs. Tusket and Medway vs. Mersey) to a highly significant ($p < 0.001$) value of 0.168 (Petitcodiac vs. Dennis). When Blueback Herring populations were included in the pairwise comparisons the global differentiation increased to 0.088. Estimates of genetic differentiation between Blueback Herring and Alewife pairwise populations were all highly significant ($p < 0.001$) and ranged from $F_{ST} = 0.254$ (Waugh Alewife vs. Orland Blueback Herring) to 0.509 (Lamprey Alewife vs. Petitcodiac Blueback Herring; Appendix 2). Estimates of pairwise genetic differentiation between Blueback Herring populations were highly significant ($p < 0.001$) and ranged from 0.033 (Mactaquac vs. Margaree) to 0.149 (Orland vs. Petitcodiac).

A PCoA of the Alewife F_{ST} values accounted for 77.2% of the genetic variation among populations (axes 1, 2), and revealed that, with a few exceptions, populations grouped according to geographic regions (i.e., New England, Bay of Fundy, Atlantic coast of Nova Scotia, Gulf of St. Lawrence; Figure 2.2). One exception was the single Massachusetts population, Nemasket, which grouped with Atlantic Canada populations. Within the Northern New England group, Dresden and Sewell were outliers that clustered together with LaHave (Atlantic coast of Nova Scotia), midway between the New England and Atlantic Canada clusters. Within the Atlantic Canada group, most Bay of Fundy populations formed a discrete cluster and were relatively divergent (Avg. $F_{ST} = 0.043$) from the populations located on the Atlantic coast of Nova Scotia and Gulf of St. Lawrence. However, one Bay of Fundy population, Mactaquac (Saint John River),

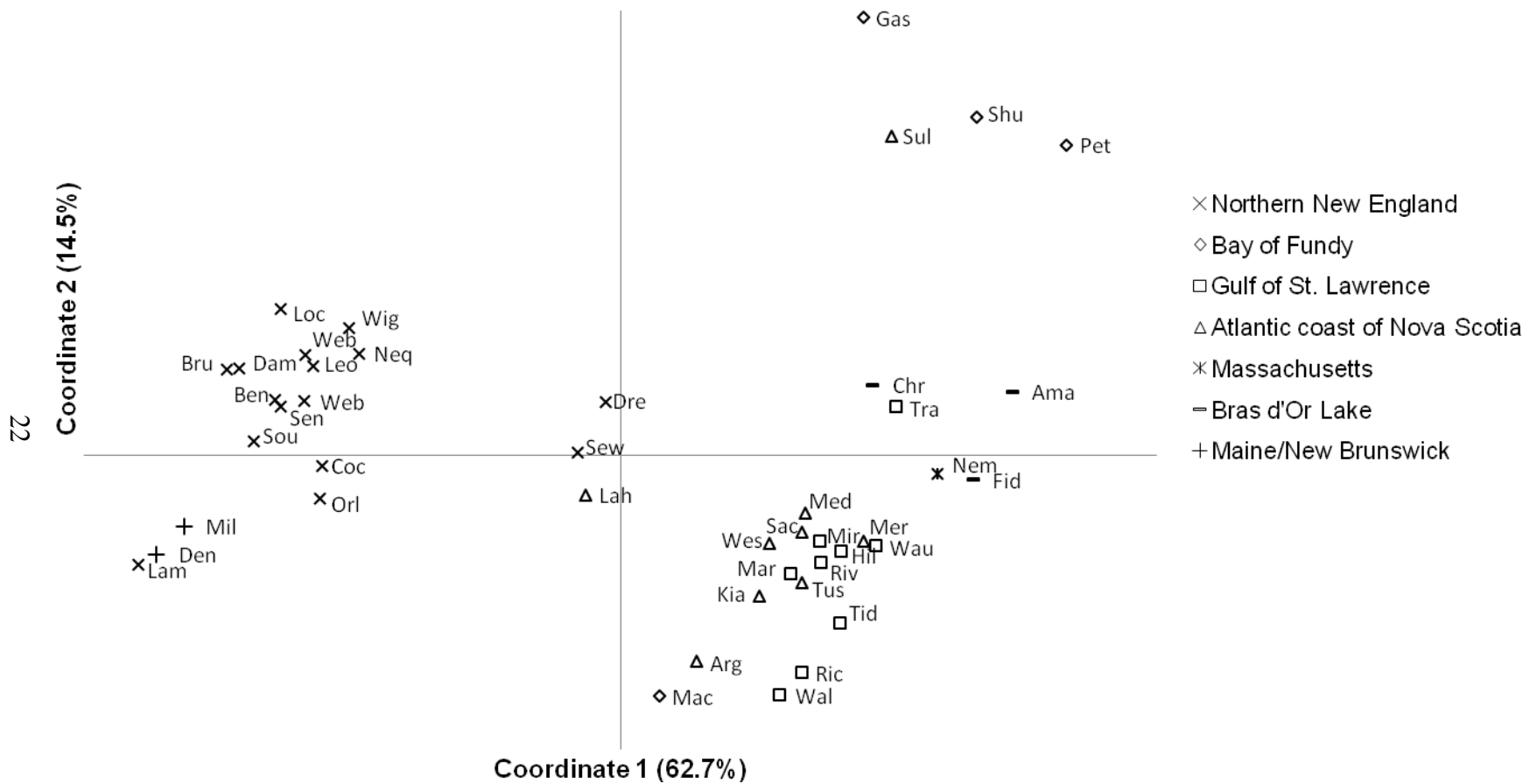


Figure 2.2: Principle coordinate analysis plot describing 77.2% of the genetic variation found in Alewife populations within the study area. The symbols indicate the geographic region in which the populations are located in. Full names are given in Table 2.1.

clustered with the Atlantic coast of Nova Scotia and Gulf of St. Lawrence populations. Additionally, Sullivan's Pond, a population located on the Atlantic coast of Nova Scotia, clustered with the Bay of Fundy populations (Figure 2.2).

2.3.2 Relationship among Populations

A neighbor-joining tree analysis, using Cavalli-Sforza and Edwards chord distances (D_c), revealed concordance between defined clusters of populations and their geographic regions of origin (Figure 2.3). Within Atlantic Canada, several population clusters had relatively strong bootstrap support ($> 50\%$). Bootstrap support for a cluster comprised of Shubenacadie and Sullivan's Pond was 67%. Within the Gulf of St. Lawrence cluster, there was strong bootstrap support (75%) for a subcluster of Bras d'Or Lake populations (Figure 2.3). Within the Northern New England cluster, bootstrap support for further subclustering was weak ($< 50\%$), except for Brunswick and Damariscotta, which were united with 52% support. Also within the Northern New England cluster, Dresden and Sewell split from all other populations in this group (Figure 2.3).

2.3.3 Population Structure

Application of Evanno's ΔK method to STRUCTURE analysis with prior location information supported $K = 2$ as the most probable number of genetically distinct populations. No genetically distinct populations were identified when prior location information was not included in the STRUCTURE analysis (i.e., $K = 1$). Although populations showed varying levels of admixture, one genetic cluster predominated strongly in all sampling locations within the Canadian portion of the range and the Massachusetts population (Figure 2.4a). The second genetic cluster dominated the remaining populations within the U.S. (i.e., Northern New England), including the Canadian-U.S. border locations (Milltown and Dresden; Figure 2.4a).

Further hierarchical analyses (Vähä *et al.* 2007) conducted on the two major population groups revealed additional population structure within them. Three clusters were identified within the Canadian-Massachusetts group. The first cluster (hereafter Bay of Fundy) was predominant in locations within the Bay of Fundy (Figure 2.4b). The second cluster (hereafter Atlantic coast) was predominant in sampling locations

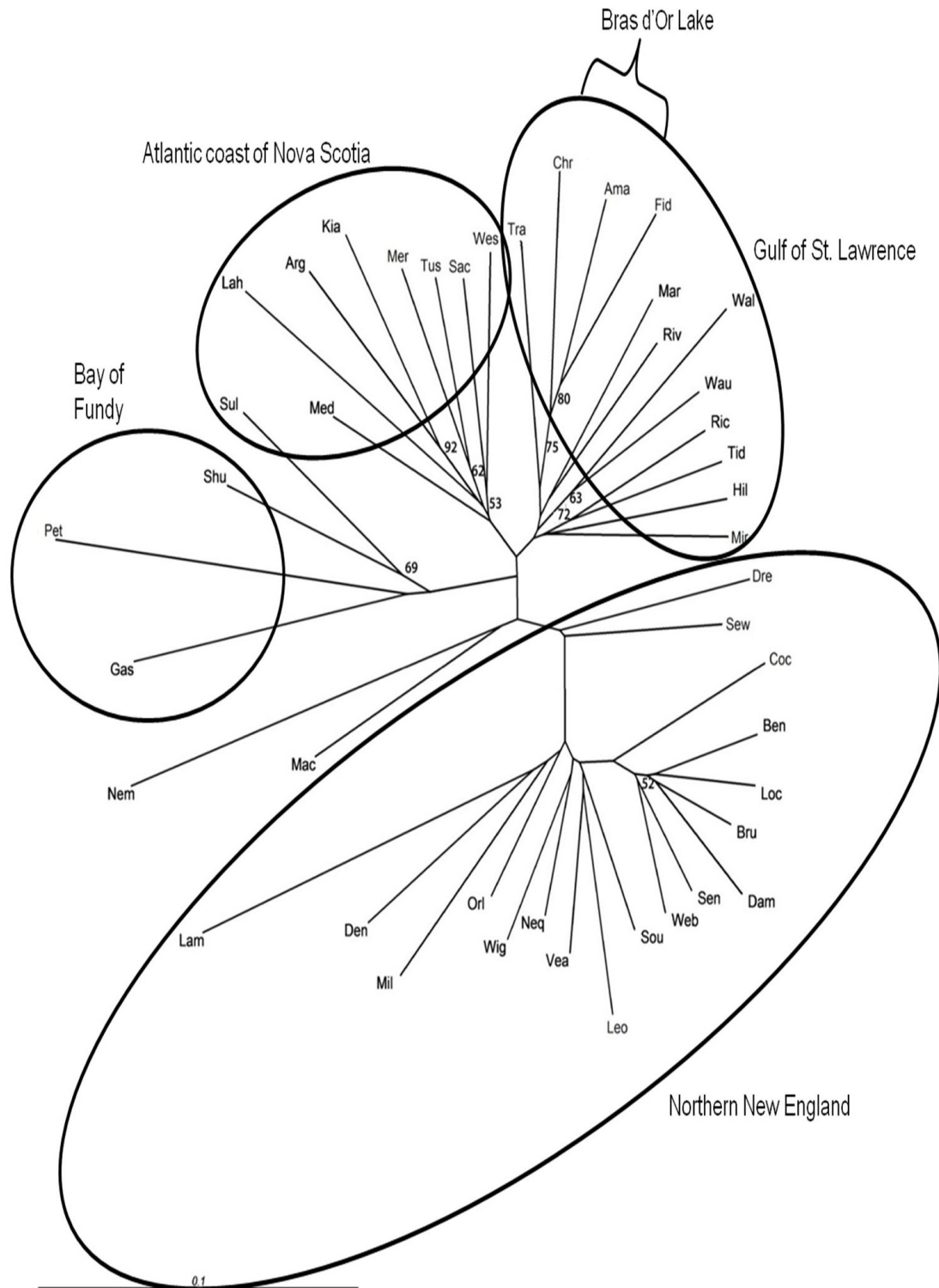


Figure 2.3: Unrooted neighbor-joining tree analysis, using Cavalli-Sforza and Edwards chord distance (D_c), displaying bootstrap support $> 50\%$. Full names of sampling locations are given in Table 2.1.

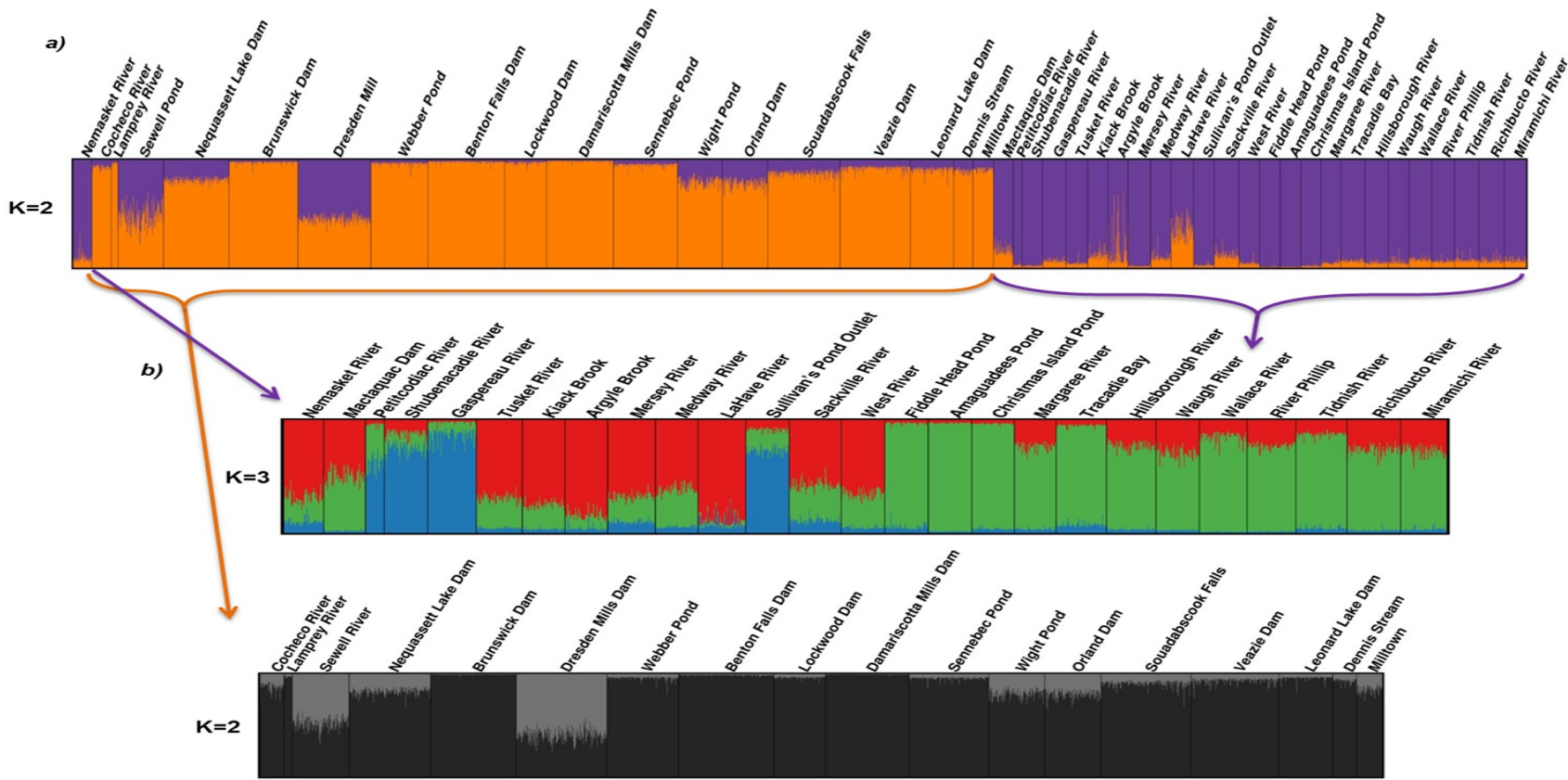


Figure 2.4: Estimated Alewife population structure inferred from the admixture analysis. $K = 2$ clusters were identified using the program STRUCTURE. The number of clusters was inferred using the Evanno method (Evanno *et al.* 2005). Each vertical line represents one individual and is partitioned into coloured blocks that indicate the estimated membership of that individual into each of the genetic clusters. (a) Results for $K = 2$ clusters identified in Bayesian analyses. Canadian locations and the Massachusetts sample location are predominantly one cluster (purple) and remaining U.S. sampling locations the second cluster (orange). (b) Bayesian clustering conducted separately on populations dominated by one of the two major clusters identified in (a) revealed $K = 3$ for the Canadian (and Nemasket) cluster and $K = 2$ for the U.S. cluster. Within the former group, the blue cluster dominates in the Bay of Fundy, the green cluster dominates in the Gulf of St. Lawrence and the red cluster dominates in the Atlantic coast of Nova Scotia. Within the latter group dark grey represents one cluster and the light grey represents the second cluster.

along the Atlantic coast of Nova Scotia (Figure 2.4b). The third cluster (hereafter Gulf of St. Lawrence) was predominant in all Gulf of St. Lawrence locations, including Bras d'Or Lake populations (Figure 2.4b). Within the Northern New England population group, two clusters were identified, one cluster only appeared as admixture within populations, and was present in relatively high proportion in only two locations, Dresden and Sewell (Figure 2.4b).

Although for the most part the distributions of genetic clusters corresponded with geographic regions, there were exceptions. The Nemasket population in Massachusetts was comprised mostly of the genetic cluster that was otherwise characteristic of the Atlantic coast. Alewives from Mactaquac appeared to be an admixture of the Atlantic coast and Gulf of St. Lawrence clusters. Finally, Sullivan's Pond was comprised mostly of the Bay of Fundy cluster (Figure 2.4b).

STRUCTURE analysis of four Blueback Herring populations with prior location information and Evanno's ΔK method revealed $K = 3$ as the most probable number of genetically distinct population clusters. When no prior location information was used, STRUCTURE analysis supported only a single genetic cluster, similar to what was observed with Alewife when no prior location information was used. Under the $K = 3$ model, one cluster was predominant in the Orland Dam location (Northern New England), a second cluster was predominant in the Petitcodiac River (Bay of Fundy) and the third cluster was predominant in both the Margaree River (Gulf of St. Lawrence) and Mactaquac Dam (Bay of Fundy; Figure 2.5).

An AMOVA conducted for Alewife revealed that 3.61% of the total genetic variation was partitioned among populations, while 96.39% ($p < 0.001$) was found within populations (Table 2.5). A hierarchical AMOVA in which rivers were grouped according to their predominant genetic cluster revealed that 3.63% ($p < 0.001$) of the total genetic variation was partitioned among clusters, while 1.16% ($p < 0.001$) was among populations within clusters, and 95.22% ($p < 0.001$) was among individuals within populations (Table 2.5). A similar analysis conducted for Blueback Herring revealed 6.97% ($p < 0.001$) of the genetic variance was partitioned among populations, while 93.03% was among individuals within populations (Table 2.5). Hierarchical AMOVA revealed 5.70% ($p < 0.05$) of the genetic variation partitioned among rivers grouped

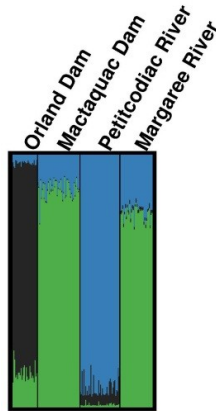


Figure 2.5: Estimated Blueback Herring population structure inferred from the admixture analysis $K = 3$ clusters identified in the program Structure. The number of clusters was inferred using the Evanno method (Evanno *et al.* 2005). Each vertical line represents one individual and is partitioned into coloured blocks that indicate the estimated membership of that individual into each of the genetic clusters. Dark grey cluster dominates the U.S. population, blue cluster dominates Petitcodiac, located in the Bay of Fundy and lastly, the green cluster dominates Mactaquac (Bay of Fundy) and Margaree (Gulf of St. Lawrence).

Table 2.5: Analysis of molecular variance among populations, within populations and among population groups defined by predominant genetic clusters identified using the program Structure for both Alewife (Ale) and Blueback Herring (Bbh) species.

Species	Populations	Variance Components	df	Sum of Squares	% total variance	<i>F</i> Statistic	<i>P</i>
Ale	All	Among populations	43	927.01	3.61	0.036	< 0.001
		Within populations	7007	21769.34	96.39		
	Clusters	Among clusters	4	573.15	3.63	0.036	< 0.001
		Among populations within clusters	39	353.86	1.16	0.012	< 0.001
		Among individuals within populations	7007	21769.34	95.22	0.048	< 0.001
Bbh	All	Among populations	3	58.31	6.97	0.070	< 0.001
		Within populations	279	847.64	93.03		
	Clusters	Among clusters	2	48.28	5.70	0.057	0.006
		Among populations within clusters	1	10.03	2.51	0.027	< 0.001
		Among individuals within populations	279	847.64	91.79	0.082	< 0.001

according to their predominant genetic clusters, 2.51% ($p < 0.001$) among populations within clusters, and 91.79% ($p < 0.001$) among individuals within populations.

2.3.4 Isolation by Distance

There was no significant difference in the IBD relationship when total or sea water was used as a distance measure in Maine, thus total distance was used. The most direct route explained more of the variation in genetic differentiation among rivers in the Bay of Fundy, thus this distance was used. Mantel tests revealed significant IBD for Alewife populations across the study area ($p < 0.001$, $R^2 = 0.129$; Figure 6a). When two notable outliers, Sullivan's Pond and Nemasket were removed from the analysis, the IBD trend strengthened ($R^2 = 0.157$, $p < 0.001$; Figure 6b).

To determine the effect that different management strategies may have had on population structure, Alewife populations within the U.S. range were separated according to their stocking history (Table 2.2). Mantel tests were conducted on all U.S. 'stocked' populations subjected to out-of-basin translocations (Table 2.2), U.S. 'non-stocked' populations (i.e., either no stocking history or recipient only of within basin translocations; Table 2.2) and Canadian non-stocked populations. Mantel tests revealed non-significant IBD relationships within both the U.S. stocked and non-stocked population groups, but the IBD trend for U.S. non-stocked population was much stronger and closer to significance ($R^2 = 0.224$; $p = 0.058$) than the stocked populations ($R^2 = 0.002$; $p = 0.354$; Figure 2.7a). The Canadian non-stocked populations did not have a strong IBD relationship ($R^2 = 0.099$), but the trend was highly significant ($p < 0.001$; Figure 2.7b). When the U.S. and Canadian non-stocked populations were combined there was a significant trend but the proportion of variation in the data explained remained low ($p < 0.001$, $R^2 = 0.113$; data not shown). A Mantel test conducted on all U.S. spawning sites (without regard to stocking history) revealed no IBD relationship ($R^2 = 0.042$, $p = 0.107$; data not shown).

When geographic regions within Atlantic Canada were analyzed separately, IBD was significant in both the Gulf of St. Lawrence region ($p < 0.001$, $R^2 = 0.535$) and Bay of Fundy ($p = 0.014$, $R^2 = 0.662$; Figure 2.8a,b). IBD was not significant within Atlantic coast region ($p = 0.312$, $R^2 = 0.012$, Figure 2.8a). Removing a significant outlier (Sullivan's Pond) had little effect on this result ($p = 0.226$, $R^2 = 0.026$, Figure 2.8b).

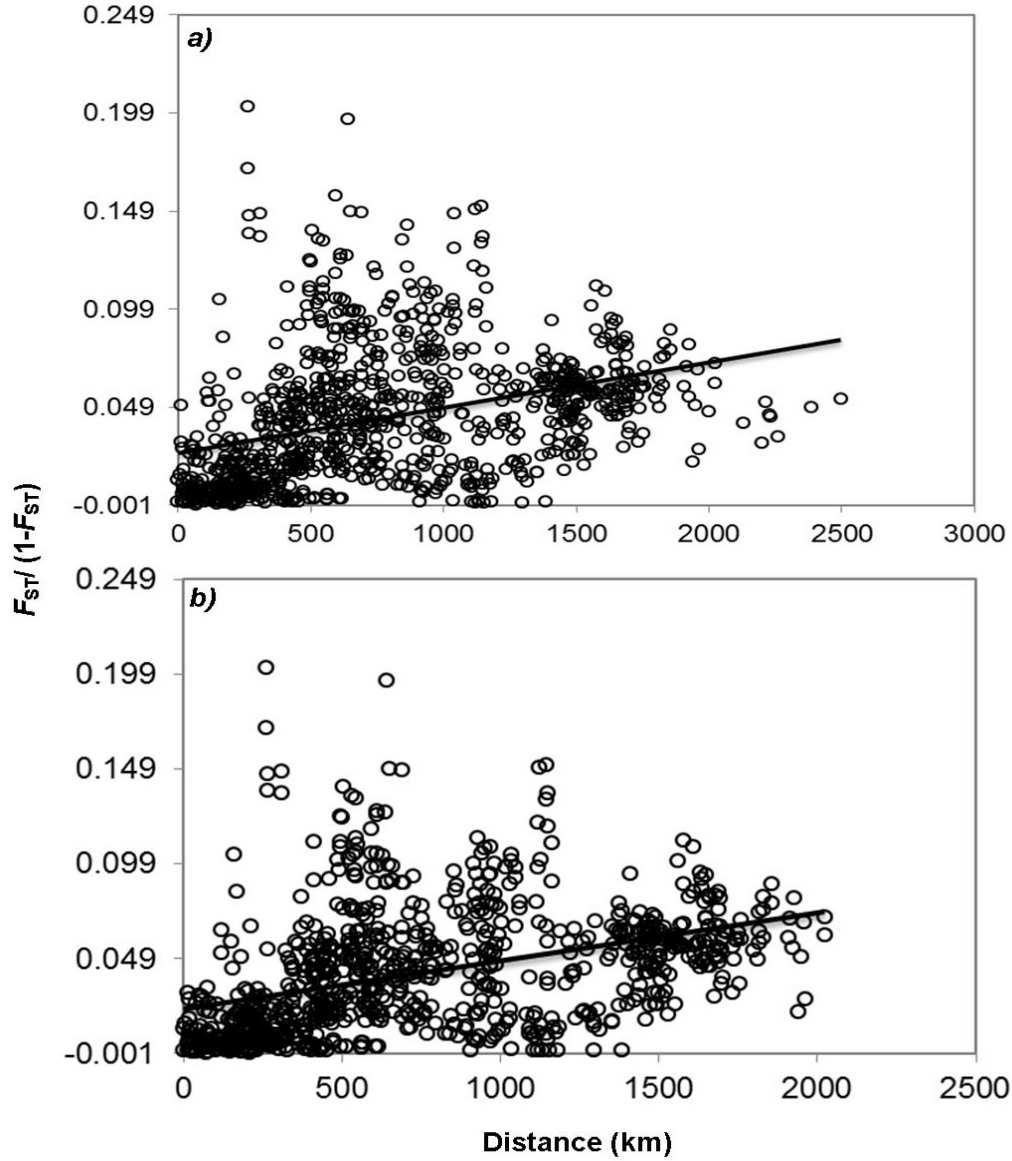


Figure 2.6: The relationship between pairwise linearized genetic distances and geographic distances for all Alewife populations in the study area. (a) Highly significant ($p < 0.001$, $R^2 = 0.129$) isolation by distance (IBD) when all study populations are included. (b) Exclusion of Nemaskett and Sullivan's Pond results in a slightly stronger IBD relationship ($p < 0.001$, $R^2 = 0.157$).

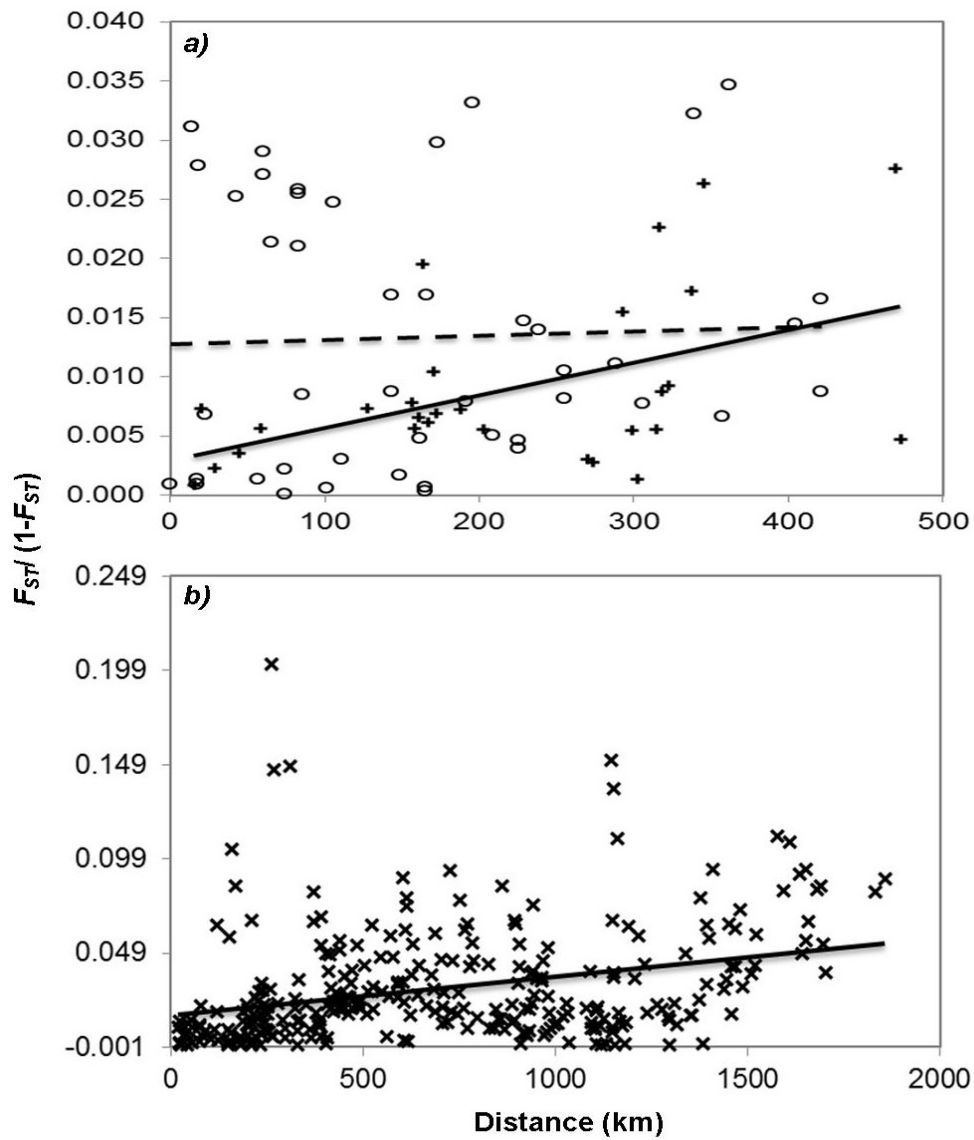


Figure 2.7: Isolation by distance for stocked and non-stocked Alewife populations. (a) U.S. populations: IBD is non-significant ($p = 0.354$, $R^2 = 0.002$, dashed line) for stocked populations (o). IBD is non-significant for non-stocked populations (+, solid line; $p = 0.058$, $R^2 = 0.224$). (b) Canadian (non-stocked) populations: IBD is highly significant ($p < 0.001$, $R^2 = 0.099$).

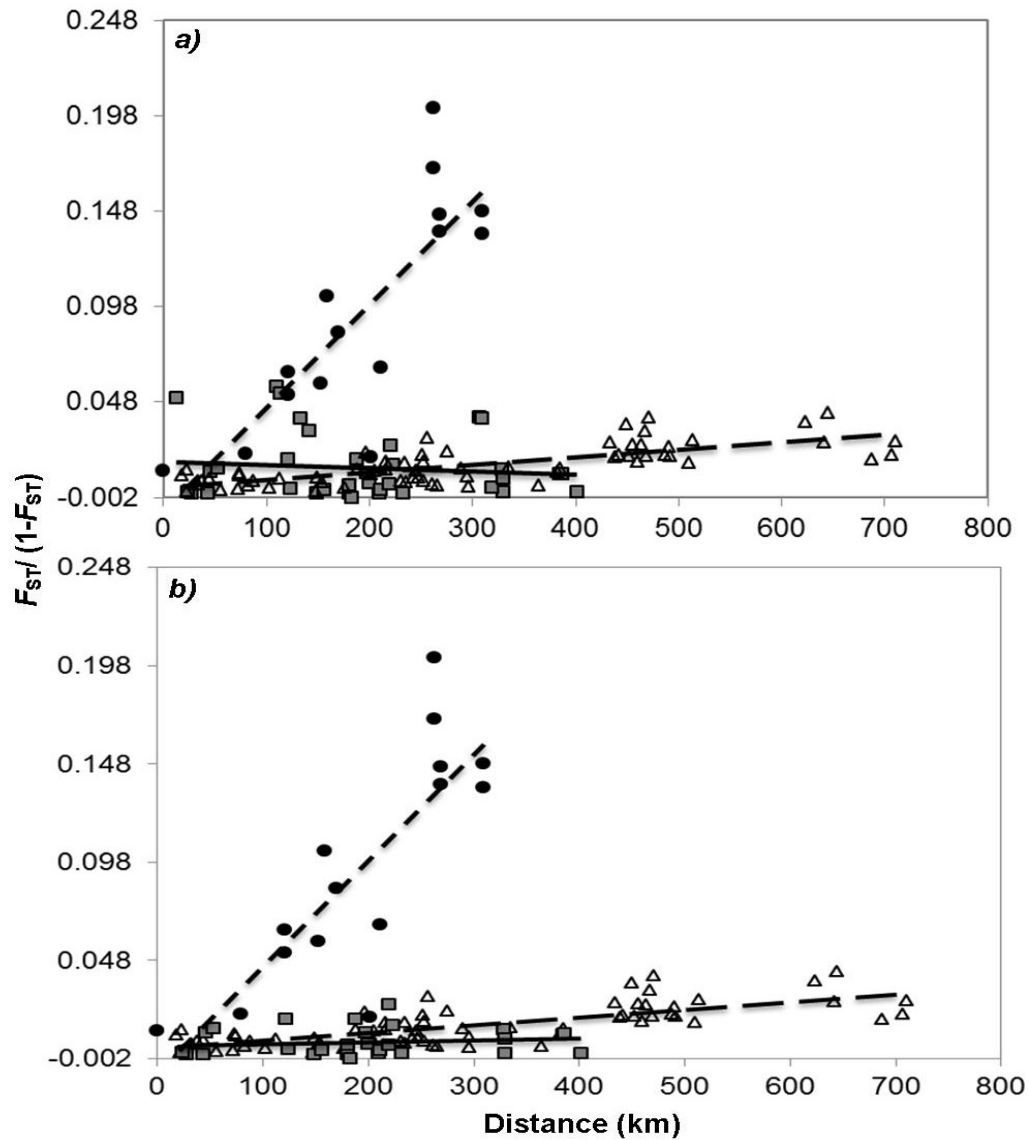


Figure 2.8: Isolation by distance for three regions in Atlantic Canada (Bay of Fundy, short dashed line, ●; Atlantic coast, solid line, □; Gulf of St. Lawrence, long dashed line, Δ). (a) IBD is significant for both the Bay of Fundy ($p = 0.014$, $R^2 = 0.662$) and Gulf of St. Lawrence ($p < 0.001$, $R^2 = 0.535$), but non-significant for the Atlantic coast ($p = 0.312$, $R^2 = 0.012$). (b) Excluding Sullivan's Pond from the Atlantic coast still results in non-significant IBD ($p = 0.231$, $R^2 = 0.026$).

2.3.5 Barrier Analysis

Five discontinuities in gene flow, supported by ≥ 7 loci, were identified within the study area using BARRIER (Figure 2.9). Four of these barriers (2, 3, 4, and 5) were located within the Bay of Fundy (Figure 2.9). The first consensus barrier (10 loci) was positioned between Nemasket and Lamprey, separating Nemasket from the rest of the range (Figure 2.9). The second consensus barrier (8 loci) was positioned between Mactaquac and Petitcodiac, while the third consensus barrier (7 loci) separated Petitcodiac from Shubenacadie. The fourth consensus barrier (7 loci) was positioned between Dennis Stream and Mactaquac, isolating Northern New England populations from Atlantic Canada. The fifth consensus barrier (7 loci) was positioned between Gaspereau and Tusket, dividing the Bay of Fundy from the rest of the Atlantic Canada regions (i.e., Atlantic coast and Gulf of St. Lawrence; Figure 2.9).

2.3.6 Bottleneck Analysis

Within the U.S. populations, where stocking is prevalent, nine of the 19 populations had fewer low frequency alleles than expected, while in Atlantic Canada seven of the 25 populations demonstrated the same trend, thus providing evidence that these locations may have undergone a recent bottleneck (Figure 2.10*a,b*). A deficit of rare alleles (frequency < 0.1) and excess of more common alleles (frequency ≈ 0.6) was observed within most populations indicating a recent bottleneck that may have been severe (Figure 2.10*a,b*); although, Tusket, Lamprey and Sennebec may have experienced a less severe recent bottleneck (frequency $\approx 0.2 - 0.4$; Figure 2.10*a,b*). Additionally, Sign tests conducted using both IAM and SMM revealed a tendency towards heterozygote excess at all populations, with some exceptions (Table 2.6). Sewell and Veazie had an even split between excess and deficiency for SMM (Table 2.6). Under the IAM, heterozygote excess was highly significant at all populations whereas under the SMM, heterozygote excess was significant in only 15 of 44 cases (Table 2.6).

2.3.7 Effective Population Size

Estimates of N_e ranged from 36 (Lamprey) to 6850 (Souadabscook), but most estimates had 95% confidence intervals that included infinity (Table 2.7), indicating that the method and markers used provided insufficient resolution for the majority of populations. However, estimates of N_e for Waugh, Wallace and Tidnish (31, 34, and 44,

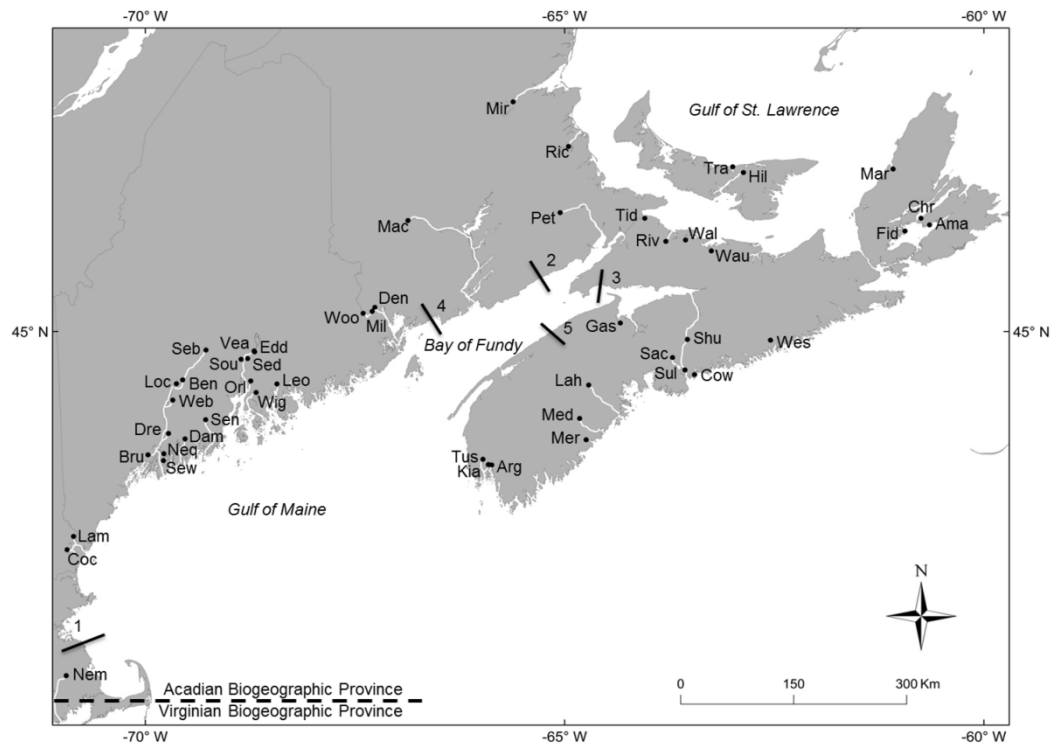


Figure 2.9: Major discontinuities in gene flow revealed by BARRIER. Identified barriers are those supported by ≥ 7 loci) and are numbered in order of importance (1 = most important; 5 = least important).

a)

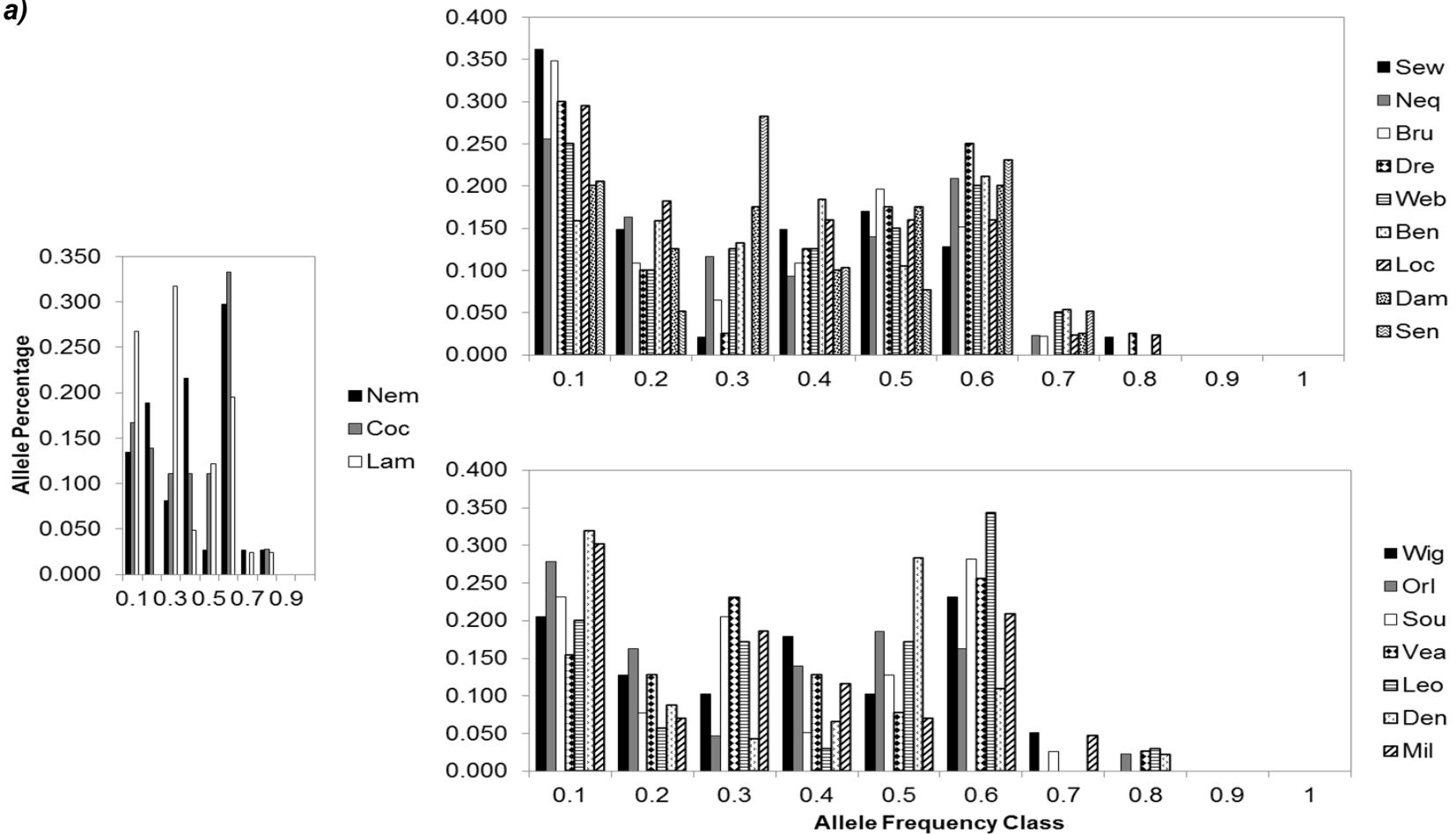


Figure 2.10a: Allele frequency distribution histograms on U.S. populations ranging from Nem to Mill. Nem, Lam, Coc, Ben, Sen, Wig, Vea, Sou and Leo demonstrated a mode shift from the normal L-distribution (frequency > 0.1) suggesting a recent bottleneck. Full names are given in Table 2.1.

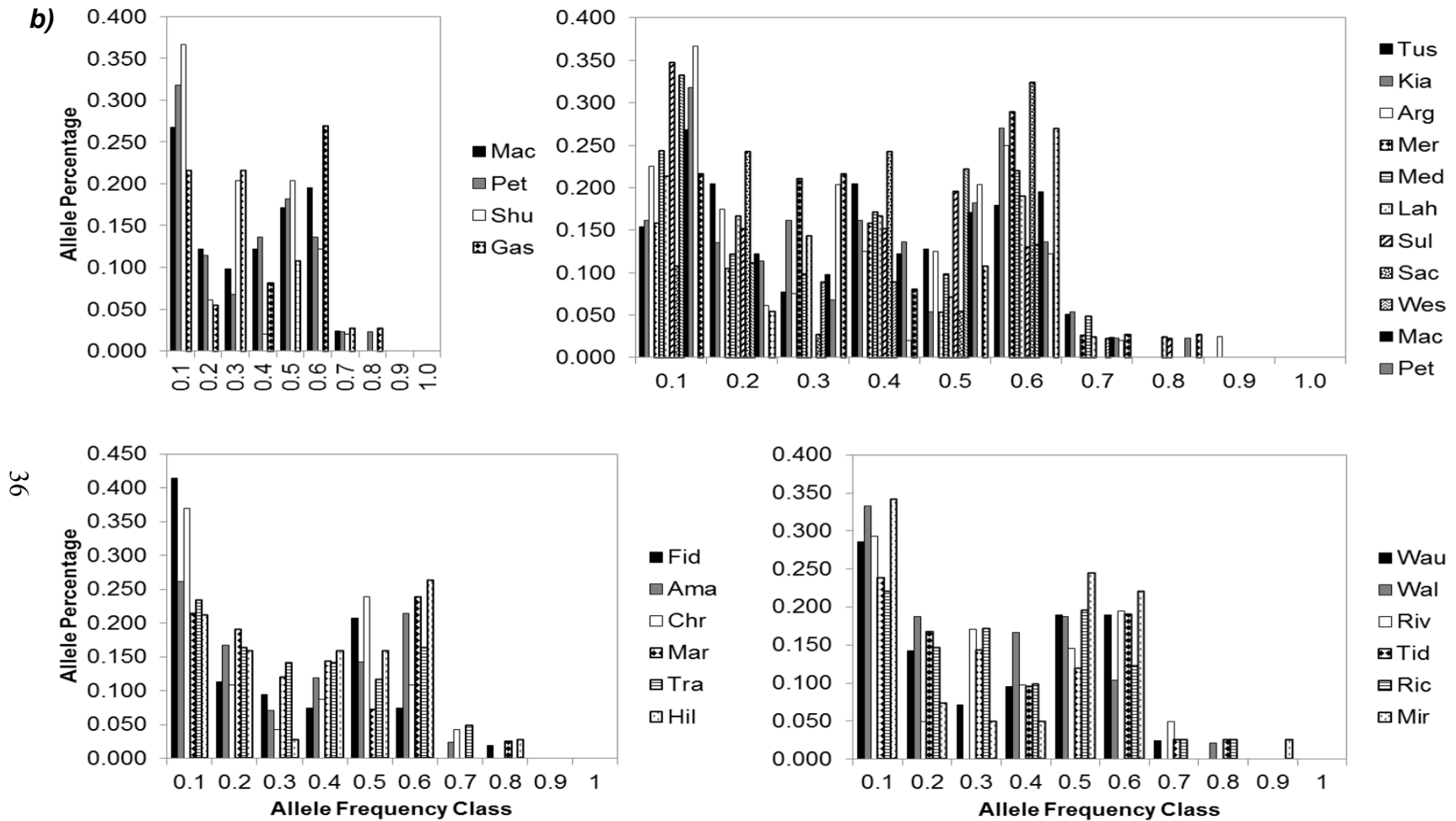


Figure 2.10b: Allele frequency distribution histograms on Atlantic Canada populations ranging from Mac to Mir. Gas, Tus, Arg, Mer, Sac, Hil, and Mar demonstrated a mode shift from the normal L-distribution (frequency > 0.1) suggesting a recent bottleneck. Full names are given in Table 2.1.

Table 2.6: Sign tests indicating the ratio of heterozygosity excess (He) to heterozygosity deficiency (Hd) and the probability (p) of deviation from expected equilibrium calculated for both the Infinite Allele Model (IAM) and Stepwise Mutation Model (SMM). Full names of sampling locations are given in Table 2.1.

Stocked	Population	<i>Sign Test</i>			
		<i>IAM</i>		<i>SMM</i>	
		He/Hd	p	He/Hd	p
N	Nem	12/0	0.000*	9/3	0.194
N	Coc	12/0	0.000*	11/1	0.015*
N	Lam	12/0	0.004*	10/2	0.056
Y	Sew	12/0	0.000*	6/6	0.394
N	Neq	12/0	0.000*	9/3	0.178
Y	Bru	12/0	0.000*	8/4	0.373
Y	Dre	12/0	0.000*	7/5	0.603
Y	Web	12/0	0.000*	8/4	0.380
Y	Ben	12/0	0.000*	8/4	0.371
Y	Loc	12/0	0.000*	11/1	0.014
N	Dam	12/0	0.000*	7/5	0.608
Y	Sen	12/0	0.000*	8/4	0.373
Y	Wig	12/0	0.000*	9/3	0.186
N	Orl	12/0	0.000*	11/1	0.017*
N	Sou	12/0	0.000*	9/3	0.179
N	Vea	12/0	0.000*	6/6	0.399
Y	Leo	12/0	0.000*	9/3	0.182
N	Den	12/0	0.000*	10/2	0.068
Y	Mil	11/1	0.004*	9/3	0.188
N	Mac	12/0	0.000*	9/3	0.152
N	Pet	12/0	0.006*	11/1	0.014*
N	Shu	12/0	0.000*	11/1	0.012*
N	Gas	12/0	0.000*	9/3	0.171
N	Tus	12/0	0.000*	10/2	0.058
N	Kia	12/0	0.000*	10/2	0.066
N	Arg	11/1	0.005*	10/2	0.065
N	Mer	12/0	0.000*	8/4	0.354
N	Med	12/0	0.000*	11/1	0.012*
N	Lah	12/0	0.000*	9/3	0.192
N	Sul	12/0	0.000*	9/3	0.196
N	Sac	12/0	0.000*	11/1	0.011*
N	Wes	12/0	0.000*	11/1	0.015*
N	Fid	12/0	0.000*	12/0	0.000*
N	Ama	12/0	0.002*	9/3	0.130
N	Chr	12/0	0.000*	11/1	0.008*
N	Mar	12/0	0.000*	10/2	0.060
N	Tra	12/0	0.000*	11/1	0.010*
N	Hil	12/0	0.000*	12/0	0.001*
N	Wau	12/0	0.000*	10/2	0.045*
N	Wal	12/0	0.003*	11/1	0.011*
N	Riv	12/0	0.000*	12/1	0.001*
N	Tid	12/0	0.000*	10/2	0.066
N	Ric	12/0	0.000*	9/3	0.191
N	Mir	12/0	0.000*	11/1	0.010*

*Significant deviation ($p < 0.05$) from equilibrium/nonbottleneck expectation (Luikart and Cornuet 1998)

Table 2.7: Effective population size for Alewife populations collected within the upper Virginian and Acadian Biogeographic Provinces calculated in the program LDNe. Full names of sampling locations are given in Table 2.1.

Population	N _e	95% C.I. (Jackknife on loci)	
		Lower	Upper
Nem	-313	141.7	Infinite
Coc	-176.5	120	Infinite
Lam	35.7	10.6	Infinite
Sew	-160	212.2	Infinite
Neq	1344.5	209.7	Infinite
Bru	2939.6	258.7	Infinite
Dre	582.5	160	Infinite
Web	-1807	293.4	Infinite
Ben	2340.6	307	Infinite
Loc	-364.3	271	Infinite
Dam	273.6	123.8	3597.3
Sen	947.8	174.7	Infinite
Wig	248.4	106.3	Infinite
Orl	251.3	105.2	3475.4
Sou	6849.5	326.4	Infinite
Vea	-793.3	505.5	Infinite
Leo	1449.9	182.4	Infinite
Den	-166.3	168.9	Infinite
Mil	-207.9	124.4	Infinite
Mac	151.3	49.5	Infinite
Pet	-189.4	25.7	Infinite
Shu	127.4	52	Infinite
Gas	-388	139.4	Infinite
Tus	183.6	61.4	Infinite
Kia	316.3	75.7	Infinite
Arg	1972.6	130.1	Infinite
Mer	224.8	74.3	Infinite
Med	395.1	84.3	Infinite
Lah	-274.3	252.4	Infinite
Sul	-108.9	-1182.5	Infinite
Sac	-4821	143.6	Infinite
Wes	-198.6	414.3	Infinite
Fid	268.1	60	Infinite
Ama	407.3	77.2	Infinite
Chr	147.1	47.7	Infinite
Mar	-468	153	Infinite
Tra	146.7	58.7	Infinite
Hil	289.1	78.6	Infinite
Wau	31.4	21.6	49.2
Wal	33.6	22.6	54.6
Riv	151.7	61	Infinite
Tid	44.2	24.7	103.2
Ric	1037.9	107.3	Infinite
Mir	121.9	52.4	Infinite

respectively), had finite and very low upper bounds of 55, 49 and 103.2, respectively (Table 2.7), suggesting accurate estimates of N_e . Estimates of N_e for Damariscotta and Orland (274 and 251, respectively) also had finite upper bounds of 792 and 3475, respectively (Table 2.7). Negative estimates of N_e were obtained for Nemasket, Sewell, Webber, Lockwood, Veazie, Dennis Stream, Milltown, Petitcodiac, Gaspereau, LaHave, Sullivan's Pond, Sackville, West River, and Margaree, which suggests that not enough information was available to form credible estimates.

2.4 Discussion

River herring are comprised of two genetically distinct species, Alewife and Blueback Herring, that are currently managed as single stocks within both U.S. and Canada (DFO 2001, AFSMC 1985). Managing a species as a single population where equal fishing pressure is applied to multiple populations that differ in biomass and resilience will potentially bring about a collapse of less productive populations (Hutchinson 2008). A loss of any populations, especially when the populations in question occupy peripheral positions in a species range could lead to an interruption in normal patterns of gene flow among populations, thus possibly resulting in an erosion of genetic diversity and a decrease in fitness by affecting various traits (e.g., developmental stability; Gautschi *et al.* 2002).

Significant declines within these two species have occurred across their whole range and have resulted in different recovery efforts within Canada and the U.S. The U.S. developed and implemented a program that transfers gravid adults within (in-basin) and between (out-of-basin) watersheds in an effort to enhance and/or restore extirpated spawning runs (AFSMC 1985), whereas no stocking has occurred in Canada. However, stock transfers among divergent source populations may unintentionally reduce population fitness and jeopardize genetically distinct populations by increasing gene flow between populations that do not normally exchange migrants (Nelson and Soule 1987, Hasselman and Limburg 2012). Current data on population structure and spatial patterns of genetic diversity for river herring across much of the upper Virginian and Acadian Biogeographic Provinces are largely unavailable. My research used 12 polymorphic loci

and 44 populations to examine the pattern of genetic diversity and differentiation over a large spatial scale (< 2500 km).

2.4.1 Spatial Patterns of Population Structure and Genetic Differentiation

Several lines of evidence, including F_{ST} values and the PCoA derived from them, Bayesian clustering, and dendrogram of chord distances revealed relatively strong population structure for Alewife populations located in the Canadian portion of the range and weak population structure within the U.S. portion of their range. However, the only U.S. population included from the Virginian biogeographic province, Nemasket, was relatively divergent from the U.S. populations to the north in the Acadian province. Barrier analysis determined that the most important barrier to gene flow was between Nemasket and other U.S. populations, which suggests that the differences in environmental conditions (e.g., temperature) between these two biogeographic provinces may act as a natural barrier to gene flow as seen in Atlantic Silverside (Mach *et al.* 2011). The relatively strong population structure within the Canada portion of the range is evidence that Alewives do return to their natal streams for spawning, but the amount of genetic admixture observed within many populations is also consistent with some dispersal and their known tendency to readily recolonize new ponds or streams and reoccupy systems from which they had been extirpated (Loesch 1987). The generally weaker population structure in the U.S. suggests the possibility that stock transfers have reduced the strength of genetic differentiation there (Bouzat *et al.* 2009). This result is in line with those obtained from other studies of stocked U.S. Alewife populations (Palkovacs *et al.* 2008), as well as American Shad populations under a similar restoration program as Alewives (Hasselman *et al.* 2010, 2013).

The hierarchical Bayesian analysis, dendrogram of chord distances, and genetic differentiation, for the most part, identified regional clusters of rivers exhibiting modest differentiation (i.e., Northern New England, Bay of Fundy, Atlantic coast of Nova Scotia, and Gulf of St. Lawrence). However, five populations, Dresden (Maine), Sewell (Maine), Nemasket (Massachusetts), Sullivan's Pond (Atlantic coast) and Mactaquac (Bay of Fundy), appeared somewhat anomalous in their genetic affinities.

Dresden and Sewell, two Maine populations, are unusually divergent from other populations in the region, despite the fact that both populations have been subject to

stocking. Therefore, it appears that these populations have resisted genetic homogenization from translocation efforts. Similar observations have been made for other anadromous fish species (e.g., Coho Salmon, Atlantic Salmon and Brown Trout) where native populations have been shown to resist genetic swamping by large releases of non-native fish and heavy exploitation (Nielsen *et al.* 2001, Hansen 2002, Eldridge *et al.* 2009). Moreover, these two populations, along with Nemasket and Mactaquac, appeared to be genetically more similar to populations further away than to nearby populations within their region. The greater gene flow between populations further away implied by this genetic similarity could reflect dispersal promoted by differences between the seasonal feeding migrations of these populations and their nearby populations (Neves 1981, Dadswell *et al.* 1987, Stone and Jessop 1992). However, more populations from the Virginian Biogeographic Province need to be examined to reach a firmer conclusion about Nemasket and its surprising genetic similarity to Canadian populations.

The Sullivan's Pond population, although located on the Atlantic coast, appeared to be closely related to the Shubenacadie River population and to a lesser extent, other Bay of Fundy populations. Sullivan's Pond is part of the Shubenacadie Canal system that connects the Bay of Fundy to the Atlantic coast by a series of locks and incline planes; hence, the genetic similarity to Bay of Fundy populations, and dissimilarity to other Atlantic coast populations strongly suggests that the Sullivan's Pond population was founded by dispersal from the Shubenacadie River population via the Shubenacadie Canal, sometime after the canal was built in 1861. However, an impassable barrier prevents Alewives from re-entering the Shubenacadie Canal from Sullivan's Pond, therefore, this population remains separated from the Shubenacadie population. The fact that this population shows very little admixture and relatively strong genetic differentiation from neighboring Atlantic coast populations provides evidence of natal homing and is consistent with the recent origin of the population.

The pattern of genetic structure observed in Canadian populations in the hierarchical Bayesian analysis suggests a possible role of historical demography in shaping contemporary genetic relationships. The genetic similarities between Bay of Fundy and Gulf of St. Lawrence populations could have been influenced by the retreating Laurentide ice sheet (Hasselman *et al.* 2010, Curry 2007). The Tantramar Marsh region,

which separates the Bay of Fundy from the Gulf of St. Lawrence, is an area of low elevation that spans the border between New Brunswick and Nova Scotia (Curry 2007). This area may have been submerged post-glacially, providing a colonization route between Bay of Fundy and Gulf of St. Lawrence (Seaman 2004, Hasselman 2010).

The PCoA revealed low genetic differentiation (mean $F_{ST} = 0.010$) between the Gulf of St. Lawrence and Atlantic coast, which may reflect dispersal promoted by seasonal migrations. Populations from the Gulf of St. Lawrence and Cape Breton mix in the Sydney Bight during the fall and then move south along the Scotian shelf following the aggregation of zooplankton (Stone and Jessop 1992) and mixing with Alewives from the Atlantic coast.

Isolation by distance analysis revealed a significant association between geographic and genetic distance across the entire study area, regardless of whether two genetic outlier populations, Nemasket and Sullivan's Pond, were included or excluded from the analysis. Significant IBD has also been found in another alosine species, American Shad over a similar geographic area (Hasselman *et al.* 2013). These results suggest that IBD, and therefore strong natal homing, are general features of alosine biology.

When the IBD analysis was restricted to Canadian populations, IBD remained significant over the Canadian portion of the study area, but the proportion of genetic variance explained by geographic distance was relatively low ($R^2 = 0.099$). Separate analyses of the three geographic regions in Atlantic Canada that corresponded to the genetic clusters identified in the structure analysis revealed distinctly different patterns in each area. First, IBD was significant in both the Gulf of St. Lawrence and Bay of Fundy, but not along the Atlantic coast. Second, the slopes of the IBD relationships differed by approximately a factor of 10 between the Gulf of St. Lawrence (0.00004) and Bay of Fundy (0.00050).

Although sampling effects may have influenced the differing IBD results obtained in the three Canadian areas, it is likely that the results also reflect real differences among regions in historical or contemporary dispersal patterns. For example, the apparent absence of IBD on the Atlantic coast might be related to re-colonization of rivers following the removal of old dams, as seen in the Kennebec River with the removal of

Edwards Dam (Crane 2009) or modification of fish ladders, as seen on the St. Croix River (Bentzen and Paterson 2005). The low slope of the IBD pattern in the Gulf of St. Lawrence could reflect dispersal promoted by Alewife migratory patterns that follow residual currents and the coastline within this large semi-enclosed region. The relatively steep IBD slope observed in the Bay of Fundy indicates very low levels of dispersal in that area, and is consistent with the number of genetic discontinuities in gene flow reported in the Barrier analysis. Similar IBD and Barrier results were found for American Shad populations in the Bay of Fundy (Hasselman *et al.* 2010). The location of these barriers suggests the possibility that hydrodynamic features and distribution patterns of zooplankton found within the inner (i.e., Minas Basin and Shepody Bay) and outer Bay of Fundy (Amos 1978, Dadswell *et al.* 1987) may influence gene flow.

Isolation by distance analysis of all U.S. populations except Nemasket revealed a non-significant association between genetic and geographic distance. IBD remained non-significant when stocked and non-stocked populations were analyzed separately. The absence of detectable IBD is consistent with results of previous studies of U.S. Alewife populations (Palkovacs *et al.* 2008, Bentzen *et al.* 2009, Labbe 2012), and suggests widespread stocking may have reduced or eliminated IBD in these populations, either through direct effects of human-mediated gene flow, or indirect effects caused by increased straying of stocked fish (Bams 1976).

2.4.2 Bottleneck and Effective Population Size

Evidence of recent population bottlenecks was found in 16 of the 44 populations in this study, including nine of 16 Maine populations and seven of 25 Atlantic Canada populations. These results differ from those obtained in a recent study of Maine Alewife populations, which found no evidence of bottlenecks (Labbe 2012). The discrepancy in results may be a result of differences in power associated with sample sizes, number of loci examined, and number of temporal replicates. Population bottlenecks identified within Atlantic Canada were in rivers that have dams within 0.5 km of the head of tide (Argyle, Mersey, Sackville and Hillsborough) or have a major river herring fishery (Gaspereau, Tuskent and Margaree), which suggests the likelihood that these bottlenecks were human-induced (Hall *et al.* 2011). Further investigation should incorporate

historical samples for U.S. populations to shed further light on bottlenecks and to determine the influence stocking may have had on allele frequency distribution

A majority of the populations had ‘infinite’ as the upper bound of the 95% confidence interval and/or a negative N_e estimate, which indicated a high uncertainty regarding their effective population size estimates. However, Damariscotta and Orland in Maine, and Tidnish, Wallace and Waugh in the Gulf of St. Lawrence had relatively low estimates with finite upper confidence limits. The tight lower and upper confidence interval surrounding the estimate of effective population size for Tidnish, Wallace and Waugh, suggests that the effects of bottlenecks have persisted more in these locations than elsewhere. Additional temporal replicates with cohort information should be obtained to clarify the effective population size estimates.

2.4.3 Blueback Herring

Bayesian clustering analysis suggested that Blueback Herring populations are genetically structured in a manner similar to that seen in Alewives within this study. Significant genetic differentiation was found in all comparisons between populations, and was similar in magnitude to the differentiation observed between Alewife populations at comparable spatial scales.

When sampled in the same river, Alewife and Blueback Herring were strongly differentiated (e.g., Mactaquac $F_{ST} = 0.429$), indicating reproductive isolation and little if any introgressive gene flow. Nonetheless, hybrids between the two species were observed, as was the case in a previous study of river herring in the same geographic region (Bentzen *et al.* 2009). In some locations, hybrids appeared relatively abundant, for example in the Petitcodiac, where 29 out of a total of 96 samples were hybrids. Hybridization between the two species is presumably promoted by temporal and spatial overlap between the species, which may vary among rivers. Hybridization rates between the two species, and the environmental factors that may be correlated with hybridization, deserve further study. In general, more Blueback Herring populations need to be studied in order to make stronger comparisons of the population structuring of the two species. Since they are co-managed with Alewives it would be interesting to investigate whether stock transfers have resulted in weaker population structure within the U.S. portion of their northern range.

Chapter 3: The Effects of Stocking on the Genetic Integrity of Alewife (*Alosa pseudoharengus*) Population Structure in Maine

3.1 Introduction

Overfishing, habitat degradation and fragmentation have been acknowledged as major sources of fish population declines globally (Jackson *et al.* 2001, Pauly *et al.* 2002, Myers *et al.* 2003), including commercially valuable anadromous fishes (Ward 2006, Neff *et al.* 2011). Stocking programs (i.e., restocking and translocation) have historically been undertaken to offset the lost production of anadromous fishes resulting from overexploitation in the marine realm (Ward 2006, Neff *et al.* 2011). Stocking programs are deemed successful when abundance is shown to increase and spawning runs are augmented (Hasselman and Limburg 2012). However, these short-term gains can have long-term unintended negative consequences for population persistence and evolutionary potential for a species (Bouzat *et al.* 2009, Hasselman and Limburg 2012). When stocking practices are implemented, genetic homogenization may result, thereby eroding genetic and life history diversity and adaptations to local spawning and nursery habitats, and thus potentially reducing the resilience of the populations to exploitation and environmental changes (Hilborn *et al.* 2003). Additionally, populations that have recently suffered a severe reduction in size (i.e., bottleneck) and a small effective population size could be increasingly at risk of extirpation, despite stocking practices, if their adaptive potential is reduced as a result of an increased rate of inbreeding, loss of genetic variation and fixation of mildly deleterious alleles (Ryman and Laikre 1991, Cornuet and Luikart 1996). Evaluating the spatiotemporal genetic structure of a species will help determine whether stocking practices have affected population structure and the genetic diversity of the species.

Alewife (*Alosa pseudoharengus*) and Blueback Herring (*A. aestivalis*) (collectively termed river herring) are closely related (Bentzen *et al.* 1993) iteroparous anadromous clupeid fishes that overlap over much of their spawning ranges from the tributaries of the Gulf of St. Lawrence (Bigelow and Schroeder 1953) to North Carolina; although, Blueback Herring extend further south to Florida (Neves 1981, Kuhn and Kornfield 2004, ASFMC 2009) and are more abundant in the middle and southern portion of their range than in their northern portion (Schmidt *et al.* 2003, Greene *et al.*

2009). River herring are sympatric in freshwater (Loesch *et al.* 1982, Jessop and Anderson 1989), estuarine (Stone and Daborn 1987), and marine (Neves 1981) habitats. They exhibit only minor differences in morphology, growth parameters, and spawning time (Bigelow and Schroeder 1953, Leim and Scott 1966, Messieh 1977), Loesch 1987, Stone and Jessop 1992) which has led to misidentification, harvesting and management of the two species as one (Alewife). Spawning runs occur in south to north chronological progression (Neves 1981) from late February (Holland and Yelverton 1973) through June (ASFMC 2009, DFO 2001) for Alewife, and as early as December (Greene *et al.* 2009) through August for Blueback Herring (Leim and Scott 1966, Greene *et al.* 2009). Peak spawning periods may differ only by 2-3 weeks (Hildebrand and Schroeder 1928) between the two species and with the considerable spatiotemporal overlap in spawning (Loesch 1987), hybridization between these species has been observed (see Chapter 2).

River herring usually return to their natal streams for spawning, but they also readily recolonize new ponds or streams and reoccupy areas from which they had been extirpated (Loesch 1987, see Chapter 2). For centuries, inland commercial fisheries have taken advantage of the predictable spring migration (DFO 2001, ASFMC 2009, Davis and Schultz 2009) with landings dating back over 350 years in some areas (NOAA 2009). However, extirpation and dramatic declines in landings (31,750 mt in 1957 to 454 mt in 2007) within U.S. waters (ASMFC 2009, Limburg *et al.* 2003) has resulted in the National Oceanic and Atmospheric Administration (NOAA) placing river herring on the Species of Concern List in 2006 (Cournane *et al.* 2012). Currently, river herring are under consideration for possible listing as ‘threatened’ under the Endangered Species Act (Cournane *et al.* 2012).

Moratoria on directed fisheries have been in place for individual U.S. state managed waters (inshore 3 NM) for the past 10 years, but in 2012 they were extended coast-wide, resulting in mandatory closures of all direct fisheries that lack a sustainable harvest plan approved by the Atlantic States Marine Fisheries Commission (ASMFC 2009, (Cournane *et al.* 2012). To date, the only states with approved sustainable harvest plans are Maine, New Hampshire, New York, North and South Carolina (Cournane *et al.* 2012). Recovery efforts also include spawning habitat reclamation (through modification of fish passages and dam removal) and stock transfers (Hasselman and Limburg 2012). In

the absence of any genetic data, a stock translocation program was implemented within the U.S. that moved stocks within (in-basin) and between (out-of-basin) watersheds to either restore extirpated populations or enhance depleted populations (ASMFC 1985). Stock transfers among divergent source populations may unintentionally reduce population fitness and increase gene flow among populations, jeopardizing genetically distinct and locally adapted populations that were created by natural reproductive barriers (Nelson and Soule 1987, Hasselman and Limburg 2012). Human mediated gene flow among populations with low natural rates of gene flow provides the opportunity for non-native genes or gene complexes to introgress into native populations and lead to the breakdown of co-adapted gene complexes within the native population (i.e., outbreeding; Edmands 2006, McClelland and Naish 2007, Bentzen *et al.* 2009). The risk to remnant populations posed by stocking may increase if the amount and/or frequency of the stocking are high (Brunner *et al.* 1998, Marie *et al.* 2010).

In the state of Maine, extensive translocation programs have been implemented by the Maine Department of Marine Resources (MeDMR) within the last century, however, complete stocking records only exist from 1983 to present with sporadic paper records dating earlier to the 1940s (Labbe 2012). The translocation program has concentrated on transferring gravid adults from three major dams on the Kennebec River and one major dam on the Androscoggin River (Bentzen *et al.* 2009) to support both within and out-of-basin productive spawning runs along with the most cost-effective and practical spawning runs to manage (Labbe 2012 and references therein). Kennebec and Androscoggin are two of the three largest watersheds located within Midcoast Maine (Bentzen *et al.* 2009).

The effects of past stocking on population structure and spatial patterns of genetic diversity in river herring are largely unknown. In chapter 2, for both species of river herring I demonstrated that there are genetically distinguishable populations, and that for Alewife populations in Canada, where stocking has not occurred, there is a significant isolation by distance (IBD) trend indicative of natal homing with some level of dispersal. In contrast, I found weak population structure and a non-significant IBD trend for Alewife populations within New England, where stocking has occurred for at least the past 30 years (Chapter 2). The goal of this chapter is to examine patterns of

genetic diversity and differentiation in Alewife populations that have been subject to stocking, at a small spatial scale (< 450 km). Specific objectives are to use temporal replicates spanning at least a three year period to a) evaluate the genetic stability of the populations and patterns of population structure, b) determine whether isolation by distance exists, and if so, whether stocking has influenced the IBD pattern, and c) test for evidence that Maine Alewife populations have experienced population bottlenecks.

3.2 Material and Methods

3.2.1 *Sample Collection*

A total of 2,407 Alewife samples were collected from 20 locations within Maine from 2005-2011 (Figure 3.1; Table 3.1). Twelve of the 20 sample locations have been directly influenced by out-of-basin stocking (Table 3.2). Sampling took place during the spring spawning migration for all populations except Soudabscook and Veazie Dam on the Penobscot River, ME, which were collected in September as young-of-year specimens in 2011. Additionally, both young-of-year and gravid adults were collected in 2009 for Sewell, located on the Kennebec River, ME. The majority of the samples were collected at fish ladders and at commercial dip-stands and weirs, while a few sampling locations were sampled using dip-nets. Collections of fin clips per sample location were obtained through the cooperation of governmental organizations and fishers. All samples were stored in 95% ethanol prior to DNA extraction.

3.2.2 *Laboratory Protocol*

Whole genomic DNA was extracted from 2,288 specimens and amplified across 14 microsatellite loci (Table 3.3) following the laboratory protocols outlined in Chapter 2.

3.2.3 *Statistical Analysis*

Not all genotyped specimens generated useable data, defined as interpretable genotypes at a minimum of seven loci. In addition, since sample size can influence estimates of genetic parameters, sample locations with a total number of individuals less than 50 were excluded from further analysis (Table 3.1; Sebasticook, Sedgeunkedunk, Eddington and Woodland) except for Dennis Stream with a sample size of 48. Sample

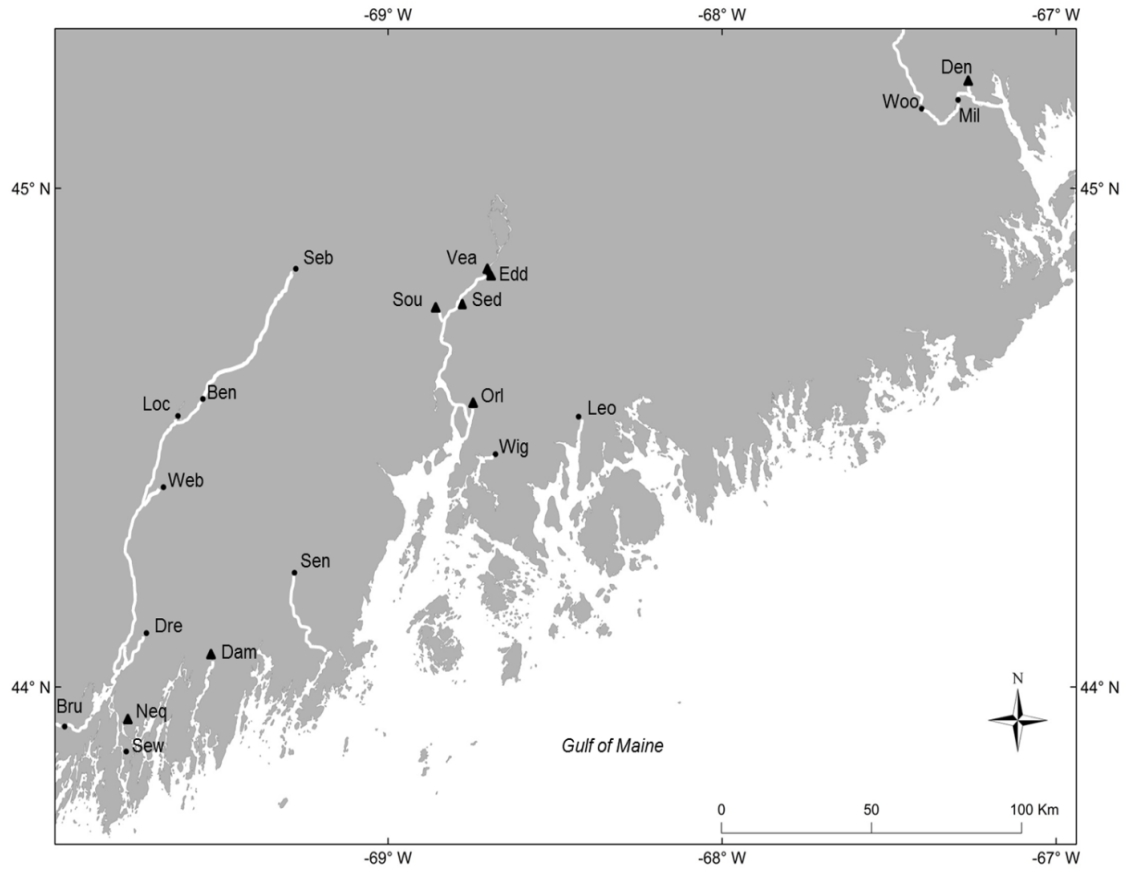


Figure 3.1: Sampling locations for Maine Alewife collections during the time period of 2005-2011. Symbols indicate whether locations are stocked (•) or unstocked (▲). Full names of sampling locations are given in Table 3.1

Table 3.1: Summary indicating the number of samples collected in Maine from 2005-2011, sample location and coordinates.

Code	Coordinates (decimal deg)		Sampling Location	2005	2008	2009	2010	2011	Total
Sew	43.869	-69.783	Sewell Pond			65		51	116
Neq	43.931	-69.779	Nequasset Lake Dam			65	60	46	171
Bru	43.92	-69.968	Brunswick Dam			60	60	51	171
Dre	44.109	-69.723	Dresden Mills Dam		63	34	60	57	214
Web	44.403	-69.672	Webber Pond			37	60	52	149
Ben	44.58	-69.554	Benton Falls Dam			65	63	60	188
Seb	44.84	-69.276	Sebasticook Lake Dam			22			22
Loc	44.546	-69.629	Lockwood Dam			66		51	117
Dam	44.061	-69.526	Damariscotta Mills Dam			60	70	51	181
Sen	44.231	-69.28	Sennebec Pond		52		63	50	165
Wig	44.469	-68.678	Wight Pond			53		55	108
Orl	44.57	-68.743	Orland Dam			5	75	53	133
Sou	44.759	-68.857	Souadabscook Falls			65	60	114	239
Sed	44.765	-68.78	Sedgeunkedunk Dam			15			15
Edd	44.824	-68.696	Eddington Bend			16			16
Vea	44.833	-68.702	Veazie Dam			70	53	52	175
Leo	44.544	-68.429	Leonard Lake Dam			66		50	116
Den	45.21	-67.263	Dennis Stream	48					48
Mil	45.176	-67.293	Milltown	56					56
Woo	45.158	-67.402	Woodland	7					7
Total				111	115	764	624	793	2407

Table 3.2: Summary of the stocking history of all Maine sample locations for 1983-2009 with some recorded events from the 1940s. The count number of stocking events by source and stock source are based on sampling locations and all locations upstream where applicable (i.e., Brunswick, Benton, Lockwood and Sennebec). The rivers or watersheds in which sampling locations are located are abbreviated as follows: A, Androscoggin River; K, Kennebec River; S, Sebasticook River; O, Orland River; M, Machias River; R, Royal River.

Location	River	Major Watershed	Stocked	Stock Source	Count No. of Stocking Events by Source
Sewell Pond	Sewell Creek	Kennebec	Y	A	A=6
Nequasset Lake Dam	Nequasset Brook	Kennebec	N		
Brunswick Dam	Androscoggin River	Androscoggin	Y	K, S, R	K=18, S=19, R=16
Dresden Mills Dam	Eastern River	Kennebec	Y	K, S	K=4, S=5
Webber Pond	Seven-mile Stream	Kennebec	Y	A, S	A=2, S=31
Benton Falls Dam	Sebasticook River	Kennebec	Y	K, A, R	K=221, A=213, R=15
Sebasticook Lake Dam	Sebasticook River	Kennebec	Y	K, A, R	K=91, A=104, R=15
Lockwood Dam	Kennebec River	Kennebec	Y	A, S, R	A=16, S=29, R=2
Damariscotta Mills Dam	Damariscotta River	Central Coastal	N		
Sennebec Pond	St. George River	Central Coastal	Y	K, A, S	K=3, A=5, S=4
Wight Pond	Bagaduce River	Penobscot	Y	O	O=1
Orland Dam	Orland River	Penobscot	N		
Soudabscook Falls	Soudabscook Stream	Penobscot	N		
Sedgeunkedunk Dam	Sedgeunkedunk Stream	Penobscot	N		
Eddington Bend	Penobscot River	Penobscot	N		
Veazie Dam	Penobscot River	Penobscot	N		
Leonard Lake Dam	Union River	Eastern Coastal	Y	O	O=1
Dennis Stream	Dennis Stream	St. Croix	N		
Milltown	St. Croix River	St. Croix	Y	M	M=1
Woodland	St. Croix River	St. Croix	Y	M	M=1

Table 3.3: Summary information for 14 microsatellite markers used in this study.

Locus	Repeat motif	Primer sequence (5'-3')	T _A (°C)	Size range (bp)	Reference	Accession #
Asa-8	(TTTG) ₈	F: TCCATTCCATTACGTAGAGCACT R: CCGGCAGGGCACAGAAC	60	132-148	Waters <i>et al.</i> 2000	AF039660
Aa082	(ACAT) ₄	F: TGCTTCCCACACAAACACAC R: ATCGAAATCGTGACACCCC	60	168-184	Labbe <i>et al.</i> 2012	JN383996
AsaC249	(CATA) ₈ (TTCT) ₁₃	F: TTATTACAACGGTGAATTGAGTG R: TAAGTGCATGTTGTGTGTGATG	60	210-254	Julian & Bartron, 2007	EF014994
MGPL- Aa093*	(ATCT) ₈	F: ACAGTGTCTCCAGTATAGATGAATCG R: AGCCTATTGTGTTGGCGATATT	60	272-284	Labbe <i>et al.</i> 2012	JN384003
Aa081	(ATCT) ₅	F: TGTCCCATGAACTGCACAAC R: GTCATTGCAGCAGCACACC	60	152-184	Labbe <i>et al.</i> 2012	JN384000
Aa070	(GATT) ₅	F: TGACAGGTTGATTGATGATTGC R: TGACCGTTGTGGCCGTAG	60	210-270	Labbe <i>et al.</i> 2012	JN383999
Aps2A	(TCAA) ₈	F: CCAGTTACGTCAGTCACACGA R: TGGGCAGACAACAGAAGTTTT	60	94-122	Bentzen & Paterson, 2005	HM190305
AsaD042	(CTAT) ₁₂	F: ACTGGTCAATTGTAAGACACCC R: CAAGATGACCAAGGGTTAAGAC	60	167-191	Julian & Bartron, 2007	EF015000
Ap010	(AATG) ₉	F: GGGGACATCGTTGATTAATTGC R: GAACTGCCATTTACTGGATAC	60	223-263	Labbe <i>et al.</i> 2012	JN383997
MGPL- Ap071	(CATT) ₈	F: TTTTCCCATATTTTGTCTATTTT R: TCGTTCGGCATAAACACAAA	62	280-292	Labbe <i>et al.</i> 2012*	JN383998
Aps-1	(CTGT) ₈	F: CTGCACGTCTGACTGTCTGC R: TATGGGATGGATGGGATCAG	62	84-104	Bentzen & Paterson, 2005	HM190304
Aa046	(GGAT) ₈	F: GTCACCTCCTTACCAGGG R: TTCCTGCCAGCATTATTGAGC	62	212-228	Labbe <i>et al.</i> 2012	JN383992
Ap058	(ACGC) ₅	F: TGCTTACCCCTTCACTGGC R: GCGGAAATGGTGTGGAAC	62	254-278	Labbe <i>et al.</i> 2012	JN383995
MGPL- Aa039*	(CTTT) ₄	F: TCGGTACGTCTGCTCATCTG R: GCCTAGGCCTTGAGTATGAAA	62	300-320	Labbe <i>et al.</i> 2012	JN384006

*Both forward and reverse primers were redesigned for locus Aa093 to increase the size range. The reverse primer was redesigned for locus Ap071 and the forward primer was redesigned for locus Aa039 to increase their size ranges.

sizes of 50 individuals have been advocated as the appropriate number to conduct analyses of population structure using microsatellites (Ruzzante 1998). Overall, the final database consisted of 16 locations and 2,213 individuals. File formatting of the database to conduct analysis was done using both CREATE (v.1.35; Coombs *et al.* 2008) and PGDSpider (v.2.0.1.7; Lischer and Excoffier 2012).

A Bayesian model-based clustering analysis was conducted on this modified dataset to identify and filter Alewife, Blueback Herring and hybrid individuals (Table 3.3; STRUCTURE v.2.2; Pritchard *et al.* 2000, Falush *et al.* 2003). A burn-in of 50,000 replicates was followed by 1,000,000 replicates of Markov Chain Monte Carlo (MCMC) simulations, employing the admixture model and correlated frequencies among populations. Ten iterations of this parameter set were performed for $K = 2$ (number of clusters). Each individual was separated and categorized based on their proportion of genotypes that represent each species (q-value). An individual was categorized as a pure Alewife if it had a q-value > 0.90 or a pure Blueback if it had $q < 0.10$, and individuals with a q-value between 0.10 and 0.90 were considered to be hybrids. Following this analysis, individuals were respectively identified as Alewife (2,147), Blueback (10) and hybrids (56; Table 3.4). Alewife samples were separated by collection years, and each location-year was treated as a separate location for the remainder of the analysis, resulting in a total of 40 locations. The Dresden 2009 sample had only 19 individuals, but was retained in the analysis in order to maintain consecutive collection years.

Microsatellite genotypes were checked for scoring errors, presence of null alleles and large allele dropouts using MICROCHECKER 2.2.3 (van Oosterhout *et al.* 2004). No scoring errors or large allele dropouts were detected, but evidence of null alleles was detected in *Aa082* and *Ap071*. These loci showed an excess of homozygotes at a few alleles that were consistent across populations therefore these loci were removed. Markers were tested for neutrality using LOSITAN: Selection Detection Workbench (Beaumont and Nichols 1996, Antao *et al.* 2008) which applies the F_{ST} outlier detection algorithm to identify loci that may be under selection. Analyses used the stepwise mutation model and 80,000 permutations and a sample size reflecting the smallest

Table 3.4: Filtered dataset indicating the number of samples per a location genetically identified using Bayesian clustering analyses as Alewife, Hybrid or Blueback Herring. Full names of sampling locations are given in Table 3.1.

Location	Year	Alewife	Hybrid	Blueback
Ben	2009	65		
	2010	62		
	2011	61		
Bru	2009	59		
	2010	60		
	2011	50		
Dam	2009	57	3	
	2010	56	2	
	2011	53		
Dre	2008	49	4	
	2009	19	4	1
	2010	56	3	
	2011	53	1	
Sen	2008	47	3	
	2010	62	1	
	2011	49	2	
Leo	2009	58	1	
	2011	49		
Loc	2009	63	1	1
	2011	35	3	8
Neq	2009	57	2	
	2010	59	1	
	2011	45		
Orl	2010	59	1	
	2011	52	1	
Wig	2009	50	2	
	2011	60		
Sew	2009	62	2	
	2011	51		
Sou	2009	57	2	
	2010	59	1	
	2011	60	1	
Vea	2009	69	2	
	2010	52		
	2011	52		
Web	2009	37		
	2010	53	7	
	2011	52		
Mil	2005	51	5	
Den	2005	47	1	
Total		2147	56	10

population size ($n = 19$). All loci were within the 95% confidence range for candidate of neutrality (Antao *et al.* 2008).

Population-specific sample size (N), number of alleles (N_a), probability of departures from HWE (P-val), observed (H_o) and expected heterozygosity (H_e) were assessed using Arlequin (v.3.5.1.3; (Excoffier *et al.* 2005). Allelic richness (A_e) per a locus and location was estimated with FSTAT (Goudet 1995) standardized to a minimum sample size of 34 individuals (Leberg 2008). Dresden (2009) was excluded from allelic richness estimations due to the low sample size. The population-specific inbreeding coefficient (F_{IS}) was estimated by GENEPOP (v.4.1.4; Raymond and Rousset 1995). Arlequin was also used to estimate linkage between loci within each location (using 100,000 permutations) and the adherence of loci and location to Hardy-Weinberg Equilibrium (HWE; 1,000,000 permutations and 100,000 dememorization steps); results were evaluated using the sequential Bonferroni method (Rice 1989) with an initial α level of 0.05.

Genic differentiation among populations was estimated in GENEPOP using default parameters (Raymond and Rousset 1995); tests were combined across all loci or collections using Fisher's method, and sequential Bonferroni adjustments were used to determine the significant levels (Rice 1989). Genetic differentiation between all possible pair of populations using the F_{ST} estimate θ (Weir and Cockerham 1984) was evaluated in Microsatellite Analyser (MSA; v.4.05; Dieringer and Schlötterer 2003) using 10,000 permutations. Sequential Bonferroni adjustments were also used to assess significance (Rice 1989).

To assess the patterns of genetic variation among locations, among years within locations and within locations, a hierarchical AMOVA was assessed with Arlequin (10,000 permutations; Excoffier *et al.* 2005).

The pairwise F_{ST} values generated by MSA were visualized in a Principal Coordinate Analysis (PCoA) using GenAlEx (v.6.41; Peakall and Smouse 2006). Genetic affinities among locations were also examined using a neighbor- adjoining (NJ) tree based on Cavalli-Sforza and Edwards chord distances (D_c) and bootstrapped over all loci (5,000 replications) was computed in POPULATIONS (v.1.2.3; Langella 1999) and visualized in TREEVIEW (Page 1996). The distance measure of D_c was chosen due to its

ability to accurately convey phylogenetic relationships with microsatellite data (Takezaki and Nei 1996).

Bayesian model-based hierarchical clustering, implemented with STRUCTURE (Pritchard *et al.* 2000, Falush *et al.* 2007), was used to infer the number of homogenous clusters. Structure analysis was conducted twice, first without location prior information and second with the location prior information. Both analyses employed the admixture model and correlated frequencies among populations with a burn in period of 50,000 steps followed by an MCMC chain of 500,000 steps. Three iterations of K (1-40) were tested, where the most likely value of K was estimated on the ΔK statistic proposed by (Evanno *et al.* 2005) and implemented by Structure Harvester (Earl and vonHoldt 2012). Results from all STRUCTURE iterations were amalgamated into a single population assignment using CLUMPP (v.1.1.2; Jakobsson and Rosenberg 2007).

Mantel tests were calculated using GenAlEx (10,000 permutations; Peakall and Smouse 2006) to determine the significance of the relationship between genetic distance and geographic distance (IBD) among all locations, stocked and non-stocked locations. Following (Rousset 1997) pairwise F_{ST} was linearized ($F_{ST}/(1-F_{ST})$). The geographic distances between sample locations were measured as the most direct route between river mouths while maintaining close proximity to the shoreline (~5km) using Google Earth v.6.2 (Bradbury *et al.* 2008). Two distance measures were calculated; (i) 'total' and (ii) sea water, where the latter measured only the distance between river mouths at head of tide, whereas the former included freshwater distances to the mouth of tributary rivers where the river herring spawn. Additionally, a regression analysis was used to examine trends exhibited by the residuals in the IBD relationships of stocked and non-stocked locations.

BayesAss (v.1.3; default parameters; Wilson and Rannala 2003) was used to calculate recent migration rates within and among sites using a Bayesian model. Due to the population limitation for BayesAss (max. 25 populations), temporal replicates were pooled for each site.

To test for recent historical bottlenecks within collections, allele frequency data were assessed in BOTTLENECK (v.1.2.02; 10,000 permutations; Cornuet and Luikart 1996). Sign tests were used to determine if the proportion of loci with heterozygosity

excess is significantly larger than expected at equilibrium (Cornuet and Luikart 1996, Luikart and Cornuet 1998). Following Luikart *et al.* (1998) the allele frequency mode shift was plotted and examined for each location to determine whether the allele frequency distribution diverged from the null hypothesis of an L-shaped distribution.

Effective population size was estimated using the linkage disequilibrium method implemented in LDNe (Waples and Do 2008). For this analysis temporal replicates were pooled for each site and the random mating option was chosen in LDNe. To reduce the possibility of parent-offspring sampling affecting the N_e estimate, Dresden and Sennebec 2008 were removed as well as Sewell 2009.

3.3 Results

3.3.1 Population Genetic Statistics

The amount of genetic polymorphism observed varied among loci and locations (Appendix 3). The number of alleles per locus ranged from five (*Aa082*, *Aps1*, *Aa046*) to 16 (*Aps2A*; Appendix 3), with five loci exhibiting ≤ 10 alleles. Allelic richness per locus and location ranged from 1.642 (*Aps 1*; Dresden '11) to 9.736 (*Ap010*; Dresden '11; Appendix 3) and observed heterozygosity varied between 0.019 (*Aps 1*; Dresden '11) and 0.865 (*Aa081*; Veazie '10). Across all 12 loci (i.e., excludes *Aa082* and *Ap071*) allelic richness ranged from 4.058 (Damariscotta '10) to 5.362 (Dresden '08), observed heterozygosity varied between 0.469 (Souadabscook'10) and 0.554 (Benton'11), and expected heterozygosity varied between 0.465 (Souadabscook'10) and 0.587 (Dresden'08; Table 3.5).

No evidence of linkage was detected among the 12 loci used across the 40 sampling locations. After sequential Bonferroni correction, five locus-location combinations were out of HWE from a possible 480 comparisons overall samples (Appendix 3). The loci involved were not consistent among locations (Appendix 3).

Pairwise tests of genic differentiation between locations revealed that out of 745 comparisons, 348 were significant (Appendix 4). Sewell, Dresden, Dennis Stream, and Damariscotta'09 were particularly differentiated from other locations (Appendix 4). Additionally, pairwise tests of genic differentiation within locations revealed Damariscotta '09 to '10 and '11, Dresden '08 to '11, Webber '09 to '10 and,

Table 3.5: Genetic diversity statistics across all 12 loci for each location. Shown are stocking history (Y/N), year, number of individuals (N), observed and expected heterozygosity (H_o , H_e), inbreeding coefficient (F_{IS}) and associated p-value (P -val) and allelic richness (A_e). Full names of sampling locations are given in Table 3.1. Bolded p-values are significant.

Stocked	Population	Year	N	H_o	H_e	F_{IS}	P -val	A_e
Y	Ben	2009	65	0.508	0.525	0.032	0.282	4.639
		2010	62	0.554	0.510	-0.087	0.316	4.313
		2011	61	0.525	0.537	0.022	0.493	4.644
Y	Bru	2009	59	0.511	0.511	0.001	0.676	4.463
		2010	60	0.518	0.516	-0.004	0.009	4.389
		2011	50	0.472	0.488	0.032	0.642	4.262
N	Dam	2009	57	0.501	0.499	-0.004	0.178	4.109
		2010	56	0.504	0.483	-0.045	0.087	4.058
		2011	53	0.491	0.502	0.023	0.171	4.357
Y	Dre	2008	49	0.572	0.587	0.026	0.452	5.362
		2009	19	0.547	0.557	0.019	0.833	NA
		2010	56	0.539	0.529	-0.019	0.089	4.974
		2011	53	0.546	0.549	0.007	0.222	5.340
Y	Leo	2009	58	0.553	0.546	-0.013	0.082	4.626
		2011	49	0.517	0.520	0.005	0.384	4.510
Y	Loc	2009	63	0.502	0.520	0.035	0.395	4.509
		2011	35	0.498	0.496	0.084	0.476	4.645
N	Neq	2009	57	0.491	0.516	0.048	0.315	4.360
		2010	59	0.526	0.522	-0.008	0.027	4.614
		2011	45	0.528	0.530	0.005	0.093	4.312
N	Orl	2010	59	0.491	0.494	0.007	0.490	4.684
		2011	52	0.526	0.511	-0.028	0.523	4.357
Y	Sen	2008	47	0.529	0.531	0.004	0.181	4.521
		2010	62	0.480	0.485	0.011	0.167	4.342
		2011	49	0.497	0.530	0.015	0.725	4.690
Y	Sew	2009	62	0.485	0.517	0.061	< 0.001	5.145
		2011	51	0.535	0.536	0.003	0.470	4.594
N	Sou	2009	57	0.491	0.484	-0.014	0.904	4.742
		2010	59	0.469	0.465	-0.009	0.068	4.278
		2011	60	0.526	0.520	-0.013	0.668	4.715
N	Vea	2009	69	0.494	0.496	0.003	0.607	4.491
		2010	52	0.504	0.495	-0.018	0.149	4.294
		2011	52	0.482	0.513	0.060	0.083	4.719
Y	Web	2009	37	0.482	0.503	0.043	< 0.001	4.535
		2010	53	0.474	0.469	-0.011	< 0.001	4.246
		2011	52	0.486	0.523	0.072	0.099	4.532
Y	Wig	2009	50	0.530	0.534	-0.009	0.898	4.627
		2011	60	0.539	0.538	-0.002	0.052	4.462
N	Den	2005	47	0.489	0.497	0.015	0.376	4.506
Y	Mil	2005	51	0.486	0.497	0.023	0.541	4.835

lastly, Webber '10 to '11 were the five out of 35 temporal comparisons that were found to be significant (Appendix 4).

The global F_{ST} was 0.012 and highly significant ($p < 0.001$). In pairwise (temporal) comparisons, F_{ST} ranged from an insignificant value of -0.005 (Damariscotta'11 vs. Brunswick'11) to a significant value of 0.067 (Dennis'05 vs. Dresden'10). Among 745 F_{ST} pairwise comparisons between locations, 242 were significant (Appendix 5). A majority of the significant pairwise comparison (185 out of 242) involved a Dresden, Sewell or Dennis Stream sample (Appendix 5). Additionally, none of the 35 pairwise F_{ST} comparisons within locations were significant (Appendix 5).

A hierarchical AMOVA revealed that of total genetic variation, 98.75% ($p < 0.001$) occurred among individuals within locations, 0.98% ($p < 0.001$) occurred among locations and 0.27% ($p < 0.001$) occurred among years within locations (Table 3.6).

3.3.2 Relationships among Collection Sites and Temporal Replicates

A two-dimensional PCoA based on F_{ST} values accounted for 69.3% of the genetic variation among populations, and revealed the close genetic similarity of most population collections, with the exception of the Dresden, Sewell and Dennis Stream collections (Figure 3.2). There was no evident tendency of samples to group more closely by watershed or by location when there were multiple collection years. Relatively strong genetic differentiation was detected between Dresden and Sewell populations and the rest of the Maine sampling sites (mean $F_{ST} = 0.028$).

A neighbor-joining tree analysis, using Cavalli-Sforza and Edwards chord distances (D_c), revealed little evidence of hierarchical structure among most locations and temporal replicates, with the exception of the relatively divergent Sewell and Dresden samples (Figure 3.3). Temporal replicates at these two divergent locations clustered together, albeit with low bootstrap support, except for Dre'09 and Dre'08, which clustered with 93% support. The remaining samples showed little tendency for temporal replicates to cluster by location, or for locations to cluster by major watershed. Instead, a relatively tight cluster of collections was formed, with one exception, by stocked collections (i.e., Brunswick, Webber, Benton, Lockwood and Sennebec) that have been stocked with each other at a relatively high frequency (Table 3.2; Figure 3.3). The exception was Damariscotta, a non-stocked collection located in close geographic

Table 3.6: Hierarchical analysis of molecular variance showing the genetic variation found among locations, among years within locations and within locations.

Variance Components	df	Sum of Squares	% of variance	F Statistics	P
Among locations	15	180.84	0.98	0.010	< 0.001
Among years within locations	24	95.06	0.27	0.003	< 0.001
Among individuals within locations	4224	13010.67	98.75	0.013	< 0.001

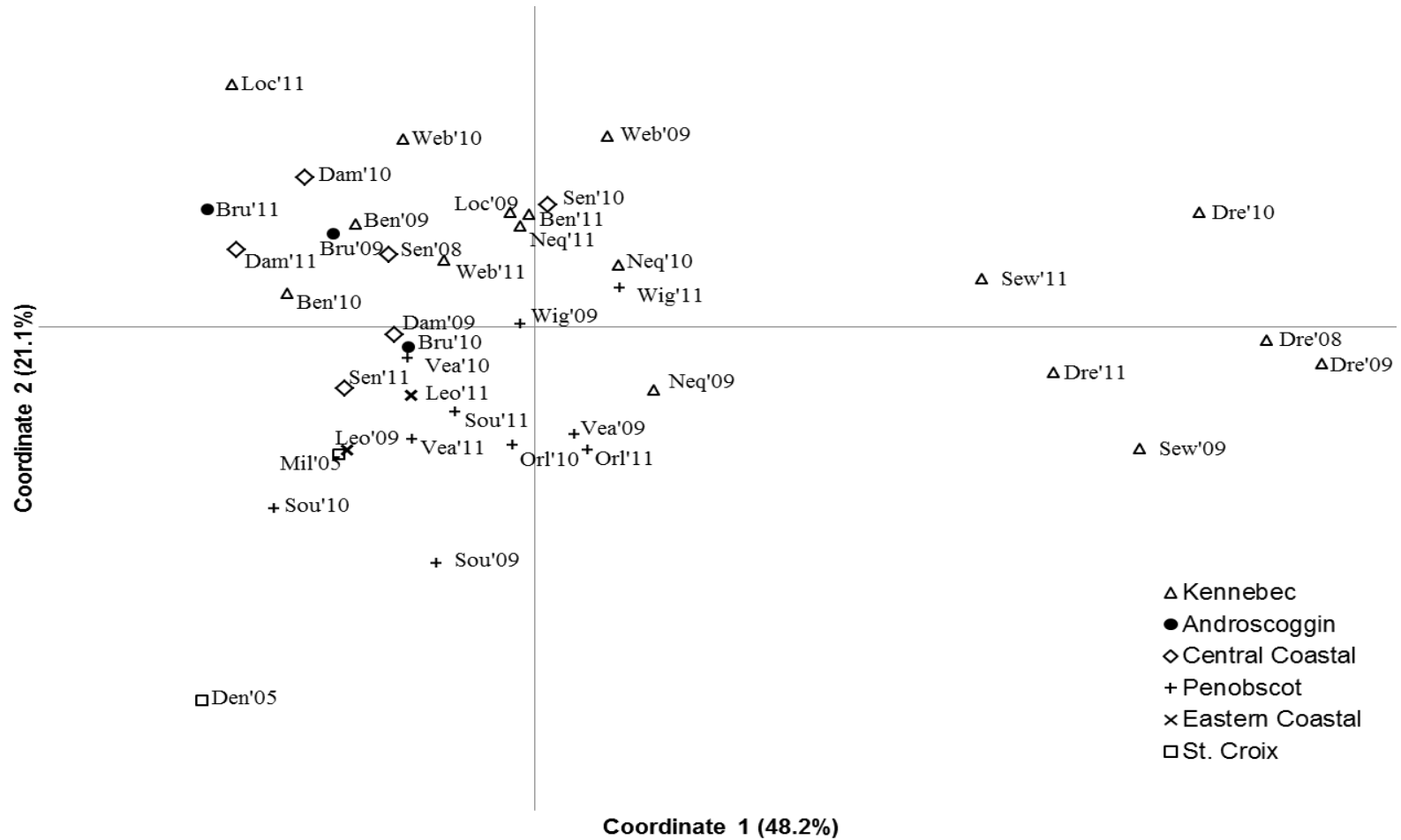


Figure 3.2: Principal coordinates analysis plot describing 69.3% of the genetic variation found in Alewife populations within Maine during the time period of 2005-2011. Symbols indicate the major watershed in which the sampling locations are situated. Full names of sampling locations are given in Table 3.1.

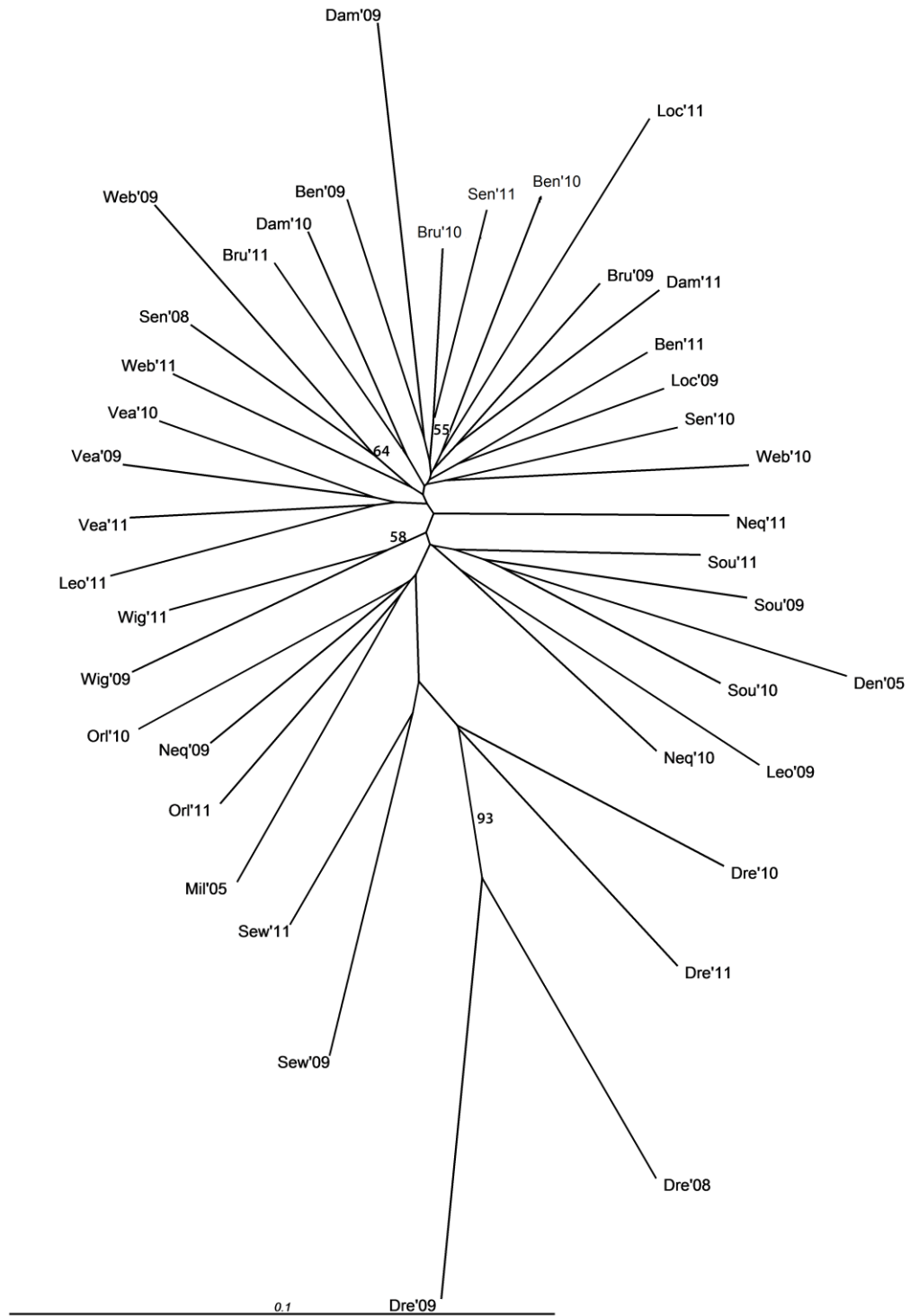


Figure 3.3: Unrooted neighbor-joining tree analysis, using Cavalli-Sforza and Edwards chord distances (D_c), displaying bootstrap support $> 50\%$. Full names of sampling locations are given in Table 3.1.

proximity to the stocked collections (i.e., Brunswick, Webber, Benton, Lockwood and Sennebec), suggesting the possibility of an indirect stocking effect.

Application of Evanno's ΔK method to STRUCTURE analysis with prior location information indicated that the most probable number of genetically distinct clusters was $K = 2$ (Figure 3.4). When no prior location information was inputted into the analysis, only a single genetic cluster was identified (i.e., $K = 1$). In the $K = 2$ analysis, one genetic cluster occurred principally in Sewall and Dresden, but always in admixture with the other genetic cluster. The second genetic cluster dominated all other locations in Maine (Figure 3.4).

3.3.3 Isolation by Distance

There was no significant difference in the IBD relationship when total or sea water was used as a distance measure, thus total distance was used. Mantel tests revealed no evidence of isolation by distance ($p = 0.063$; $R^2 = 0.023$; Figure 3.5a) when all locations were considered; however, excluding Dresden and Sewell resulted in a highly significant IBD relationship ($p < 0.001$; $R^2 = 0.232$; Figure 3.5b). When locations were separated into stocked and non-stocked locations, Mantel tests for non-stocked locations revealed a highly significant ($p < 0.001$) and stronger ($R^2 = 0.521$) relationship between genetic and geographic distance. When only stocked locations were considered, IBD was not significant when Dresden and Sewell were included ($p = 0.512$; $R^2 = 0.000$; Figure 3.6a), but it was significant when these two populations were excluded from the analysis ($p < 0.001$; $R^2 = 0.152$; Figure 3.6b).

Regression of residuals from the IBD pattern for all Maine samples, except Dresden and Sewell, on geographic distance revealed a significant negative slope for comparisons involving stocked locations ($p = 0.003$) and a significant positive slope for comparisons of non-stocked locations ($p = 0.009$), but a non-significant positive slope for stocked to non-stocked location pairs ($p = 0.249$; Figure 3.7).

3.3.4 Migration between Populations

Bayesian analysis suggested some migration between populations, but not always in accordance with stocking history (Table 3.7). For example, both Brunswick and Lockwood were key sites where gravid adults were collected and transferred into a

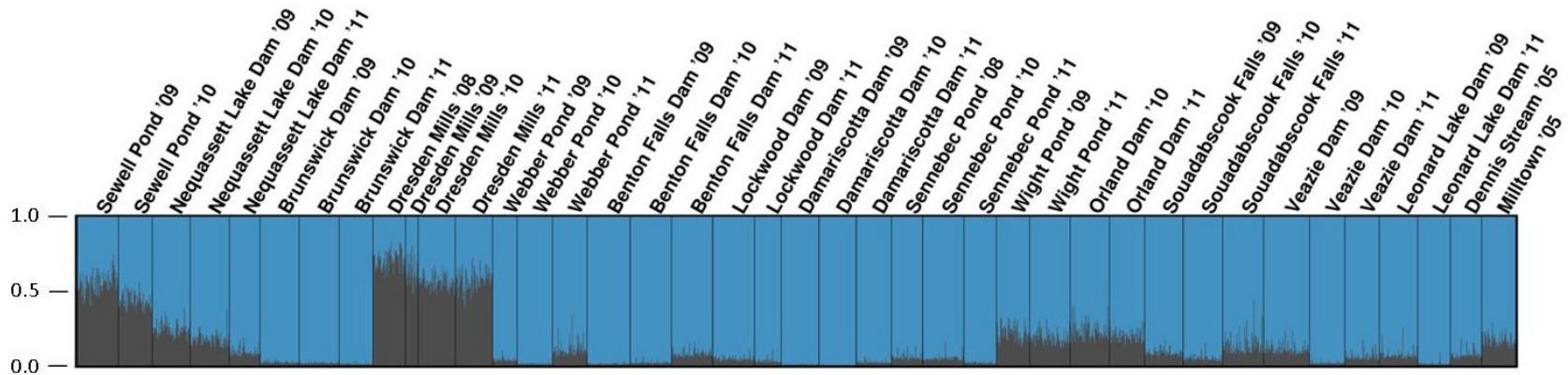


Figure 3.4: Estimated population structure inferred from the admixture analysis $K = 2$ clusters identified using the program Structure. The number of clusters was inferred using the Evanno method (Evanno *et al.* 2005). Each vertical line represents one individual and is partitioned into coloured blocks that indicate the estimated membership of that individual into each of two genetic clusters, where blue is one cluster and grey is the second.

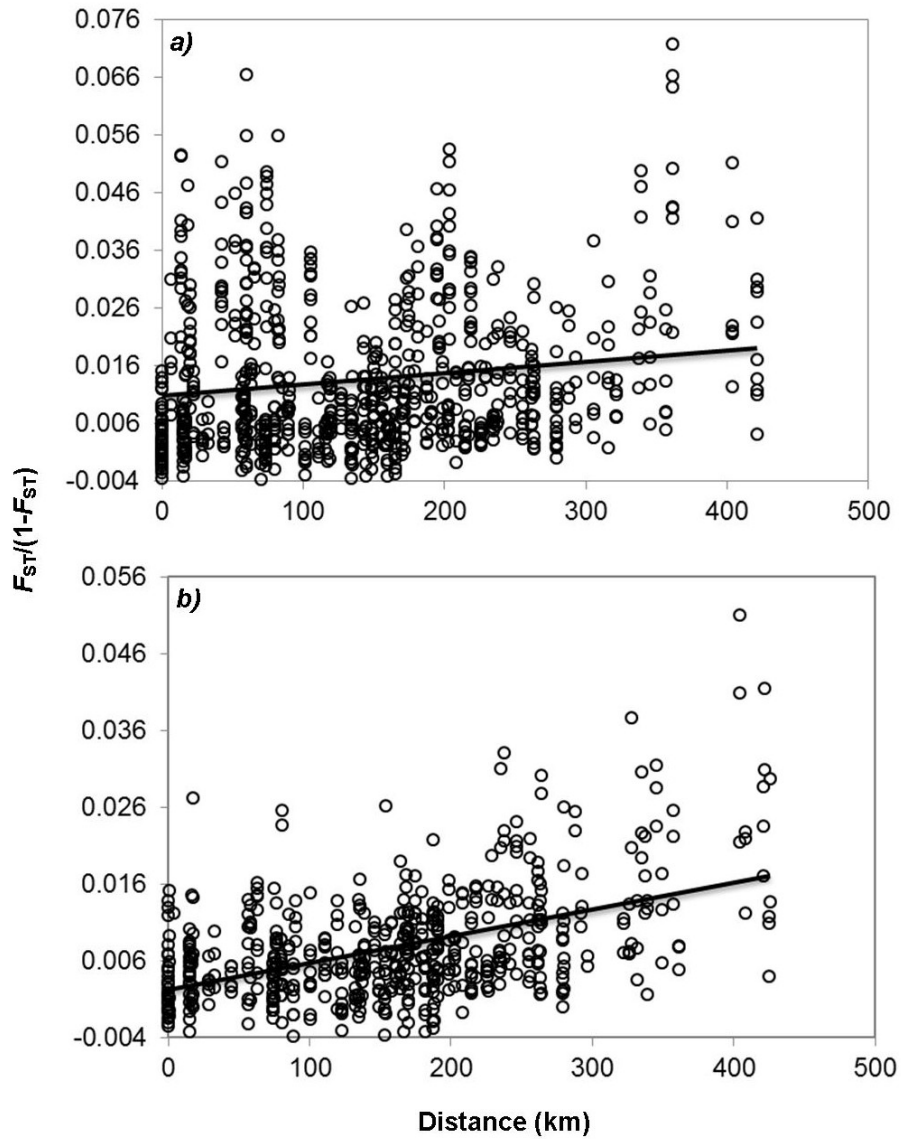


Figure 3.5: The relationship between pairwise linearized genetic distances and geographic distances for Maine Alewife populations. (a) There is no relationship ($R^2 = 0.023$, $p = 0.063$) between genetic distances and geographic distances when Dresden and Sewell are included. (b) Excluding Dresden and Sewell results in a highly significant relationship ($p < 0.001$, $R^2 = 0.232$) between genetic distances and geographic distances.

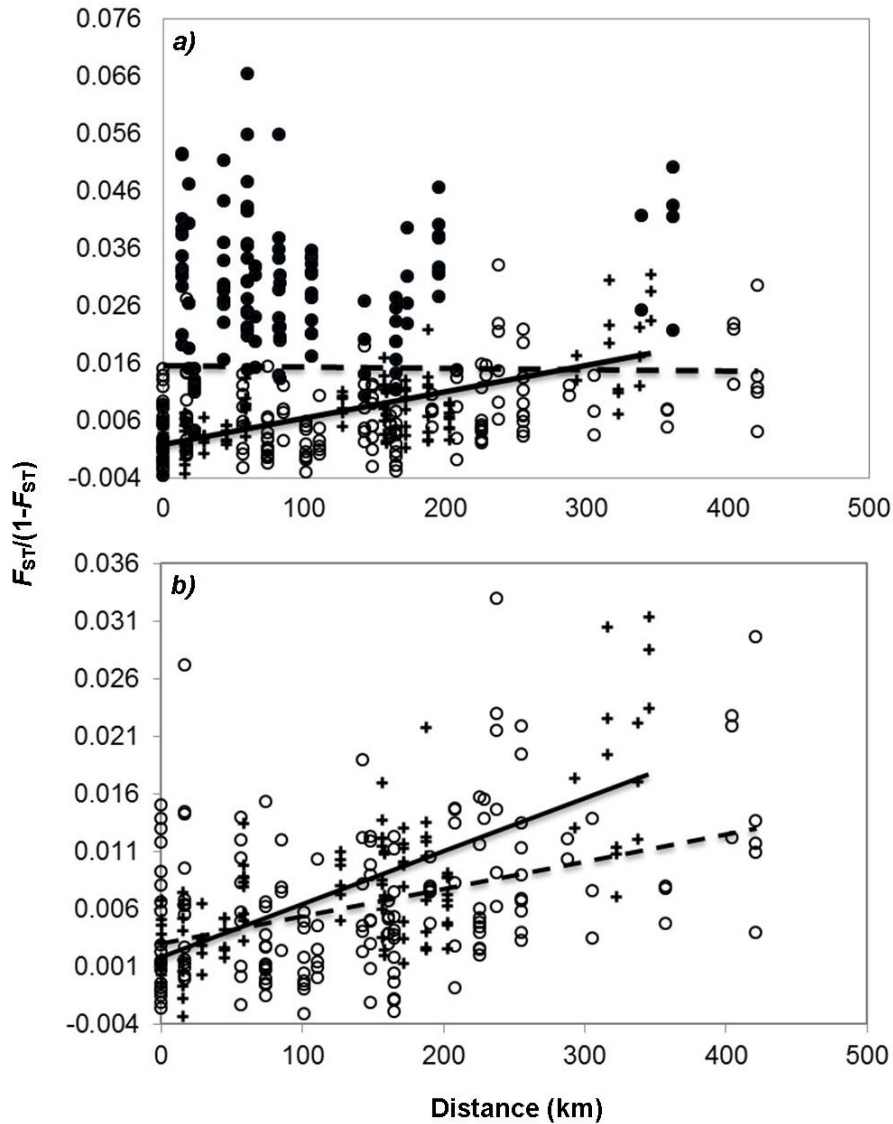


Figure 3.6: Isolation by distance for stocked and non-stocked Maine Alewife populations. (a) Isolation by distance is non-significant ($R^2 = 0.000$, $p = 0.512$; dashed line) for stocked locations (○), where comparisons involving two relatively divergent stocked populations, Dresden and Sewell, are highlighted (●), but IBD is highly significant ($p < 0.001$, $R^2 = 0.521$; solid line) for non-stocked locations (+). (b) Excluding Dresden and Sewell results in a highly significant IBD relationship ($p < 0.001$, $R^2 = 0.152$; dashed line) for the remaining stocked populations.

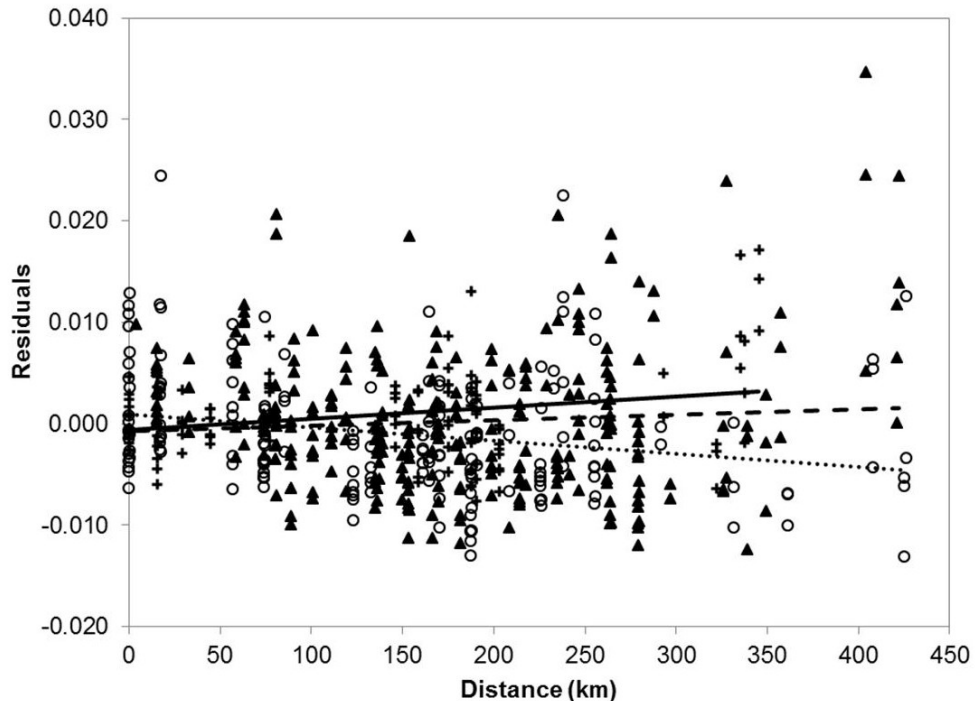


Figure 3.7: Plot of residuals for stocked (\circ ; dotted line), non-stocked ($+$; solid line) and stocked to non-stocked pairwise population comparisons (\blacktriangle ; small dashed line) from IBD relationship shown in Figure 3.5b. The slopes for both stocked and non-stocked residuals are significant ($p = 0.003$ and $p = 0.009$, respectively), while the stocked to non-stocked slope is non-significant ($p = 0.249$).

Table 3.7: Migration rates within and among sites estimated using BayesAss. The bolded values indicate migration rates that differ significantly from zero, based on 95% Confidence Interval (C.I.). The diagonal values are non-immigrants at each site and the starred (*) values indicate migration rates that are more uncertain than the other migration rates, based on the 95% C.I. Full names of sampling locations are given in Table 3.1.

<i>Migration To</i>	<i>Migration From</i>															
	Sew	Neq	Bru	Dre	Web	Ben	Loc	Dam	Sen	Wig	Orl	Sou	Vea	Leo	Den	Mil
Sew	0.761*	0.019	0.004	0.008	0.005	0.072*	0.002	0.002	0.002	0.002	0.020	0.009	0.089*	0.002	0.001	0.001
Neq	0.004	0.671	0.007	0.002	0.005	0.176*	0.001	0.004	0.008	0.003	0.017	0.029	0.069*	0.002	0.001	0.001
Bru	0.001	0.002	0.678	0.002	0.001	0.307	0.001	0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.000	0.000
Dre	0.049*	0.021	0.020	0.692	0.005	0.022	0.002	0.005	0.004	0.003	0.084	0.019	0.072	0.002	0.001	0.001
Web	0.001	0.002	0.003	0.001	0.672	0.292*	0.001	0.001	0.002	0.001	0.005	0.004	0.011	0.001	0.001	0.001
Ben	0.004	0.001	0.005	0.001	0.002	0.928	0.001	0.033	0.006	0.002	0.006	0.002	0.006	0.001	0.000	0.001
Loc	0.002	0.002	0.003	0.001	0.003	0.306	0.670	0.001	0.002	0.001	0.002	0.003	0.002	0.001	0.001	0.001
Dam	0.001	0.001	0.003	0.001	0.003	0.305	0.001	0.671	0.002	0.001	0.002	0.002	0.004	0.001	0.001	0.000
Sen	0.001	0.002	0.004	0.001	0.002	0.279*	0.001	0.002	0.674	0.001	0.002	0.006	0.022	0.001	0.001	0.001
Wig	0.003	0.005	0.009	0.002	0.004	0.179*	0.002	0.003	0.012	0.671	0.014	0.004	0.087*	0.002	0.001	0.001
Orl	0.004	0.004	0.013	0.003	0.007	0.093*	0.002	0.003	0.013	0.003	0.677	0.008	0.166*	0.002	0.001	0.001
Sou	0.003	0.003	0.022	0.002	0.003	0.192*	0.001	0.005	0.004	0.002	0.003	0.672	0.083*	0.003	0.001	0.001
Vea	0.005	0.002	0.013	0.002	0.003	0.224*	0.002	0.001	0.008	0.002	0.001	0.013	0.719*	0.003	0.001	0.001
Leo	0.003	0.005	0.021	0.002	0.002	0.214*	0.002	0.008	0.005	0.003	0.004	0.019	0.037	0.672	0.001	0.002
Den	0.004	0.003	0.170*	0.003	0.005	0.034	0.002	0.007	0.003	0.003	0.007	0.068*	0.010	0.004	0.673	0.003
Mil	0.006	0.008	0.085*	0.003	0.007	0.023	0.002	0.009	0.008	0.003	0.018	0.007	0.143*	0.003	0.002	0.673

number of locations on the Kennebec and Sebasticook Rivers (e.g., Benton), but migration estimates for sites on these rivers do not reflect this human-mediated migration (Table 3.7). Nor did migration among populations always reflect geographic proximity; in some cases populations further apart appeared to be linked by relatively high migration rates (e.g., Benton and Veazie, Leonard, Souadabscook; Table 3.7). Confidence intervals (95%) for many migration estimates (e.g., Veazie and Benton) were relatively high, suggesting limited ability of BayesAss to resolve patterns of recent migration in weakly differentiated Alewife populations.

3.3.5 Bottleneck Analysis

The allele frequency mode shift indicated 22 of the 40 total locations had fewer low frequency alleles than expected, thus providing evidence that these locations may have undergone a recent bottleneck (Figure 3.8). Within these locations, a deficit of rare alleles (frequency <0.1) and excess of more common alleles (frequency ≈ 0.6) was observed (Figure 3.8). Allele frequency distributions varied slightly among temporal replicates within locations, and as a consequence, identification of bottleneck events at times varied among the years within locations (e.g., Brunswick, Damariscotta, Dresden, Nequassett, Orland and Veazie), suggesting variable effects associated with stocking and location (i.e., location and timing during spawning migration; Figure 3.8). Additionally, Sign tests conducted using both IAM and SMM revealed a tendency towards heterozygote excess at all locations. Under the IAM, heterozygote excess was highly significant at all locations, whereas under the SMM, heterozygote excess was significant in only 12 of 40 cases (Table 3.8). Overall these tests suggest that most locations may have experienced a bottleneck within the recent past.

3.3.6 Effective Population Size

Estimates of effective population size ranged from 248 (Wight) to 6850 (Souadabscook), but most estimates had 95% confidence intervals that included infinity (Table 3.9), which suggests stocking and a mixture of age-classes may be influencing the resolution of the estimate. However, estimates of N_e for Damariscotta and Orland (274 and 251, respectively), had finite upper bounds of 792 and 3475, respectively (Table 3.9). Negative estimates of effective population sizes were obtained for Webber, Veazie,

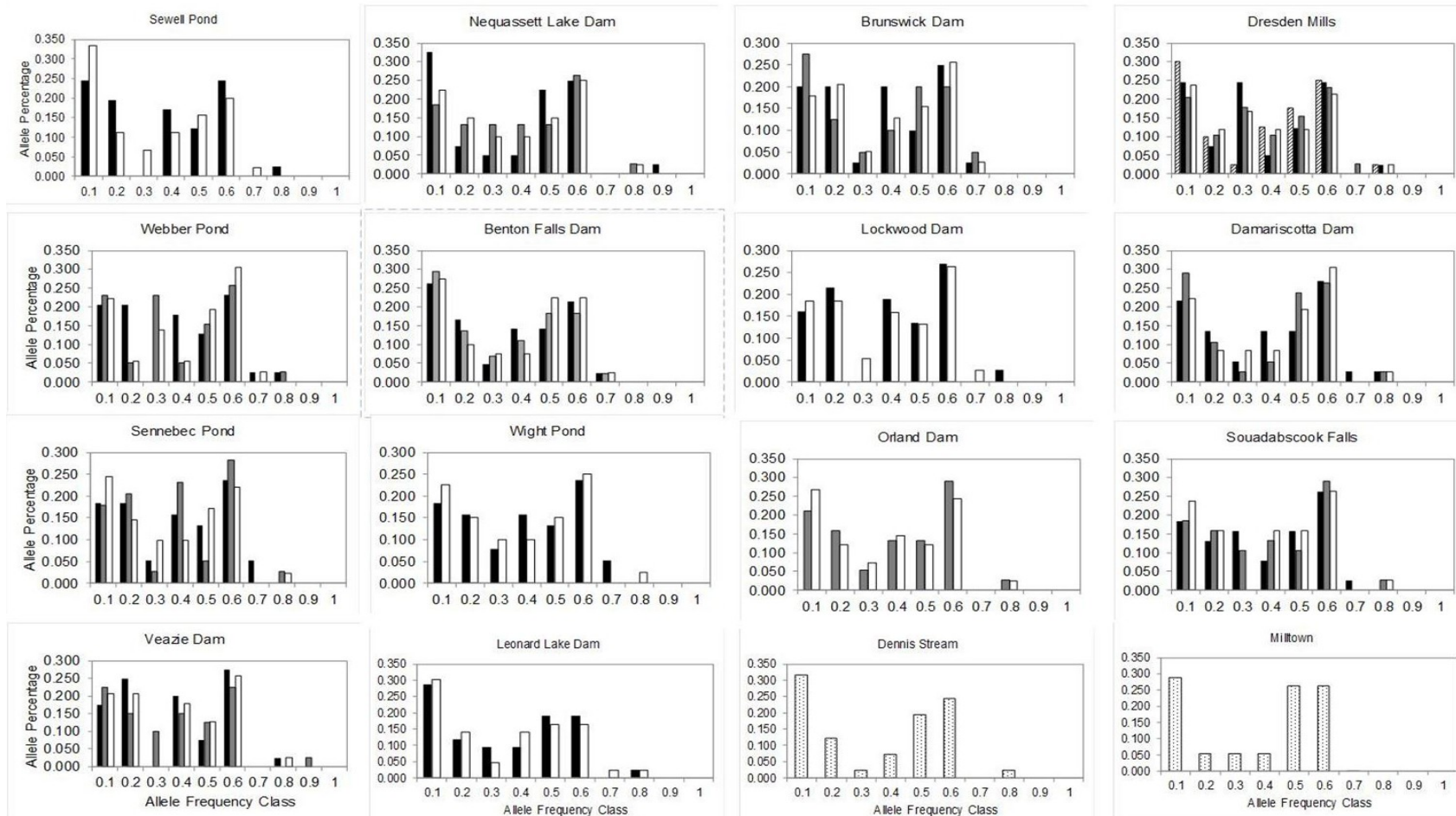


Figure 3.8: Allele frequency distribution histograms for each sampling location. Most populations have samples collected in 2009, 2010 and 2011 represented in the histograms by colours (black, gray and white, respectively). Dresden has samples collected from 2008 (striped pattern) and both Milltown and Dennis Stream were collected in 2005 (dotted pattern).

Table 3.8: Sign tests indicating the ratio of heterozygosity excess (He) to heterozygosity deficiency (Hd) and the probability (p) of deviation from expected equilibrium calculated for both the Infinite Allele Model (IAM) and Stepwise Mutation Model (SMM). Full names of sampling locations are given in Table 3.1.

Stocked	Location	Year	<i>Sign Test</i>			
			<i>IAM</i>		<i>SMM</i>	
			He/Hd	p	He/Hd	p
Y	Sew	2009	12/0	0.000*	7/5	0.612
		2011	12/0	0.000*	10/2	0.057
N	Neq	2009	12/0	0.000*	10/2	0.056
		2010	12/0	0.000*	9/3	0.191
		2011	12/0	0.000*	11/1	0.012*
Y	Bru	2009	12/0	0.000*	10/2	0.058
		2010	12/0	0.000*	10/2	0.007*
		2011	12/0	0.000*	11/1	0.015*
Y	Dre	2008	12/0	0.000*	9/3	0.190
		2009	12/0	0.000*	11/1	0.006*
		2010	12/0	0.000*	9/3	0.165
		2011	11/1	0.004*	9/3	0.167
Y	Ben	2009	12/0	0.000*	4/8	0.385
		2010	12/0	0.000*	10/2	0.067
		2011	12/0	0.000*	11/1	0.007*
Y	Web	2009	12/0	0.000*	10/2	0.069
		2010	12/0	0.000*	9/3	0.171
		2011	12/0	0.000*	11/1	0.010*
Y	Loc	2009	12/0	0.000*	11/1	0.015*
		2011	12/0	0.000*	11/1	0.016*
N	Dam	2009	12/0	0.000*	9/3	0.007*
		2010	12/0	0.000*	12/0	0.001*
		2011	12/0	0.000*	9/3	0.068
Y	Sen	2009	12/0	0.000*	10/2	0.057
		2010	12/0	0.000*	4/8	0.394
		2011	12/0	0.000*	4/8	0.393
Y	Wig	2009	12/0	0.000*	10/2	0.068
		2011	12/0	0.000*	10/2	0.067
N	Orl	2009	12/0	0.000*	9/3	0.194
		2011	12/0	0.000*	11/1	0.015*
N	Sou	2009	12/0	0.000*	10/2	0.056
		2010	12/0	0.000*	10/2	0.056
		2011	12/0	0.000*	10/2	0.068
N	Vea	2009	12/0	0.000*	11/1	0.014*
		2010	12/0	0.000*	4/8	0.354
		2011	12/0	0.000*	9/3	0.192
Y	Leo	2009	12/0	0.000*	9/3	0.192
		2011	12/0	0.000*	10/2	0.070
N	Den	2005	12/0	0.000*	10/2	0.069
Y	Mil	2005	11/1	0.004*	9/3	0.193

*Significant deviation ($p < 0.05$) from equilibrium/nonbottleneck expectation (Luikart and Cornuet 1998)

Table 3.9: Effective population size for all Maine locations calculated using the program LDNe. Full names of sampling locations are given in Table 3.1.

Location	Ne	95% C.I. (Jackknife on Loci)	
		Lower	Upper
Ben	2340.6	309.1	Infinite
Bru	2939.6	259.2	Infinite
Dam	273.6	123.9	2528.3
Den	-166.3	168.9	Infinite
Dre	582.5	160	Infinite
Leo	1449.9	182.4	Infinite
Loc	-364.3	271	Infinite
Mil	-207.9	124.4	Infinite
Neq	1153.8	204.2	Infinite
Orl	251.3	105.2	3475.4
Sen	947.8	174.7	Infinite
Sew	-160	212.2	Infinite
Sou	6849.5	326.4	Infinite
Vea	-793.3	505.5	Infinite
Web	-1807.4	293.4	Infinite
Wig	248.4	106.3	Infinite

Milltown, Lockwood, Sewell, and Dennis Stream, which suggests that not enough information was available to form credible estimates.

3.4 Discussion

Overexploitation, habitat destruction and fragmentation have reduced river herring abundance to historically low levels along the east coast of North America (DFO 2001, ASFMC 2009, Davis and Schultz 2009). In response, the U.S. developed and implemented a stock translocation program that essentially moved stocks within (in-basin) and between (out-of-basin) watersheds for the re-establishment of extirpated populations in an attempt to increase abundance and recover spawning runs (ASFMC 1985). Specifically in Maine, the MeDMR, within the last century, has concentrated on transferring gravid adults from two of the three largest watersheds (Kennebec and Androscoggin) to not only support both within and out-of-basin productive spawning runs, but also those spawning runs that are practical and most cost-effective to manage (Bentzen *et al.* 2009, Labbe 2012 and references therein). Unfortunately, complete stocking records only exist from 1983 to present with sporadic paper records dating earlier to the 1940s (Labbe 2012).

This restoration strategy overlooks the possible negative implications it can have on the genetic structure of populations. Repetitive stock transfers among divergent populations can reduce the level of genetic differentiation among populations (*Oncorhynchus kisutch*; Eldridge and Naish 2007) and jeopardize the persistence of genetically distinct populations by increasing the gene flow between populations (Evans and Willox 1991, Hansen *et al.* 2000, Hasselman and Limburg 2012). The effects of past stocking events on population structure and spatial patterns of genetic diversity in river herring are largely unknown. My research used temporally replicated samples spanning at least a three year period to examine the pattern of genetic diversity and differentiation in Maine Alewife populations that have been subject to stocking for at least the past 30 years, and at a small spatial scale (< 450 km).

3.4.1 Structure and Genetic Stability of Populations

I found evidence of weak population structure for Maine Alewife populations, in line with results obtained from other stocked U.S. populations of alosine fishes (Alewife:

Palkovacs *et al.* 2008; American Shad, *Alosa sapidissima*: Hasselman *et al.* 2013). However, the dendrogram of chord distances as well as the PCoA, IBD, and pairwise genic analyses all indicated that two populations, Dresden and Sewell, are unusually divergent and have apparently resisted genetic homogenization from translocation efforts. Both of these populations spawn earlier than other Alewife populations in the region (T. Willis, personal comm.), suggesting that spawning time differences may be the reason that stocking has failed to make these two populations genetically similar to the rest of the Maine populations. Similar observations have been made in other anadromous fish species (e.g., *Oncorhynchus kisutch*, *Salmo salar* L., and *Salmo trutta*) where native populations have been shown to resist genetic swamping by large releases of non-native fish and heavy exploitation (Nielsen *et al.* 2001, Hansen 2002, Eldridge *et al.* 2009).

The dendrogram of chord distances along with the PCoA indicated no evident tendency of samples to group more closely by watershed or by location when there were multiple collection years. These results suggest some instability of allele frequencies within locations among years. On the other hand, the hierarchical AMOVA analysis revealed only a very small proportion of genetic variation (0.27%) occurred among years within locations. Overall, these results suggest that the limited genetic instability observed could be attributed to drift associated with small population sizes, sampling effects, and/or consequences of stocking. Repetitive stocking can slowly change the genetic composition of populations over time as introgression between native and non-native individuals occurs (Laikre *et al.* 2010). In populations of a closely related species, American Shad that were not subject to stocking, Hasselman *et al.* (2010) found genetic stability among years.

3.4.2 Isolation by Distance

Dresden and Sewell are unusually divergent populations, and when these two populations were retained in the IBD analysis the results revealed a non-significant association between geographic and genetic distance among Maine populations. However, exclusion of these two outlier populations resulted in a significant IBD relationship for all remaining populations. This result differs from previous studies that failed to find evidence of IBD among U.S. Alewife populations (Palkovacs *et al.* 2008,

Bentzen *et al.* 2009, Labbe 2012). The difference in results may be due to increased resolution and power associated with the relatively large sample sizes, number of loci, and number of temporal replicates in the present study.

The slope of the IBD relationship provides an indication of the amount of gene flow occurring among populations (Bradbury and Bentzen 2007), which may be attributed to natural and/or human-mediated migration. Very little gene flow among populations would result in a steep slope with a substantial increase in genetic distance over a short geographic distance, as seen for the Alewife populations in the Bay of Fundy where the slope was 0.00050 (Figure 2.8; see Chapter 2). The slope of the Maine IBD relationship (0.00003) suggests a level of gene flow similar to the Alewife populations in the Gulf of St. Lawrence (slope = 0.00004), and less gene flow than among Alewife populations located on the Atlantic coast of Nova Scotia, where IBD was non-significant (slope \approx 0.00000; Figure 2.8; see Chapter 2). The apparent difference in levels of gene flow between Maine and Atlantic coast of Nova Scotia might simply reflect differences in resolution and power associated with the large number of populations and temporal replicates examined in Maine. Significant IBD relationships in two of three Canadian regions (Bay of Fundy and Gulf of St. Lawrence), where stocking has not occurred, indicates that IBD may occur as a consequence of natural dispersal among Alewife populations. Therefore, evidence of IBD in Maine indicates that stocking has not eliminated evidence of natural dispersal among populations.

In this study, the strongest association between geographic and genetic distance was found among non-stocked populations, suggesting that these populations are exchanging migrants at a rate inversely proportional to their geographic distance (Wright 1943).

As noted above, Dresden and Sewell were two stocked populations that were relatively divergent, even from nearby populations. These results are not in accord with the theory that when populations are subjected to stock transfers they tend to have lower genetic differentiation than would be predicted based on geographic distance alone (Hasselman and Limburg 2012). Although IBD was significant when non-stocked and stocked Maine populations (excluding Sewell and Dresden) were considered together, analysis of residuals suggested a dampening effect of stocking on the IBD relationship.

Regression of residuals from the IBD relationship on geographic distance revealed a negative slope for pairwise comparisons in which both populations were stocked, and a positive slope for pairwise comparisons involving only non-stocked populations. Thus, genetic differentiation of stocked populations tended to be less than expected on the basis of geographic distance alone, and this effect became more evident with increasing geographic separation. These results suggest that human-mediated migration has altered the genetic relationships of Maine Alewife populations, and supports the notion that stocking increases gene flow among populations relative to what would be expected based on geographic distance alone (Hasselman and Limburg 2012, Laikre *et al.* 2010 and references therein). In this regard, my results are consistent with those obtained in other studies of stocked Alewife populations (e.g., Labbe 2012) or other alosine populations influenced by stock transfers (e.g., American Shad, Hasselman *et al.* 2013).

3.4.3 Bottleneck and Effective Population Size

Evidence of recent bottlenecks was found in a majority of the populations, but was not always consistent among temporal replicates (years) within populations, suggesting the possibility of variable effects associated with stocking or sampling (i.e., location and timing during spawning migration) on the results of the bottleneck analysis. Although evidence for population bottlenecks was variable, these results differ from those obtained in a recent study conducted on Maine Alewife populations (Labbe 2012), where no evidence of bottlenecks was found. The discrepancy in results may be a result of differences in power associated with sample sizes, number of loci examined, and number of temporal replicates.

A majority of the populations had ‘infinite’ as the upper bound of the 95% confidence interval and/or a negative N_e estimate, which indicated a high uncertainty regarding their effective population size estimates. However, Damariscotta and Orland had relatively low estimates with finite upper confidence limits. As in other analyses, stocking and sampling effects may have interfered with the resolution of bottleneck results. Further investigation should incorporate historical samples to clarify the bottleneck and effective population size estimates and to determine whether stocking is affecting the allele frequency distributions.

3.4.4 Concluding Remarks

The restoration strategy implemented by Maine for at least the last 30 years, has included large-scale transfers of gravid adults between watersheds. Genetic data collected from 16 populations with temporal replicates spanning at least a three year period revealed that stocking has influenced the genetic structure of Maine populations. Analysis of residuals from IBD patterns showed a general tendency of populations subjected to stocking to be less genetically differentiated than non-stocked populations at comparable levels of geographic separation. Nonetheless, the fact that evidence of IBD was detected for the majority of Maine Alewife populations examined indicates that although stocking has influenced genetic structure, it has not entirely obliterated natural patterns of genetic differentiation. Moreover, two unusually divergent populations, Dresden and Sewell, have apparently resisted genetic homogenization, despite having been subjected to stocking from other populations, perhaps because their early spawning time period has provided a barrier to introgression. The fact that natural homing behaviour occurs in Alewife, and that some populations are both phenotypically (i.e., spawning time) and genetically divergent suggests that stocking could risk disrupting local adaptations. Therefore, the long-term viability of Alewife populations may best be served by a shift in the management focus from stocking to habitat restoration efforts, such as dam removal.

Chapter 4: Conclusion

My research on Alewife and Blueback Herring, conducted on varying spatial scales using neutral microsatellite markers, revealed insights regarding the patterns of genetic differentiation and structure within the northern portion of their ranges, as well as how these patterns may be influenced by different management strategies. Within Canada, where Alewife populations are not subject to stocking, the population structure and spatial pattern of genetic differentiation revealed three somewhat admixed genetic clusters that correspond to particular regions in Atlantic Canada: Bay of Fundy, Atlantic coast of Nova Scotia and Gulf of St. Lawrence. Patterns of admixture within these genetic clusters along with the IBD patterns within the three regions suggest that patterns of neutral genetic differentiation in Alewife may reflect both historical biogeographical factors and the influence of contemporary dispersal patterns. Within the U.S., weak population structure and IBD patterns suggest stocking has influenced the genetic differentiation among populations, but yet has not entirely obliterated natural patterns of genetic differentiation. Moreover, two divergent populations, Dresden and Sewell, appeared to have resisted homogenization, despite being subject to stocking. Recent population bottlenecks were identified in both U.S. and Canadian populations. Among Canadian populations bottlenecks seem associated with rivers that have either a dam 0.5 km from head of tide or a major river herring fishery, suggesting human-induced bottlenecks. Strong evidence that Alewife home to natal rivers, along with evidence that some populations are both phenotypically and genetically divergent on relatively small spatial scales suggests that stocking could disrupt local adaptations, thus the long-term viability of Alewife populations may best be served by a shift in management focus from stocking to habitat restoration, such as dam removal.

My results suggest Blueback Herring populations are genetically structured in a manner similar to that seen in Alewives. Significant genetic differentiation was observed between all populations and was similar in magnitude to the differentiation observed between Alewife populations at comparable spatial scales. Genetic differentiation between Alewife and Blueback Herring was strong, and commensurate with species-level differences, but nonetheless hybrids were observed. Hybridization rates should be

investigated further to better evaluate the prevalence and potential conservation significance of this phenomenon. In addition, more Blueback Herring populations should be studied to determine whether stock transfers have weakened their population structure within the U.S.

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Appendix 1: Genetic diversity statistics per locus across populations include number of alleles (A) and range in allele size (in parentheses). Statistics per locus and location include the number of individuals (N), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity, inbreeding coefficient (F_{IS}), probability of departures from HWE (P -val) and allelic richness (A_e). Bolded values indicate significant departures from HWE after sequential Bonferroni correction and ML indicates loci that are monomorphic in specific populations. Full names are given in Table 2.1.

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A	
Ale		<i>A</i>	6	5	12	8	13	8	16	
		<i>Range</i>	(114-134)	(148-164)	(193-237)	(249-281)	(188-260)	(126-158)	(68-140)	
	Ama		<i>N</i>	50	50	50	50	50	50	50
			N_a	5	5	8	3	8	4	5
			H_o	0.349	0.221	0.259	0.313	0.616	0.679	0.720
			H_e	0.374	0.004	0.072	1.000	0.042	0.458	0.506
			F_{IS}	-0.088	0.277	0.076	-0.088	0.027	0.029	0.112
			<i>P-val</i>	0.341	0.005	0.048	1.000	0.029	0.454	0.502
	Arg		A_e	4.770	4.513	7.235	3.000	7.513	3.998	5.000
			<i>N</i>	50	46	50	50	50	50	50
			N_a	5	3	8	3	5	5	8
			H_o	0.500	0.152	0.660	0.320	0.520	0.660	0.580
			H_e	0.486	0.232	0.741	0.277	0.496	0.748	0.691
			F_{IS}	-0.029	0.347	0.110	-0.156	-0.050	0.119	0.162
	Mar		<i>P-val</i>	0.223	0.018	0.604	0.689	0.352	0.088	0.357
			A_e	4.780	2.848	7.780	2.953	4.560	4.953	7.340
			<i>N</i>	49	44	49	49	49	49	49
			N_a	5	3	10	3	7	4	7
			H_o	0.510	0.091	0.490	0.469	0.571	0.653	0.531
			H_e	0.521	0.170	0.453	0.440	0.604	0.688	0.643
Ben		F_{IS}	0.021	0.469	-0.081	-0.067	0.054	0.051	0.176	
		<i>P-val</i>	0.207	0.001	0.501	0.496	0.375	0.552	0.059	
		A_e	4.993	2.988	9.299	3.000	6.590	4.000	6.592	
		<i>N</i>	188	183	187	188	187	188	188	
		N_a	5	3	11	5	7	6	10	
		H_o	0.548	0.246	0.578	0.495	0.273	0.718	0.686	
	H_e	0.533	0.351	0.569	0.540	0.269	0.755	0.654		
	F_{IS}	-0.029	0.300	-0.016	0.084	-0.014	0.050	-0.049		
	<i>P-val</i>	0.396	0.000	0.995	0.626	0.908	0.268	0.270		
	A_e	4.203	2.381	7.804	3.810	4.881	5.190	6.788		

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039
Ale	<i>A</i>		7	14	8	5	5	7	10
	<i>Range</i>		(150-178)	(203-243)	(249-277)	(79-103)	(190-210)	(231-247)	(265-305)
Ama	<i>N</i>		50	50	50	50	50	50	48
	<i>N_a</i>		4	8	4	2	2	3	7
	<i>H_o</i>		0.454	0.713	0.474	0.132	0.489	0.305	0.783
	<i>H_e</i>		0.343	0.218	0.258	1.000	0.560	1.000	0.828
	<i>F_{IS}</i>		0.121	0.103	0.096	-0.065	-0.106	-0.051	-0.011
	<i>P-val</i>		0.343	0.196	0.263	1.000	0.561	1.000	0.825
	<i>A_e</i>		3.780	7.677	3.796	2.000	2.000	2.953	6.812
Arg	<i>N</i>		50	49	50	50	50	50	50
	<i>N_a</i>		6	10	4	2	2	5	6
	<i>H_o</i>		0.420	0.694	0.540	0.080	0.400	0.600	0.420
	<i>H_e</i>		0.600	0.760	0.515	0.078	0.492	0.661	0.526
	<i>F_{IS}</i>		0.302	0.087	-0.049	-0.032	0.189	0.093	0.204
	<i>P-val</i>		0.027	0.006	0.967	1.000	0.247	0.383	0.036
	<i>A_e</i>		5.907	9.502	3.780	1.998	2.000	4.733	5.779
Mar	<i>N</i>		48	49	48	49	49	49	49
	<i>N_a</i>		5	9	4	2	2	4	6
	<i>H_o</i>		0.667	0.776	0.542	0.367	0.449	0.551	0.735
	<i>H_e</i>		0.690	0.769	0.615	0.303	0.444	0.554	0.756
	<i>F_{IS}</i>		0.034	-0.008	0.120	-0.215	-0.011	0.005	0.028
	<i>P-val</i>		0.327	0.165	0.017	0.328	1.000	0.100	0.821
	<i>A_e</i>		4.999	8.877	3.966	2.000	2.000	4.584	5.993
Ben	<i>N</i>		187	187	186	188	188	188	187
	<i>N_a</i>		6	7	4	2	3	3	7
	<i>H_o</i>		0.711	0.775	0.414	0.138	0.569	0.548	0.305
	<i>H_e</i>		0.730	0.729	0.492	0.147	0.511	0.505	0.343
	<i>F_{IS}</i>		0.026	-0.065	0.158	0.061	-0.114	-0.084	0.111
	<i>P-val</i>		0.011	0.972	0.000	0.326	0.039	0.172	0.162
	<i>A_e</i>		5.372	6.300	3.689	1.999	2.607	2.503	5.358

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Ale	Bru	N	168	165	169	166	167	169	168
		N_a	5	3	10	4	4	6	9
		H_o	0.512	0.255	0.580	0.446	0.204	0.651	0.565
		H_e	0.515	0.382	0.552	0.531	0.198	0.738	0.604
		F_{IS}	0.005	0.335	-0.050	0.161	-0.030	0.118	0.064
		$P\text{-val}$	0.569	0.000	0.994	0.177	0.092	0.107	0.533
		A_e	4.879	2.556	7.397	3.605	3.350	5.074	5.883
	Chr	N	49	48	49	49	49	49	49
		N_a	4	4	7	3	6	5	6
		H_o	0.265	0.208	0.306	0.531	0.510	0.612	0.510
		H_e	0.261	0.315	0.312	0.485	0.465	0.669	0.589
		F_{IS}	-0.017	0.341	0.020	-0.095	-0.098	0.085	0.135
		$P\text{-val}$	0.661	0.006	0.520	0.060	0.962	0.145	0.440
		A_e	3.997	3.625	6.380	3.000	5.755	4.796	5.592
	Coc	N	48	47	47	47	39	48	48
		N_a	5	2	7	3	5	5	9
		H_o	0.500	0.277	0.574	0.298	0.231	0.813	0.583
		H_e	0.531	0.326	0.504	0.556	0.339	0.748	0.627
		F_{IS}	0.059	0.153	-0.141	0.467	0.322	-0.088	0.070
		$P\text{-val}$	0.558	0.362	0.598	0.000	0.001	0.185	0.371
		A_e	4.812	2.000	6.801	3.000	5.000	4.812	8.431
	Dam	N	166	163	160	153	165	165	166
		N_a	5	2	11	4	5	5	8
		H_o	0.458	0.264	0.569	0.477	0.164	0.667	0.639
		H_e	0.461	0.342	0.523	0.509	0.154	0.725	0.629
		F_{IS}	0.006	0.229	-0.089	0.063	-0.066	0.080	-0.015
		$P\text{-val}$	0.260	0.005	0.920	0.584	1.000	0.003	0.289
		A_e	4.226	2.000	7.549	2.843	3.312	4.887	6.051
Den	N	47	43	47	47	47	47	47	
	N_a	5	2	8	2	6	4	7	
	H_o	0.447	0.163	0.574	0.447	0.277	0.723	0.596	
	H_e	0.517	0.291	0.567	0.431	0.303	0.648	0.649	
	F_{IS}	0.137	0.444	-0.013	-0.037	0.089	-0.118	0.082	
	$P\text{-val}$	0.213	0.012	0.880	1.000	0.257	0.703	0.429	
	A_e	4.945	2.000	7.489	2.000	5.489	4.000	6.651	

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039
Ale	Bru	N	169	168	167	169	169	168	168
		N_a	6	7	4	2	2	3	7
		H_o	0.722	0.738	0.413	0.154	0.562	0.560	0.333
		H_e	0.729	0.706	0.537	0.152	0.499	0.502	0.349
		F_{IS}	0.009	-0.046	0.231	-0.010	-0.127	-0.115	0.044
		$P\text{-val}$	0.592	0.884	0.000	0.607	0.122	0.139	0.074
		A_e	5.545	6.283	3.949	2.000	2.000	2.232	5.346
	Chr	N	49	48	49	49	49	49	49
		N_a	5	5	3	2	2	3	6
		H_o	0.673	0.771	0.306	0.163	0.490	0.510	0.857
		H_e	0.550	0.696	0.293	0.151	0.470	0.469	0.763
		F_{IS}	-0.227	-0.108	-0.045	-0.079	-0.044	-0.090	-0.124
		$P\text{-val}$	0.234	0.527	1.000	1.000	1.000	0.761	0.209
		A_e	4.584	4.966	2.796	2.000	2.000	3.000	5.960
	Coc	N	48	48	46	48	48	48	47
		N_a	6	8	4	2	2	2	5
		H_o	0.521	0.813	0.354	0.167	0.542	0.542	0.213
		H_e	0.715	0.720	0.540	0.154	0.474	0.502	0.236
		F_{IS}	0.274	-0.130	0.346	-0.081	-0.145	-0.081	0.099
		$P\text{-val}$	0.021	0.778	0.010	1.000	0.367	0.771	0.190
		A_e	5.812	7.619	3.966	2.000	2.000	2.000	4.798
Dam	N	164	166	162	164	166	166	166	
	N_a	5	7	5	2	3	3	9	
	H_o	0.720	0.681	0.426	0.152	0.596	0.554	0.325	
	H_e	0.713	0.705	0.519	0.141	0.553	0.502	0.339	
	F_{IS}	-0.010	0.035	0.180	-0.080	-0.078	-0.104	0.042	
	$P\text{-val}$	0.871	0.874	0.000	0.602	0.000	0.179	0.548	
	A_e	4.980	6.079	3.988	1.999	2.995	2.235	5.613	
Den	N	47	47	47	47	47	47	47	
	N_a	5	7	4	2	2	3	7	
	H_o	0.596	0.660	0.489	0.128	0.511	0.574	0.340	
	H_e	0.635	0.724	0.557	0.121	0.494	0.474	0.396	
	F_{IS}	0.063	0.089	0.123	-0.058	-0.034	-0.215	0.142	
	$P\text{-val}$	0.043	0.081	0.195	1.000	1.000	0.239	0.214	
	A_e	4.802	6.829	3.830	2.000	2.000	2.996	6.481	

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Ale	Dre	<i>N</i>	177	173	171	175	176	177	176
		<i>N_a</i>	5	4	10	6	8	6	13
		<i>H_o</i>	0.429	0.173	0.673	0.423	0.443	0.763	0.619
		<i>H_e</i>	0.464	0.251	0.688	0.478	0.438	0.725	0.621
		<i>F_{IS}</i>	0.076	0.311	0.022	0.116	-0.012	-0.052	0.003
		<i>P-val</i>	0.030	0.000	0.126	0.254	0.411	0.774	0.534
		<i>A_e</i>	4.804	3.510	8.060	3.663	5.534	5.087	8.870
	Fid	<i>N</i>	50	49	50	50	50	50	49
		<i>N_a</i>	5	4	5	3	4	4	7
		<i>H_o</i>	0.440	0.122	0.120	0.340	0.540	0.600	0.714
		<i>H_e</i>	0.491	0.258	0.117	0.357	0.541	0.715	0.679
		<i>F_{IS}</i>	0.105	0.528	-0.030	0.049	0.002	0.162	-0.053
		<i>P-val</i>	0.297	0.000	1.000	0.165	0.605	0.341	0.843
		<i>A_e</i>	4.770	3.953	4.467	3.000	4.000	4.000	6.592
	Gas	<i>N</i>	56	50	56	56	56	56	56
		<i>N_a</i>	4	2	8	2	5	4	5
		<i>H_o</i>	0.321	0.040	0.661	0.554	0.304	0.518	0.536
		<i>H_e</i>	0.302	0.114	0.620	0.485	0.299	0.679	0.596
		<i>F_{IS}</i>	-0.066	0.651	-0.066	-0.143	-0.016	0.239	0.102
		<i>P-val</i>	0.841	0.005	0.912	0.403	1.000	0.014	0.092
		<i>A_e</i>	3.973	2.000	7.399	2.000	4.729	4.000	4.992
	Hil	<i>N</i>	57	53	57	57	57	57	57
		<i>N_a</i>	5	4	8	3	6	6	5
		<i>H_o</i>	0.456	0.189	0.526	0.368	0.509	0.684	0.649
<i>H_e</i>		0.438	0.394	0.474	0.435	0.486	0.668	0.691	
<i>F_{IS}</i>		-0.042	0.523	-0.110	0.154	-0.046	-0.024	0.061	
<i>P-val</i>		0.601	0.000	0.885	0.077	0.509	0.764	0.196	
<i>A_e</i>		4.684	3.915	7.863	3.000	5.586	5.368	4.970	
Kia	<i>N</i>	50	49	50	50	50	50	50	
	<i>N_a</i>	4	3	8	3	6	4	8	
	<i>H_o</i>	0.340	0.143	0.580	0.420	0.560	0.680	0.740	
	<i>H_e</i>	0.457	0.171	0.605	0.364	0.570	0.698	0.755	
	<i>F_{IS}</i>	0.257	0.168	0.042	-0.156	0.018	0.025	0.020	
	<i>P-val</i>	0.032	0.333	0.201	0.519	0.518	0.784	0.510	
	<i>A_e</i>	4.000	2.960	7.776	3.000	5.770	4.000	7.731	

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039
Ale	Dre	<i>N</i>	175	172	176	176	177	177	168
		<i>N_a</i>	6	10	5	3	3	4	9
		<i>H_o</i>	0.737	0.773	0.580	0.131	0.548	0.514	0.554
		<i>H_e</i>	0.739	0.735	0.608	0.130	0.488	0.510	0.650
		<i>F_{IS}</i>	0.003	-0.053	0.048	-0.009	-0.124	-0.007	0.149
		<i>P-val</i>	0.992	0.414	0.017	0.388	0.219	0.868	0.028
		<i>A_e</i>	5.683	8.994	4.224	2.852	2.714	3.355	6.629
	Fid	<i>N</i>	50	50	50	50	50	50	47
		<i>N_a</i>	4	7	4	2	2	3	6
		<i>H_o</i>	0.540	0.640	0.440	0.120	0.420	0.440	0.851
		<i>H_e</i>	0.521	0.680	0.482	0.114	0.416	0.444	0.771
		<i>F_{IS}</i>	-0.037	0.060	0.088	-0.054	-0.010	0.009	-0.106
		<i>P-val</i>	0.693	0.224	0.051	1.000	1.000	1.000	0.458
		<i>A_e</i>	3.780	6.513	3.953	2.000	2.000	3.000	5.996
	Gas	<i>N</i>	56	55	56	56	56	56	56
		<i>N_a</i>	5	7	4	2	2	3	5
		<i>H_o</i>	0.464	0.818	0.429	0.161	0.286	0.125	0.714
		<i>H_e</i>	0.623	0.748	0.478	0.149	0.296	0.150	0.717
		<i>F_{IS}</i>	0.257	-0.094	0.104	-0.078	0.035	0.170	0.004
		<i>P-val</i>	0.006	0.350	0.139	1.000	1.000	0.294	0.396
		<i>A_e</i>	4.670	6.603	3.910	2.000	2.000	2.696	5.000
	Hil	<i>N</i>	57	57	56	57	57	57	53
		<i>N_a</i>	3	9	3	2	2	3	7
		<i>H_o</i>	0.474	0.772	0.625	0.175	0.491	0.596	0.660
		<i>H_e</i>	0.467	0.790	0.512	0.161	0.391	0.542	0.600
		<i>F_{IS}</i>	-0.014	0.023	-0.222	-0.087	-0.258	-0.103	-0.101
		<i>P-val</i>	0.526	0.804	0.224	1.000	0.082	0.697	0.534
<i>A_e</i>		2.997	8.357	3.000	2.000	2.000	3.000	6.467	
Kia	<i>N</i>	49	50	50	50	50	50	50	
	<i>N_a</i>	6	9	4	2	2	3	7	
	<i>H_o</i>	0.592	0.700	0.540	0.020	0.400	0.580	0.540	
	<i>H_e</i>	0.560	0.758	0.586	0.059	0.492	0.597	0.599	
	<i>F_{IS}</i>	-0.057	0.077	0.079	0.662	0.189	0.029	0.099	
	<i>P-val</i>	0.229	0.505	0.541	0.030	0.245	0.552	0.244	
	<i>A_e</i>	5.991	8.722	3.998	1.990	2.000	3.000	6.340	

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Ale	Lah	N	52	48	52	52	52	52	52
		N_a	5	3	8	3	5	6	8
		H_o	0.596	0.125	0.731	0.442	0.192	0.692	0.654
		H_e	0.675	0.257	0.718	0.428	0.215	0.796	0.675
		F_{IS}	0.118	0.516	-0.018	-0.034	0.106	0.132	0.031
		$P\text{-val}$	0.219	0.000	0.418	1.000	0.398	0.099	0.279
		A_e	5.000	2.966	7.686	2.750	4.675	5.986	7.439
	Lam	N	16	16	16	16	16	16	16
		N_a	5	2	3	5	2	4	5
		H_o	0.500	0.125	0.188	0.500	0.125	0.625	0.625
		H_e	0.575	0.444	0.331	0.581	0.121	0.732	0.645
		F_{IS}	0.134	0.725	0.441	0.143	-0.035	0.150	0.032
		$P\text{-val}$	0.133	0.007	0.756	0.403	1.000	0.693	0.626
		A_e	NA	NA	NA	NA	NA	NA	NA
	Leo	N	107	105	107	107	107	107	106
		N_a	5	3	10	4	6	6	10
		H_o	0.570	0.219	0.654	0.664	0.159	0.757	0.613
		H_e	0.630	0.376	0.653	0.507	0.168	0.719	0.665
		F_{IS}	0.096	0.419	-0.002	-0.312	0.055	-0.054	0.078
		$P\text{-val}$	0.010	0.000	0.452	0.002	0.580	0.945	0.085
		A_e	4.596	2.754	8.876	3.110	4.531	5.110	7.734
Loc	N	98	98	98	97	95	98	98	
	N_a	5	3	9	4	5	5	8	
	H_o	0.459	0.306	0.612	0.515	0.263	0.653	0.571	
	H_e	0.547	0.397	0.595	0.547	0.275	0.723	0.584	
	F_{IS}	0.161	0.229	-0.029	0.058	0.045	0.097	0.021	
	$P\text{-val}$	0.117	0.056	0.913	0.192	0.494	0.751	0.387	
	A_e	4.397	2.871	7.574	3.745	4.531	4.871	6.209	
Mac	N	48	47	48	48	48	48	48	
	N_a	5	2	8	3	6	5	8	
	H_o	0.688	0.064	0.500	0.438	0.396	0.583	0.750	
	H_e	0.604	0.062	0.443	0.434	0.453	0.702	0.682	
	F_{IS}	-0.140	0.022	-0.130	-0.008	0.127	0.171	-0.101	
	$P\text{-val}$	0.135	1.000	0.904	0.055	0.163	0.337	0.500	
	A_e	4.999	1.996	7.558	3.000	5.960	4.812	7.745	

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039	
Ale	Lah	N	52	52	52	52	52	52	52	
		N_a	5	8	4	2	2	3	6	
		H_o	0.558	0.673	0.538	0.038	0.462	0.519	0.481	
		H_e	0.670	0.730	0.558	0.038	0.457	0.593	0.601	
		F_{IS}	0.169	0.079	0.036	-0.010	-0.010	0.125	0.201	
		$P\text{-val}$	0.246	0.009	0.776	1.000	1.000	0.001	0.034	
		A_e	4.939	7.189	4.000	1.939	2.000	3.000	5.939	
	Lam	N	16	16	16	16	16	16	16	15
		N_a	5	5	3	1	3	2	3	
		H_o	0.563	0.500	0.500	ML	0.375	0.375	0.133	
		H_e	0.665	0.550	0.526	ML	0.506	0.484	0.131	
		F_{IS}	0.159	0.094	0.051	ML	0.265	0.231	-0.018	
		$P\text{-val}$	0.105	0.117	0.492	ML	0.549	0.591	1.000	
		A_e	NA	NA	NA	NA	NA	NA	NA	
	Leo	N	107	107	106	107	107	107	107	105
		N_a	6	6	5	2	3	3	7	
		H_o	0.626	0.626	0.425	0.206	0.477	0.542	0.543	
		H_e	0.664	0.655	0.563	0.185	0.527	0.506	0.523	
		F_{IS}	0.057	0.044	0.247	-0.110	0.095	-0.073	-0.038	
		$P\text{-val}$	0.143	0.294	0.001	0.597	0.079	0.810	0.765	
		A_e	5.557	5.596	3.969	2.000	2.937	2.745	6.686	
Loc	N	98	98	97	98	98	98	98	97	
	N_a	6	9	6	2	3	4	6		
	H_o	0.714	0.694	0.474	0.122	0.480	0.439	0.299		
	H_e	0.734	0.750	0.501	0.133	0.518	0.472	0.284		
	F_{IS}	0.027	0.075	0.054	0.082	0.074	0.070	-0.052		
	$P\text{-val}$	0.609	0.496	0.847	0.393	0.206	0.238	1.000		
	A_e	5.395	7.451	4.819	1.999	2.784	2.796	4.664		
Mac	N	48	48	48	48	48	48	48	48	
	N_a	5	8	4	2	2	3	6		
	H_o	0.604	0.854	0.563	0.063	0.292	0.458	0.625		
	H_e	0.651	0.816	0.585	0.061	0.357	0.509	0.623		
	F_{IS}	0.073	-0.047	0.039	-0.022	0.185	0.101	-0.004		
	$P\text{-val}$	0.528	0.888	0.743	1.000	0.228	0.458	0.110		
	A_e	4.812	7.773	3.812	1.994	2.000	2.813	5.933		

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Ale	Med	<i>N</i>	50	47	50	50	50	50	50
		<i>N_a</i>	5	2	10	3	6	6	8
		<i>H_o</i>	0.440	0.021	0.600	0.480	0.500	0.740	0.660
		<i>H_e</i>	0.573	0.102	0.600	0.469	0.440	0.725	0.687
		<i>F_{IS}</i>	0.234	0.793	0.000	-0.023	-0.139	-0.021	0.040
		<i>P-val</i>	0.005	0.002	0.607	0.745	0.881	0.091	0.862
		<i>A_e</i>	4.990	2.000	9.638	3.000	5.778	5.733	7.559
	Mer	<i>N</i>	56	52	56	56	53	56	56
		<i>N_a</i>	5	3	9	3	6	7	7
		<i>H_o</i>	0.393	0.058	0.625	0.357	0.528	0.696	0.589
		<i>H_e</i>	0.557	0.146	0.577	0.338	0.497	0.760	0.646
		<i>F_{IS}</i>	0.297	0.608	-0.084	-0.058	-0.065	0.085	0.088
		<i>P-val</i>	0.006	0.000	0.934	0.414	0.545	0.751	0.224
		<i>A_e</i>	4.998	2.985	8.351	3.000	5.979	6.644	6.605
	Mil	<i>N</i>	51	44	51	48	51	51	51
		<i>N_a</i>	5	3	8	3	5	5	7
		<i>H_o</i>	0.451	0.114	0.569	0.500	0.176	0.725	0.725
		<i>H_e</i>	0.544	0.258	0.587	0.516	0.168	0.742	0.628
		<i>F_{IS}</i>	0.173	0.562	0.032	0.031	-0.050	0.023	-0.157
		<i>P-val</i>	0.020	0.001	0.186	1.000	1.000	0.957	0.242
		<i>A_e</i>	4.946	2.886	7.759	2.813	4.688	4.988	6.739
	Mir	<i>N</i>	54	48	54	54	54	54	53
		<i>N_a</i>	5	3	8	3	5	5	5
		<i>H_o</i>	0.370	0.125	0.296	0.481	0.426	0.741	0.698
		<i>H_e</i>	0.433	0.382	0.289	0.534	0.405	0.676	0.687
		<i>F_{IS}</i>	0.146	0.675	-0.027	0.099	-0.052	-0.097	-0.016
		<i>P-val</i>	0.144	0.000	0.388	0.646	1.000	0.593	0.049
<i>A_e</i>		4.975	3.000	7.451	3.000	4.702	4.722	4.983	
Nem	<i>N</i>	47	44	46	47	43	47	46	
	<i>N_a</i>	5	3	7	4	6	4	11	
	<i>H_o</i>	0.255	0.114	0.804	0.234	0.698	0.702	0.565	
	<i>H_e</i>	0.366	0.190	0.755	0.235	0.618	0.705	0.662	
	<i>F_{IS}</i>	0.304	0.406	-0.067	0.002	-0.132	0.004	0.148	
	<i>P-val</i>	0.007	0.015	0.468	0.169	0.591	0.923	0.074	
	<i>A_e</i>	4.802	2.999	6.848	3.830	5.907	4.000	10.478	

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039
Ale	Med	N	50	45	50	50	50	50	50
		N_a	5	9	6	2	2	4	7
		H_o	0.700	0.756	0.620	0.120	0.380	0.540	0.640
		H_e	0.618	0.770	0.676	0.149	0.398	0.583	0.664
		F_{IS}	-0.134	0.018	0.083	0.195	0.046	0.074	0.036
		$P\text{-val}$	0.925	0.260	0.376	0.261	0.730	0.364	0.359
		A_e	5.000	8.733	5.560	2.000	2.000	3.780	6.340
	Mer	N	56	56	56	56	56	56	56
		N_a	5	9	5	2	3	3	6
		H_o	0.607	0.750	0.661	0.125	0.464	0.571	0.714
		H_e	0.602	0.776	0.623	0.118	0.464	0.597	0.711
		F_{IS}	-0.009	0.034	-0.060	-0.058	-0.001	0.043	-0.005
		$P\text{-val}$	0.279	0.837	0.555	1.000	1.000	0.958	0.016
		A_e	4.992	8.490	4.696	2.000	2.910	3.000	5.908
	Mil	N	51	51	51	51	51	51	51
		N_a	6	9	4	2	2	4	5
		H_o	0.725	0.667	0.529	0.059	0.471	0.412	0.353
		H_e	0.683	0.660	0.544	0.058	0.495	0.486	0.399
		F_{IS}	-0.063	-0.007	0.027	-0.020	0.051	0.155	0.116
		$P\text{-val}$	0.471	0.409	0.928	1.000	0.779	0.297	0.287
		A_e	5.965	9.133	3.946	1.988	2.000	3.529	4.943
	Mir	N	54	54	54	54	54	54	54
		N_a	6	8	4	2	2	4	8
		H_o	0.519	0.630	0.426	0.167	0.537	0.444	0.796
		H_e	0.521	0.796	0.485	0.154	0.455	0.624	0.663
		F_{IS}	0.005	0.211	0.112	-0.082	-0.183	0.290	-0.203
		$P\text{-val}$	0.780	0.010	0.061	1.000	0.232	0.000	0.345
A_e		5.702	7.642	3.722	2.000	2.000	3.722	7.147	
Nem	N	47	47	46	47	47	47	45	
	N_a	5	7	4	1	2	4	7	
	H_o	0.617	0.787	0.348	ML	0.340	0.532	0.489	
	H_e	0.717	0.796	0.599	ML	0.286	0.550	0.589	
	F_{IS}	0.141	0.011	0.422	ML	-0.195	0.034	0.171	
	$P\text{-val}$	0.120	0.222	0.000	ML	0.319	0.059	0.134	
	A_e	5.000	6.829	4.000	1.000	2.000	3.830	6.699	

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Ale	Neq	<i>N</i>	161	160	161	158	161	160	161
		<i>N_a</i>	5	2	9	3	8	7	9
		<i>H_o</i>	0.484	0.131	0.615	0.443	0.224	0.725	0.652
		<i>H_e</i>	0.486	0.215	0.647	0.536	0.218	0.740	0.644
		<i>F_{IS}</i>	0.003	0.390	0.050	0.174	-0.024	0.021	-0.013
		<i>P-val</i>	0.090	0.000	0.325	0.041	0.804	0.092	0.835
		<i>A_e</i>	4.921	2.000	7.610	2.969	4.743	5.409	6.088
	Orl	<i>N</i>	111	105	110	104	111	111	111
		<i>N_a</i>	5	5	8	5	8	5	7
		<i>H_o</i>	0.514	0.210	0.500	0.567	0.270	0.766	0.595
		<i>H_e</i>	0.539	0.351	0.478	0.560	0.271	0.722	0.596
		<i>F_{IS}</i>	0.048	0.405	-0.047	-0.014	0.002	-0.061	0.003
		<i>P-val</i>	0.440	0.000	0.672	0.170	0.386	0.558	0.155
		<i>A_e</i>	4.991	3.966	6.579	4.120	5.582	4.825	5.815
	Pet	<i>N</i>	22	20	21	21	22	20	22
		<i>N_a</i>	5	2	8	2	5	4	4
		<i>H_o</i>	0.318	0.050	0.619	0.190	0.545	0.450	0.500
		<i>H_e</i>	0.359	0.050	0.690	0.251	0.538	0.581	0.479
		<i>F_{IS}</i>	0.117	NA	0.105	0.245	-0.014	0.230	-0.045
		<i>P-val</i>	0.638	1.000	0.591	0.338	0.321	0.180	0.425
		<i>A_e</i>	NA	NA	NA	NA	NA	NA	NA
	Ric	<i>N</i>	63	56	63	63	63	63	63
		<i>N_a</i>	5	4	10	3	8	5	7
		<i>H_o</i>	0.651	0.071	0.476	0.460	0.476	0.730	0.683
		<i>H_e</i>	0.559	0.280	0.448	0.468	0.509	0.710	0.636
		<i>F_{IS}</i>	-0.166	0.746	-0.064	0.018	0.065	-0.029	-0.074
		<i>P-val</i>	0.580	0.000	0.862	1.000	0.116	0.587	0.501
<i>A_e</i>		4.566	3.606	8.869	3.000	7.022	4.993	6.231	
Riv	<i>N</i>	57	49	57	57	57	57	57	
	<i>N_a</i>	5	5	9	3	7	4	7	
	<i>H_o</i>	0.579	0.204	0.544	0.456	0.456	0.684	0.649	
	<i>H_e</i>	0.490	0.474	0.551	0.388	0.506	0.698	0.648	
	<i>F_{IS}</i>	-0.184	0.572	0.014	-0.177	0.099	0.021	-0.001	
	<i>P-val</i>	0.428	0.000	0.703	0.565	0.179	0.519	0.150	
	<i>A_e</i>	4.994	4.795	8.261	3.000	6.338	4.000	6.368	

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039	
Ale	Neq	N	161	160	161	161	161	161	159	
		N_a	5	7	4	2	2	3	7	
		H_o	0.627	0.744	0.528	0.155	0.621	0.472	0.409	
		H_e	0.689	0.726	0.588	0.154	0.497	0.489	0.449	
		F_{IS}	0.090	-0.024	0.102	-0.008	-0.250	0.035	0.090	
		$P\text{-val}$	0.151	0.561	0.102	1.000	0.002	0.501	0.323	
		A_e	4.985	6.183	3.955	2.000	2.000	2.242	5.591	
	Orl	N	111	111	110	111	111	111	111	110
		N_a	6	9	4	3	2	3	5	
		H_o	0.685	0.748	0.482	0.063	0.486	0.523	0.373	
		H_e	0.716	0.693	0.590	0.062	0.488	0.513	0.390	
		F_{IS}	0.044	-0.080	0.184	-0.020	0.002	-0.018	0.044	
		$P\text{-val}$	0.053	0.330	0.081	1.000	1.000	1.000	0.531	
		A_e	5.344	7.382	3.982	2.555	2.000	2.729	4.810	
	Pet	N	22	20	22	22	21	22	16	
		N_a	3	5	3	4	3	2	5	
		H_o	0.227	0.450	0.409	0.364	0.524	0.136	0.500	
		H_e	0.210	0.623	0.458	0.318	0.452	0.130	0.758	
		F_{IS}	-0.083	0.283	0.109	-0.147	-0.164	-0.050	0.348	
		$P\text{-val}$	1.000	0.033	0.133	1.000	0.729	1.000	0.015	
		A_e	NA	NA	NA	NA	NA	NA	NA	
Ric	N	62	63	63	63	63	63	63		
	N_a	5	10	4	2	3	4	7		
	H_o	0.355	0.762	0.333	0.206	0.444	0.603	0.619		
	H_e	0.426	0.807	0.348	0.211	0.401	0.562	0.604		
	F_{IS}	0.167	0.056	0.042	0.024	-0.108	-0.075	-0.025		
	$P\text{-val}$	0.042	0.365	0.801	1.000	0.647	0.319	0.743		
	A_e	4.932	9.075	3.617	2.000	2.619	3.618	6.556		
Riv	N	57	57	57	57	57	57	57		
	N_a	5	8	4	2	2	4	6		
	H_o	0.474	0.807	0.421	0.351	0.561	0.544	0.579		
	H_e	0.509	0.791	0.453	0.292	0.492	0.541	0.648		
	F_{IS}	0.070	-0.020	0.072	-0.204	-0.143	-0.005	0.108		
	$P\text{-val}$	0.768	0.417	0.129	0.184	0.414	0.546	0.593		
	A_e	4.843	7.586	3.675	2.000	2.000	3.683	5.655		

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Ale	Sac	N	60	55	60	60	60	60	60
		N_a	5	3	9	3	5	4	6
		H_o	0.550	0.091	0.517	0.350	0.367	0.733	0.717
		H_e	0.518	0.089	0.568	0.357	0.406	0.684	0.693
		F_{IS}	-0.061	-0.027	0.091	0.020	0.098	-0.073	-0.034
		$P\text{-val}$	0.997	1.000	0.406	1.000	0.320	0.823	0.886
		A_e	4.995	2.894	7.936	2.996	4.838	4.000	5.959
	Sen	N	158	157	157	151	157	158	155
		N_a	5	3	10	4	5	5	9
		H_o	0.538	0.217	0.573	0.497	0.268	0.715	0.561
		H_e	0.539	0.299	0.565	0.537	0.254	0.744	0.604
		F_{IS}	0.001	0.277	-0.014	0.076	-0.054	0.039	0.071
		$P\text{-val}$	0.000	0.000	0.036	0.592	0.436	0.477	0.184
		A_e	4.679	2.248	7.142	3.658	4.497	4.983	6.369
	Sew	N	113	111	113	112	112	113	113
		N_a	5	4	10	3	7	5	8
		H_o	0.407	0.099	0.628	0.420	0.277	0.805	0.531
		H_e	0.424	0.259	0.625	0.507	0.276	0.711	0.548
		F_{IS}	0.040	0.618	-0.005	0.174	-0.003	-0.134	0.030
		$P\text{-val}$	0.235	0.000	0.967	0.019	0.630	0.114	0.135
		A_e	4.705	3.710	7.894	3.000	5.454	4.570	6.553
Shu	N	50	47	50	50	50	50	50	
	N_a	5	3	7	3	7	4	7	
	H_o	0.320	0.064	0.740	0.400	0.640	0.720	0.520	
	H_e	0.406	0.063	0.641	0.469	0.569	0.660	0.516	
	F_{IS}	0.213	-0.015	-0.157	0.149	-0.126	-0.091	-0.008	
	$P\text{-val}$	0.029	1.000	0.973	0.249	0.504	0.491	0.386	
	A_e	4.907	2.802	6.943	3.000	6.467	4.000	6.724	
Sou	N	176	171	176	152	176	175	176	
	N_a	5	3	9	3	8	5	9	
	H_o	0.597	0.140	0.538	0.395	0.256	0.669	0.653	
	H_e	0.515	0.235	0.533	0.526	0.232	0.701	0.611	
	F_{IS}	-0.159	0.404	-0.009	0.250	-0.101	0.046	-0.070	
	$P\text{-val}$	0.063	0.000	0.096	0.001	0.865	0.681	0.699	
	A_e	4.903	2.405	7.564	2.910	4.613	4.716	6.101	

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039
Ale	Sac	N	60	60	60	60	60	60	59
		N_a	5	9	5	2	2	4	6
		H_o	0.500	0.700	0.550	0.083	0.483	0.500	0.644
		H_e	0.549	0.726	0.684	0.081	0.442	0.544	0.683
		F_{IS}	0.089	0.036	0.197	-0.035	-0.093	0.081	0.057
		$P\text{-val}$	0.165	0.783	0.032	1.000	0.562	0.444	0.505
		A_e	4.985	8.288	4.650	1.996	2.000	4.300	5.999
	Sen	N	158	158	154	158	157	155	155
		N_a	6	10	4	3	2	2	7
		H_o	0.778	0.671	0.513	0.139	0.567	0.471	0.316
		H_e	0.745	0.692	0.597	0.152	0.501	0.499	0.332
		F_{IS}	-0.045	0.031	0.142	0.084	-0.132	0.056	0.047
		$P\text{-val}$	0.604	0.268	0.004	0.303	0.112	0.519	0.088
		A_e	5.574	6.924	3.960	2.246	2.000	2.000	5.437
	Sew	N	113	113	112	113	113	112	111
		N_a	6	8	4	4	3	4	7
		H_o	0.646	0.779	0.420	0.204	0.292	0.518	0.586
		H_e	0.686	0.787	0.649	0.185	0.454	0.518	0.604
		F_{IS}	0.058	0.011	0.355	-0.099	0.358	0.000	0.031
		$P\text{-val}$	0.599	0.004	0.000	0.664	0.000	0.017	0.195
		A_e	5.769	7.856	4.000	2.690	2.345	3.301	6.019
Shu	N	50	50	50	50	50	50	50	
	N_a	5	8	4	2	2	3	7	
	H_o	0.540	0.840	0.540	0.180	0.380	0.180	0.720	
	H_e	0.576	0.786	0.593	0.198	0.358	0.167	0.740	
	F_{IS}	0.064	-0.070	0.090	0.091	-0.063	-0.078	0.028	
	$P\text{-val}$	0.639	0.010	0.041	0.460	1.000	1.000	0.344	
	A_e	4.953	7.723	3.990	2.000	2.000	2.780	6.768	
Sou	N	176	175	174	176	176	175	176	
	N_a	5	9	4	3	3	3	8	
	H_o	0.653	0.646	0.477	0.102	0.517	0.577	0.358	
	H_e	0.664	0.656	0.612	0.098	0.476	0.518	0.356	
	F_{IS}	0.016	0.015	0.222	-0.046	-0.086	-0.115	-0.005	
	$P\text{-val}$	0.803	0.291	0.001	1.000	0.364	0.209	0.809	
	A_e	4.973	7.155	3.984	2.378	2.222	2.782	6.069	

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Ale	Sul	N	51	45	51	51	51	51	51
		N_a	5	4	8	3	5	5	7
		H_o	0.588	0.089	0.588	0.373	0.627	0.706	0.451
		H_e	0.586	0.129	0.573	0.396	0.621	0.596	0.538
		F_{IS}	-0.003	0.313	-0.026	0.059	-0.010	-0.187	0.163
		$P\text{-val}$	0.221	0.037	0.595	0.620	0.731	0.523	0.422
		A_e	5.000	3.951	7.869	3.000	4.935	4.753	6.517
	Tid	N	55	52	55	55	54	55	55
		N_a	5	4	8	4	5	4	8
		H_o	0.417	0.105	0.350	0.500	0.525	0.783	0.683
		H_e	0.491	0.150	0.398	0.440	0.487	0.690	0.716
		F_{IS}	0.133	0.298	0.098	-0.164	-0.073	-0.141	0.074
		$P\text{-val}$	0.229	0.034	0.064	0.661	0.427	0.718	0.299
		A_e	4.707	3.735	6.828	3.709	4.990	4.000	7.335
	Tra	N	59	52	59	58	59	59	59
		N_a	6	4	9	3	8	5	7
		H_o	0.418	0.115	0.364	0.509	0.537	0.782	0.673
		H_e	0.482	0.164	0.403	0.438	0.501	0.686	0.726
		F_{IS}	-0.076	0.702	-0.018	0.078	-0.077	-0.012	-0.024
		$P\text{-val}$	0.908	0.000	0.420	0.564	0.886	0.587	0.732
		A_e	5.535	3.985	8.272	3.000	7.197	4.887	6.318
	Tus	N	54	53	54	54	54	54	54
		N_a	5	2	8	3	8	6	8
		H_o	0.537	0.057	0.630	0.352	0.444	0.741	0.519
		H_e	0.494	0.056	0.638	0.335	0.463	0.735	0.642
		F_{IS}	-0.088	-0.020	0.014	-0.050	0.041	-0.009	0.194
		$P\text{-val}$	0.251	1.000	0.661	0.434	0.610	0.788	0.123
		A_e	4.995	1.983	7.444	3.000	7.293	5.444	7.404
Vea	N	172	167	168	172	173	172	170	
	N_a	5	4	10	4	7	7	8	
	H_o	0.471	0.216	0.560	0.506	0.231	0.820	0.582	
	H_e	0.508	0.315	0.568	0.545	0.229	0.725	0.615	
	F_{IS}	0.073	0.316	0.015	0.073	-0.010	-0.132	0.054	
	$P\text{-val}$	0.023	0.000	0.808	0.290	0.568	0.135	0.002	
	A_e	4.896	3.081	7.059	3.750	4.817	5.434	5.997	

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039
Ale	Sul	<i>N</i>	51	51	51	51	51	51	51
		<i>N_a</i>	4	7	4	2	2	2	7
		<i>H_o</i>	0.667	0.824	0.431	0.216	0.431	0.118	0.706
		<i>H_e</i>	0.604	0.801	0.533	0.194	0.489	0.112	0.691
		<i>F_{IS}</i>	-0.105	-0.029	0.193	-0.111	0.119	-0.053	-0.022
		<i>P-val</i>	0.309	0.396	0.060	1.000	0.560	1.000	0.624
		<i>A_e</i>	3.999	6.944	3.988	2.000	2.000	2.000	6.765
	Tid	<i>N</i>	55	52	54	55	55	54	51
		<i>N_a</i>	5	9	5	2	3	3	7
		<i>H_o</i>	0.600	0.719	0.373	0.267	0.450	0.576	0.768
		<i>H_e</i>	0.573	0.786	0.459	0.233	0.391	0.529	0.703
		<i>F_{IS}</i>	-0.052	0.054	0.192	-0.137	-0.107	-0.085	-0.101
		<i>P-val</i>	0.300	0.195	0.005	0.579	0.778	0.761	0.184
		<i>A_e</i>	4.971	8.436	4.647	2.000	2.709	2.980	6.762
	Tra	<i>N</i>	59	56	57	59	59	59	57
		<i>N_a</i>	5	6	5	2	2	3	6
		<i>H_o</i>	0.600	0.750	0.389	0.255	0.418	0.574	0.784
		<i>H_e</i>	0.570	0.792	0.480	0.224	0.378	0.529	0.713
		<i>F_{IS}</i>	0.108	0.086	0.056	-0.221	-0.224	-0.064	-0.078
		<i>P-val</i>	0.804	0.416	0.193	0.187	0.101	0.876	0.137
		<i>A_e</i>	4.883	5.974	4.368	2.000	2.000	2.887	5.684
	Tus	<i>N</i>	54	54	54	54	54	54	54
		<i>N_a</i>	5	10	4	2	2	5	6
		<i>H_o</i>	0.426	0.759	0.481	0.148	0.463	0.741	0.611
		<i>H_e</i>	0.575	0.764	0.596	0.138	0.442	0.645	0.675
		<i>F_{IS}</i>	0.261	0.007	0.194	-0.071	-0.047	-0.150	0.096
		<i>P-val</i>	0.004	0.958	0.038	1.000	1.000	0.538	0.209
<i>A_e</i>		4.979	9.081	3.995	2.000	2.000	4.702	5.905	
Vea	<i>N</i>	172	172	172	173	173	173	170	
	<i>N_a</i>	6	7	4	2	2	3	7	
	<i>H_o</i>	0.663	0.657	0.483	0.121	0.457	0.468	0.388	
	<i>H_e</i>	0.713	0.626	0.589	0.114	0.488	0.502	0.380	
	<i>F_{IS}</i>	0.071	-0.050	0.181	-0.062	0.065	0.067	-0.023	
	<i>P-val</i>	0.188	0.738	0.000	1.000	0.436	0.488	0.272	
	<i>A_e</i>	5.723	5.453	3.981	1.996	2.000	2.225	5.706	

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Ale	Wal	N	51	46	50	50	48	51	51
		N_a	5	2	9	3	7	5	6
		H_o	0.490	0.130	0.620	0.260	0.583	0.529	0.765
		H_e	0.502	0.161	0.539	0.323	0.536	0.598	0.771
		F_{IS}	0.023	0.189	-0.152	0.197	-0.090	0.115	0.008
		$P\text{-val}$	0.285	0.282	0.990	0.001	0.890	0.566	0.004
		A_e	4.764	2.000	8.749	2.998	6.778	4.976	5.946
	Wau	N	46	38	46	46	45	46	46
		N_a	5	4	9	4	7	6	8
		H_o	0.652	0.256	0.609	0.196	0.511	0.565	0.739
		H_e	0.568	0.393	0.530	0.239	0.550	0.699	0.700
		F_{IS}	-0.151	0.351	-0.150	0.184	0.072	0.193	-0.057
		$P\text{-val}$	0.703	0.000	0.961	0.147	0.083	0.182	0.657
		A_e	4.978	4.000	8.777	3.848	6.967	5.696	7.543
	Web	N	142	141	142	138	142	142	142
		N_a	6	4	9	4	5	6	8
		H_o	0.479	0.305	0.507	0.333	0.218	0.775	0.676
		H_e	0.493	0.380	0.510	0.541	0.223	0.716	0.589
		F_{IS}	0.028	0.197	0.007	0.385	0.020	-0.082	-0.148
		$P\text{-val}$	0.000	0.051	0.887	0.000	0.200	0.039	0.637
		A_e	5.166	2.754	7.079	3.670	3.551	5.171	5.766
Wes	N	51	50	51	51	51	51	50	
	N_a	5	5	7	3	6	4	8	
	H_o	0.510	0.160	0.549	0.412	0.392	0.706	0.860	
	H_e	0.547	0.170	0.536	0.465	0.346	0.747	0.753	
	F_{IS}	0.068	0.058	-0.024	0.116	-0.136	0.056	-0.144	
	$P\text{-val}$	0.438	0.059	0.560	0.580	1.000	0.839	0.135	
	A_e	4.997	4.340	6.711	3.000	5.527	4.000	7.550	
Wig	N	110	107	110	110	110	110	110	
	N_a	4	4	8	4	6	5	8	
	H_o	0.445	0.234	0.564	0.445	0.327	0.718	0.627	
	H_e	0.509	0.383	0.578	0.549	0.317	0.732	0.606	
	F_{IS}	0.125	0.391	0.025	0.189	-0.034	0.019	-0.036	
	$P\text{-val}$	0.254	0.000	0.321	0.064	0.485	0.364	0.979	
	A_e	3.999	3.110	6.995	3.343	5.547	4.829	6.466	

Specie	Population		AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039
Ale	Wal	<i>N</i>	51	50	51	51	51	50	48
		<i>N_a</i>	5	8	2	2	2	4	6
		<i>H_o</i>	0.510	0.800	0.294	0.098	0.510	0.640	0.667
		<i>H_e</i>	0.520	0.781	0.353	0.094	0.489	0.551	0.538
		<i>F_{IS}</i>	0.019	-0.025	0.168	-0.042	-0.043	-0.163	-0.241
		<i>P-val</i>	0.885	0.852	0.245	1.000	0.781	0.371	0.123
		<i>A_e</i>	4.476	7.733	2.000	1.999	2.000	3.778	5.772
	Wau	<i>N</i>	46	46	46	46	46	46	44
		<i>N_a</i>	6	8	4	2	2	4	7
		<i>H_o</i>	0.696	0.739	0.435	0.196	0.630	0.500	0.591
		<i>H_e</i>	0.609	0.795	0.468	0.178	0.452	0.487	0.607
		<i>F_{IS}</i>	-0.143	0.071	0.072	-0.098	-0.402	-0.026	0.027
		<i>P-val</i>	0.193	0.249	0.011	1.000	0.008	0.366	0.391
		<i>A_e</i>	5.845	7.823	3.848	2.000	2.000	4.543	6.874
	Web	<i>N</i>	140	139	138	142	142	142	136
		<i>N_a</i>	6	8	6	2	3	2	8
		<i>H_o</i>	0.657	0.612	0.442	0.169	0.507	0.521	0.316
		<i>H_e</i>	0.711	0.702	0.592	0.178	0.509	0.490	0.358
		<i>F_{IS}</i>	0.076	0.129	0.255	0.053	0.003	-0.064	0.117
		<i>P-val</i>	0.380	0.087	0.000	0.625	0.618	0.492	0.048
		<i>A_e</i>	5.274	6.041	4.198	2.000	2.475	2.000	6.332
Wes	<i>N</i>	51	51	51	51	51	51	51	
	<i>N_a</i>	5	9	4	2	2	3	6	
	<i>H_o</i>	0.588	0.784	0.510	0.176	0.569	0.471	0.667	
	<i>H_e</i>	0.576	0.740	0.594	0.162	0.477	0.537	0.728	
	<i>F_{IS}</i>	-0.022	-0.061	0.143	-0.087	-0.194	0.124	0.085	
	<i>P-val</i>	0.807	0.178	0.308	1.000	0.235	0.592	0.544	
	<i>A_e</i>	4.765	8.687	3.999	2.000	2.000	3.000	5.946	
Wig	<i>N</i>	110	110	110	110	110	110	110	
	<i>N_a</i>	6	7	4	2	3	3	7	
	<i>H_o</i>	0.764	0.773	0.427	0.145	0.473	0.536	0.600	
	<i>H_e</i>	0.733	0.701	0.573	0.135	0.504	0.486	0.534	
	<i>F_{IS}</i>	-0.043	-0.102	0.256	-0.074	0.062	-0.105	-0.124	
	<i>P-val</i>	0.122	0.561	0.000	1.000	0.628	0.223	0.207	
	<i>A_e</i>	5.354	6.083	3.584	1.999	2.355	2.355	6.541	

Specie	Population		Asa8	Aa082	AsaC249	Aa093	Aa070	Aa081	Aps2A
Bbh		<i>A</i>	5	9	6	6	5	5	9
		<i>Range</i>	(114-130)	(148-184)	(189-209)	(253-281)	(224-240)	(142-170)	(76-108)
Mac		<i>N</i>	9	48	48	48	48	47	48
		<i>N_a</i>	3	9	5	6	4	4	8
		<i>H_o</i>	0.444	0.750	0.333	0.625	0.438	0.340	0.792
		<i>H_e</i>	0.386	0.707	0.435	0.624	0.370	0.376	0.711
		<i>F_{IS}</i>	-0.164	-0.061	0.235	-0.002	-0.185	0.095	-0.115
		<i>P-val</i>	1.000	0.927	0.071	0.864	0.231	0.706	0.742
		<i>A_e</i>	NA	NA	NA	NA	NA	NA	NA
Mar		<i>N</i>	30	37	36	28	33	37	37
		<i>N_a</i>	5	6	4	5	3	4	7
		<i>H_o</i>	0.600	0.351	0.361	0.536	0.333	0.595	0.595
		<i>H_e</i>	0.580	0.555	0.483	0.662	0.369	0.492	0.686
		<i>F_{IS}</i>	-0.035	0.371	0.255	0.193	0.097	-0.212	0.135
		<i>P-val</i>	0.372	0.003	0.000	0.241	0.736	0.545	0.558
		<i>A_e</i>	NA	NA	NA	NA	NA	NA	NA
Orl		<i>N</i>	26	29	29	25	24	28	27
		<i>N_a</i>	4	3	2	4	4	3	6
		<i>H_o</i>	0.346	0.690	0.414	0.760	0.375	0.393	0.778
		<i>H_e</i>	0.307	0.508	0.436	0.703	0.364	0.340	0.683
		<i>F_{IS}</i>	-0.131	-0.368	0.051	-0.083	-0.030	-0.160	-0.141
		<i>P-val</i>	1.000	0.053	1.000	0.030	0.538	1.000	0.392
		<i>A_e</i>	NA	NA	NA	NA	NA	NA	NA
Pet		<i>N</i>	4	44	44	42	33	44	43
		<i>N_a</i>	3	5	2	4	3	4	5
		<i>H_o</i>	0.750	0.545	0.159	0.762	0.727	0.386	0.465
		<i>H_e</i>	0.607	0.545	0.148	0.567	0.537	0.406	0.487
		<i>F_{IS}</i>	-0.286	0.000	-0.075	-0.349	-0.363	0.048	0.046
		<i>P-val</i>	1.000	0.120	1.000	0.043	0.000	0.003	0.376
		<i>A_e</i>	NA	NA	NA	NA	NA	NA	NA

Specie	Population	AsaD042	Ap010	Ap071	Aps1	Aa046	Ap058	Aa039	
Bbh	<i>A</i>	10	4	10	4	7	10	6	
	<i>Range</i>	(150-190)	(203-218)	(257-293)	(83-111)	(194-218)	(235-259)	(269-305)	
	Mac	<i>N</i>	48	48	48	48	46	41	48
		<i>N_a</i>	8	4	10	2	6	5	2
		<i>H_o</i>	0.813	0.250	0.854	0.104	0.543	0.341	0.042
		<i>H_e</i>	0.798	0.348	0.860	0.100	0.613	0.666	0.041
		<i>F_{IS}</i>	-0.018	0.284	0.007	-0.044	0.115	0.496	-0.011
		<i>P-val</i>	0.455	0.084	0.253	1.000	0.569	0.000	1.000
		<i>A_e</i>	NA	NA	NA	NA	NA	NA	NA
	Mar	<i>N</i>	37	37	37	37	37	32	37
		<i>N_a</i>	7	3	9	2	5	7	3
		<i>H_o</i>	0.838	0.243	0.703	0.216	0.405	0.688	0.081
		<i>H_e</i>	0.774	0.220	0.842	0.311	0.432	0.801	0.080
		<i>F_{IS}</i>	-0.084	-0.110	0.168	0.308	0.063	0.171	-0.019
		<i>P-val</i>	0.654	1.000	0.859	0.090	0.056	0.343	1.000
		<i>A_e</i>	NA	NA	NA	NA	NA	NA	NA
	Orl	<i>N</i>	24	28	8	29	24	10	17
		<i>N_a</i>	7	3	5	4	7	4	3
		<i>H_o</i>	0.833	0.179	0.625	0.552	0.667	0.500	0.647
		<i>H_e</i>	0.763	0.168	0.783	0.555	0.699	0.737	0.501
		<i>F_{IS}</i>	-0.094	-0.063	0.214	0.006	0.047	0.338	-0.304
		<i>P-val</i>	0.990	1.000	0.470	0.728	0.089	0.212	0.423
		<i>A_e</i>	NA	NA	NA	NA	NA	NA	NA
	Pet	<i>N</i>	36	44	21	43	41	21	42
		<i>N_a</i>	5	1	4	4	5	4	5
		<i>H_o</i>	0.889	ML	0.714	0.233	0.317	0.619	0.285
		<i>H_e</i>	0.640	ML	0.661	0.235	0.280	0.596	0.262
		<i>F_{IS}</i>	-0.396	ML	-0.083	0.012	-0.134	-0.040	-0.090
<i>P-val</i>		0.005	ML	0.370	0.193	1.000	0.002	1.000	
<i>A_e</i>		NA	NA	NA	NA	NA	NA	NA	

Appendix 2: Matrix of pairwise F_{ST} estimates (θ ; Weir and Cockerham 1984; below diagonal) and associated P-values (above diagonal) for both species, Alewife (within the vertical and horizontal dotted line) and Blueback Herring (outside the vertical and horizontal dotted line). Bolded F_{ST} values indicate non-significant p-values. Full names are given in Table 2.1.

N	47	48	16	113	161	169	177	142	189	98	166	158	110	111	176	173	107
Location	Nem	Coc	Lam	Sew	Neq	Bru	Dre	Web	Ben	Loc	Dam	Sen	Wig	Orl	Sou	Vea	Leo
Nem	.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Coc	0.082	.	0.124	0.000	0.005	0.017	0.000	0.246	0.066	0.258	0.008	0.618	0.016	0.093	0.014	0.012	0.000
Lam	0.136	0.007	.	0.000	0.002	0.039	0.000	0.009	0.012	0.000	0.041	0.024	0.003	0.264	0.331	0.060	0.007
Sew	0.037	0.016	0.042	.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Neq	0.079	0.006	0.019	0.014	.	0.000	0.000	0.000	0.001	0.010	0.000	0.000	0.094	0.001	0.000	0.000	0.000
Bru	0.105	0.005	0.010	0.027	0.005	.	0.000	0.089	0.402	0.144	0.832	0.215	0.001	0.000	0.000	0.000	0.000
Dre	0.032	0.021	0.048	0.007	0.017	0.030	.	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Web	0.092	0.001	0.015	0.021	0.006	0.001	0.024	.	0.145	0.308	0.085	0.061	0.001	0.000	0.000	0.000	0.000
Ben	0.095	0.003	0.014	0.025	0.004	0.000	0.026	0.001	.	0.487	0.119	0.370	0.000	0.000	0.000	0.000	0.000
Loc	0.093	0.001	0.021	0.023	0.003	0.001	0.026	0.000	0.000	.	0.049	0.423	0.037	0.000	0.000	0.000	0.000
Dam	0.108	0.006	0.010	0.029	0.006	-0.001	0.033	0.001	0.001	0.002	.	0.012	0.000	0.000	0.000	0.000	0.000
Sen	0.089	-0.001	0.011	0.021	0.005	0.001	0.024	0.038	0.000	0.000	0.003	.	0.017	0.006	0.001	0.035	0.000
Wig	0.081	0.006	0.018	0.017	0.002	0.005	0.016	0.002	0.005	0.003	0.006	0.003	.	0.001	0.000	0.002	0.000
Orl	0.087	0.003	0.003	0.016	0.006	0.005	0.019	0.007	0.006	0.009	0.007	0.004	0.006	.	0.045	0.007	0.000
Sou	0.100	0.005	0.001	0.023	0.007	0.007	0.031	0.010	0.008	0.009	0.008	0.004	0.008	0.002	.	0.217	0.000
Vea	0.088	0.005	0.009	0.020	0.005	0.005	0.024	0.008	0.006	0.006	0.007	0.002	0.004	0.003	0.001	.	0.000
Leo	0.097	0.017	0.016	0.029	0.010	0.008	0.032	0.014	0.008	0.009	0.010	0.009	0.008	0.012	0.007	0.005	.
Den	0.129	0.027	0.005	0.044	0.026	0.019	0.053	0.031	0.022	0.029	0.022	0.021	0.024	0.015	0.010	0.017	0.016
Mil	0.115	0.014	0.006	0.031	0.010	0.007	0.034	0.014	0.009	0.014	0.008	0.008	0.011	0.006	0.008	0.009	0.014
Mac	0.042	0.028	0.049	0.014	0.037	0.053	0.021	0.046	0.045	0.049	0.055	0.043	0.042	0.028	0.041	0.043	0.051
Pet	0.063	0.112	0.164	0.071	0.094	0.118	0.060	0.105	0.112	0.111	0.122	0.111	0.097	0.110	0.119	0.101	0.099
Shu	0.037	0.082	0.129	0.044	0.070	0.094	0.038	0.082	0.089	0.082	0.098	0.087	0.070	0.084	0.094	0.082	0.084
Gas	0.073	0.089	0.129	0.053	0.069	0.095	0.054	0.088	0.095	0.086	0.099	0.092	0.072	0.087	0.091	0.085	0.083
Tus	0.031	0.041	0.070	0.016	0.041	0.058	0.017	0.050	0.053	0.054	0.062	0.047	0.040	0.040	0.051	0.043	0.051
Kia	0.040	0.041	0.067	0.024	0.039	0.053	0.021	0.046	0.046	0.049	0.056	0.044	0.039	0.038	0.049	0.040	0.047
Arg	0.042	0.041	0.068	0.027	0.041	0.055	0.023	0.051	0.048	0.053	0.059	0.044	0.043	0.040	0.051	0.041	0.051

N	47	48	16	113	161	169	177	142	189	98	166	158	110	111	176	173	107
Location	Nem	Coc	Lam	Sew	Neq	Bru	Dre	Web	Ben	Loc	Dam	Sen	Wig	Orl	Sou	Ve	Leo
Mer	0.031	0.048	0.082	0.021	0.047	0.065	0.022	0.057	0.059	0.058	0.070	0.054	0.046	0.047	0.060	0.052	0.056
Med	0.034	0.039	0.068	0.016	0.036	0.054	0.019	0.049	0.048	0.047	0.060	0.046	0.039	0.037	0.048	0.043	0.042
Lah	0.054	0.025	0.047	0.016	0.020	0.038	0.017	0.036	0.032	0.029	0.041	0.028	0.024	0.025	0.032	0.028	0.029
Sul	0.060	0.074	0.118	0.051	0.065	0.084	0.044	0.070	0.079	0.069	0.090	0.078	0.061	0.079	0.089	0.076	0.078
Sac	0.041	0.042	0.073	0.020	0.039	0.056	0.023	0.051	0.050	0.051	0.061	0.046	0.044	0.038	0.049	0.043	0.047
Wes	0.046	0.046	0.072	0.020	0.038	0.055	0.023	0.051	0.047	0.051	0.059	0.047	0.005	0.040	0.051	0.044	0.041
Fid	0.058	0.073	0.108	0.048	0.072	0.090	0.043	0.081	0.084	0.087	0.094	0.081	0.068	0.072	0.085	0.077	0.080
Ama	0.059	0.089	0.130	0.057	0.078	0.098	0.051	0.089	0.091	0.094	0.101	0.090	0.077	0.086	0.098	0.086	0.087
Chr	0.052	0.064	0.092	0.033	0.053	0.070	0.032	0.062	0.070	0.072	0.072	0.068	0.050	0.060	0.073	0.064	0.069
Mar	0.045	0.039	0.064	0.021	0.042	0.057	0.020	0.050	0.052	0.056	0.061	0.049	0.040	0.038	0.055	0.051	0.055
Tra	0.039	0.054	0.091	0.025	0.046	0.065	0.026	0.054	0.060	0.061	0.067	0.060	0.049	0.054	0.069	0.060	0.064
Hil	0.041	0.043	0.071	0.024	0.043	0.059	0.028	0.049	0.054	0.057	0.061	0.053	0.049	0.042	0.053	0.048	0.054
Wau	0.031	0.042	0.086	0.022	0.043	0.062	0.022	0.051	0.053	0.053	0.066	0.052	0.049	0.045	0.061	0.053	0.058
Wal	0.048	0.048	0.073	0.031	0.048	0.064	0.032	0.056	0.055	0.063	0.067	0.058	0.060	0.047	0.063	0.059	0.063
Riv	0.042	0.043	0.075	0.019	0.039	0.054	0.024	0.047	0.048	0.051	0.058	0.048	0.043	0.042	0.056	0.049	0.049
Tid	0.031	0.047	0.075	0.021	0.048	0.064	0.026	0.056	0.058	0.064	0.066	0.057	0.051	0.044	0.058	0.054	0.057
Ric	0.047	0.046	0.069	0.028	0.052	0.062	0.034	0.056	0.056	0.063	0.066	0.055	0.055	0.043	0.055	0.050	0.053
Mir	0.051	0.051	0.075	0.030	0.046	0.059	0.034	0.051	0.054	0.058	0.059	0.056	0.048	0.045	0.058	0.052	0.054
Orl (Bbh)	0.335	0.364	0.378	0.346	0.365	0.377	0.325	0.373	0.370	0.303	0.386	0.364	0.354	0.372	0.386	0.376	0.355
Mac (Bbh)	0.424	0.436	0.450	0.425	0.438	0.445	0.402	0.444	0.438	0.373	0.455	0.435	0.427	0.444	0.457	0.447	0.426
Pet (Bbh)	0.469	0.482	0.509	0.462	0.470	0.477	0.433	0.479	0.470	0.409	0.489	0.467	0.464	0.480	0.488	0.478	0.461
Mar (Bbh)	0.345	0.359	0.370	0.352	0.367	0.377	0.331	0.375	0.370	0.303	0.387	0.365	0.357	0.372	0.386	0.377	0.355

N	47	51	48	22	50	56	54	50	50	56	50	52	51	60	51	50
Location	Den	Mil	Mac	Pet	Shu	Gas	Tus	Kia	Arg	Mer	Med	Lah	Sul	Sac	Wes	Fid
Mer	0.085	0.072	0.021	0.044	0.030	0.051	-0.002	0.005	0.013	.	0.819	0.000	0.000	0.157	0.042	0.000
Med	0.072	0.066	0.016	0.045	0.028	0.041	0.005	0.011	0.021	-0.002	.	0.001	0.000	0.417	0.191	0.000
Lah	0.052	0.037	0.022	0.082	0.054	0.057	0.014	0.024	0.020	0.013	0.011	.	0.000	0.000	0.000	0.000
Sul	0.124	0.107	0.066	0.032	0.014	0.049	0.038	0.038	0.056	0.032	0.038	0.053	.	0.000	0.000	0.000
Sac	0.078	0.068	0.022	0.044	0.037	0.051	0.008	0.012	0.022	0.002	0.000	0.018	0.048	.	0.145	0.000
Wes	0.070	0.061	0.025	0.058	0.043	0.056	0.010	0.010	0.025	0.005	0.002	0.017	0.050	0.003	.	0.000
Fid	0.120	0.106	0.038	0.043	0.038	0.061	0.025	0.032	0.051	0.018	0.019	0.051	0.045	0.020	0.023	.
Ama	0.131	0.117	0.059	0.035	0.037	0.062	0.032	0.032	0.058	0.023	0.030	0.064	0.043	0.024	0.028	0.001
Chr	0.099	0.082	0.041	0.049	0.034	0.051	0.022	0.033	0.048	0.023	0.027	0.047	0.038	0.034	0.032	0.010
Mar	0.073	0.059	0.010	0.055	0.041	0.059	0.012	0.021	0.026	0.012	0.012	0.029	0.045	0.016	0.019	0.017
Tra	0.100	0.081	0.032	0.031	0.023	0.046	0.017	0.022	0.038	0.014	0.014	0.041	0.028	0.020	0.021	0.011
Hil	0.083	0.073	0.019	0.038	0.033	0.054	0.008	0.010	0.024	0.008	0.005	0.031	0.038	0.009	0.016	0.017
Wau	0.093	0.073	0.014	0.040	0.030	0.061	0.008	0.010	0.019	0.005	0.006	0.023	0.028	0.009	0.016	0.018
Wal	0.089	0.069	0.019	0.067	0.061	0.085	0.021	0.018	0.026	0.022	0.019	0.040	0.062	0.018	0.027	0.031
Riv	0.076	0.063	0.020	0.041	0.035	0.062	0.007	0.010	0.017	0.007	0.005	0.026	0.036	0.008	0.009	0.020
Tid	0.080	0.072	0.010	0.056	0.033	0.054	0.012	0.017	0.028	0.010	0.008	0.033	0.049	0.013	0.014	0.013
Ric	0.075	0.070	0.015	0.053	0.046	0.076	0.013	0.015	0.021	0.014	0.010	0.038	0.056	0.015	0.020	0.026
Mir	0.081	0.073	0.028	0.051	0.037	0.062	0.016	0.015	0.029	0.012	0.010	0.037	0.044	0.019	0.017	0.020
Orl (Bbh)	0.374	0.378	0.350	0.291	0.342	0.363	0.349	0.343	0.346	0.341	0.338	0.361	0.336	0.359	0.344	0.382
Mac (Bbh)	0.446	0.451	0.429	0.378	0.426	0.448	0.432	0.429	0.430	0.422	0.418	0.433	0.421	0.438	0.424	0.450
Pet (Bbh)	0.492	0.497	0.473	0.424	0.471	0.492	0.475	0.473	0.474	0.462	0.459	0.474	0.460	0.479	0.467	0.497
Mar (Bbh)	0.370	0.376	0.345	0.287	0.344	0.367	0.350	0.348	0.351	0.340	0.335	0.357	0.340	0.356	0.341	0.366

N	50	49	47	59	57	46	51	57	55	63	54	29	48	45	37
Location	Ama	Chr	Mar	Tra	Hil	Wau	Wal	Riv	Tid	Ric	Mir	Orl (Bbh)	Mac (Bbh)	Pet (Bbh)	Mar (Bbh)
Mer	0.000	0.000	0.000	0.000	0.003	0.036	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Med	0.000	0.000	0.001	0.000	0.026	0.028	0.000	0.035	0.006	0.001	0.002	0.000	0.000	0.000	0.000
Lah	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sul	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sac	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Wes	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Fid	0.283	0.005	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ama	.	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chr	0.013	.	0.000	0.072	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mar	0.029	0.016	.	0.006	0.000	0.004	0.000	0.024	0.055	0.000	0.000	0.000	0.000	0.000	0.000
Tra	0.013	0.004	0.008	.	0.004	0.031	0.000	0.050	0.006	0.000	0.000	0.000	0.000	0.000	0.000
Hil	0.025	0.020	0.012	0.008	.	0.169	0.003	0.050	0.268	0.094	0.071	0.000	0.000	0.000	0.000
Wau	0.024	0.025	0.010	0.006	0.002	.	0.042	0.444	0.040	0.004	0.000	0.000	0.000	0.000	0.000
Wal	0.038	0.035	0.017	0.020	0.009	0.005	.	0.008	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Riv	0.024	0.019	0.006	0.004	0.004	0.000	0.008	.	0.033	0.013	0.003	0.000	0.000	0.000	0.000
Tid	0.028	0.017	0.005	0.008	0.001	0.005	0.011	0.005	.	0.207	0.014	0.000	0.000	0.000	0.000
Ric	0.041	0.036	0.013	0.021	0.003	0.008	0.015	0.006	0.002	.	0.004	0.000	0.000	0.000	0.000
Mir	0.027	0.018	0.019	0.012	0.004	0.013	0.022	0.008	0.007	0.008	.	0.000	0.000	0.000	0.000
Orl (Bbh)	0.372	0.380	0.322	0.356	0.365	0.254	0.345	0.351	0.364	0.378	0.352	.	0.000	0.000	0.000
Mac (Bbh)	0.446	0.452	0.401	0.428	0.437	0.328	0.413	0.419	0.433	0.440	0.425	0.079	.	0.000	0.000
Pet (Bbh)	0.494	0.503	0.444	0.474	0.482	0.367	0.459	0.466	0.475	0.484	0.471	0.149	0.076	.	0.000
Mar (Bbh)	0.360	0.368	0.316	0.346	0.355	0.246	0.332	0.340	0.348	0.358	0.342	0.062	0.033	0.060	.

Appendix 3: Genetic diversity statistics per locus across collections, including number of alleles (A) and range in allele size (in parentheses). Statistics per locus and location include the number of individuals (N), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity, inbreeding coefficient (F_{IS}), probability of departures from Hardy-Weinberg Equilibrium (P-val) and allelic richness (A_e). Bolded values indicate significant departures from HWE after sequential Bonferroni correction and ML indicates loci that are monomorphic in specific populations. Full names are given in Table 3.1.

Locus	A (range)		Ben			Bru			Dam		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
<i>Asa8</i>	6 (114-134)	<i>N</i>	65	62	61	59	59	50	57	56	53
		<i>N_a</i>	5	4	4	5	5	5	4	5	4
		<i>H_o</i>	0.492	0.694	0.459	0.458	0.525	0.560	0.421	0.482	0.472
		<i>H_e</i>	0.515	0.585	0.497	0.491	0.502	0.557	0.414	0.442	0.525
		<i>F_{IS}</i>	0.045	-0.187	0.076	0.068	-0.046	-0.006	-0.018	-0.092	0.103
		<i>P-val</i>	0.467	0.110	0.084	0.432	0.085	0.597	0.357	0.080	0.162
		<i>A_e</i>	4.512	3.999	3.993	4.821	4.817	4.990	3.999	4.546	3.998
<i>Aa082</i>	5 (148-164)	<i>N</i>	64	59	60	59	58	48	56	55	52
		<i>N_a</i>	2	3	3	2	3	3	2	2	2
		<i>H_o</i>	0.141	0.288	0.317	0.237	0.310	0.208	0.304	0.218	0.269
		<i>H_e</i>	0.297	0.360	0.399	0.365	0.415	0.366	0.369	0.276	0.379
		<i>F_{IS}</i>	0.529	0.200	0.207	0.352	0.255	0.433	0.179	0.212	0.291
		<i>P-val</i>	0.000	0.197	0.054	0.011	0.029	0.000	0.267	0.136	0.058
		<i>A_e</i>	2.000	2.576	2.567	2.000	2.586	2.917	2.000	2.000	2.000
<i>AsaC249</i>	12 (193-237)	<i>N</i>	65	61	61	59	60	50	56	51	53
		<i>N_a</i>	10	7	8	8	8	7	10	6	7
		<i>H_o</i>	0.569	0.590	0.574	0.627	0.583	0.520	0.607	0.510	0.585
		<i>H_e</i>	0.584	0.557	0.569	0.605	0.558	0.485	0.525	0.462	0.575
		<i>F_{IS}</i>	0.026	-0.061	-0.007	-0.037	-0.046	-0.074	-0.158	-0.104	-0.017
		<i>P-val</i>	0.952	0.628	0.632	0.978	0.878	0.900	0.972	0.959	0.253
		<i>A_e</i>	8.816	6.458	7.094	7.630	7.098	6.671	8.552	5.981	6.827
<i>Aa093</i>	7 (249-281)	<i>N</i>	65	62	61	59	57	50	57	43	53
		<i>N_a</i>	5	2	4	4	4	4	3	3	3
		<i>H_o</i>	0.446	0.548	0.492	0.492	0.351	0.500	0.491	0.326	0.585
		<i>H_e</i>	0.548	0.504	0.572	0.532	0.540	0.530	0.499	0.517	0.517
		<i>F_{IS}</i>	0.187	-0.090	0.141	0.077	0.352	0.058	0.016	0.374	-0.133
		<i>P-val</i>	0.192	0.609	0.613	0.906	0.016	0.832	1.000	0.013	0.633
		<i>A_e</i>	4.192	2.000	3.949	3.645	3.776	3.580	2.596	2.791	2.874

Locus	A (range)		Ben			Bru			Dam		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
<i>Aa070</i>	11 (188-260)	<i>N</i>	65	62	60	58	59	50	57	56	52
		<i>N_a</i>	5	6	6	4	4	3	3	3	4
		<i>H_o</i>	0.262	0.226	0.333	0.207	0.237	0.160	0.158	0.125	0.212
		<i>H_e</i>	0.286	0.211	0.310	0.191	0.244	0.151	0.150	0.119	0.196
		<i>F_{IS}</i>	0.087	-0.072	-0.076	-0.081	0.028	-0.060	-0.057	-0.049	-0.081
		<i>P-val</i>	0.523	1.000	0.790	1.000	0.020	1.000	1.000	1.000	1.000
		<i>A_e</i>	4.284	4.939	5.022	3.417	3.645	2.899	2.933	2.604	3.536
<i>Aa081</i>	7 (134-158)	<i>N</i>	65	62	61	59	60	50	56	56	53
		<i>N_a</i>	5	6	5	5	5	5	5	5	4
		<i>H_o</i>	0.769	0.710	0.672	0.661	0.583	0.720	0.554	0.768	0.679
		<i>H_e</i>	0.778	0.754	0.720	0.721	0.729	0.764	0.706	0.747	0.715
		<i>F_{IS}</i>	0.011	0.060	0.066	0.084	0.202	0.058	0.217	-0.029	0.051
		<i>P-val</i>	0.029	0.729	0.770	0.591	0.118	0.622	0.088	0.053	0.057
		<i>A_e</i>	4.998	5.531	4.963	4.576	4.814	4.997	4.607	4.999	4.000
<i>Aps2A</i>	16 (68-140)	<i>N</i>	65	62	61	59	60	49	57	56	53
		<i>N_a</i>	6	6	9	7	6	6	6	5	7
		<i>H_o</i>	0.708	0.677	0.672	0.593	0.617	0.469	0.649	0.625	0.642
		<i>H_e</i>	0.613	0.626	0.722	0.594	0.619	0.607	0.655	0.593	0.643
		<i>F_{IS}</i>	-0.155	-0.083	0.070	0.001	0.004	0.229	0.010	-0.055	0.002
		<i>P-val</i>	0.268	0.928	0.265	0.881	0.414	0.081	0.772	0.652	0.022
		<i>A_e</i>	5.822	5.514	7.712	6.049	5.552	5.602	5.838	4.934	6.500
<i>AsaD042</i>	6 (150-170)	<i>N</i>	65	61	61	59	60	50	55	56	53
		<i>N_a</i>	6	6	5	6	6	5	4	5	5
		<i>H_o</i>	0.677	0.705	0.7541	0.780	0.767	0.600	0.691	0.732	0.736
		<i>H_e</i>	0.741	0.714	0.73676	0.755	0.735	0.692	0.709	0.710	0.719
		<i>F_{IS}</i>	0.087	0.013	-0.0237	-0.032	-0.044	0.1343	0.025	-0.031	-0.024
		<i>P-val</i>	0.536	0.154	0.0317	0.019	0.964	0.2269	0.847	0.596	0.941
		<i>A_e</i>	5.521	5.555	4.999	5.576	5.814	4.997	4.000	4.996	4.999
<i>Ap010</i>	13 (203-243)	<i>N</i>	65	61	62	58	60	50	57	56	53
		<i>N_a</i>	6	7	7	7	6	6	5	6	7
		<i>H_o</i>	0.708	0.820	0.803	0.707	0.783	0.720	0.632	0.768	0.642
		<i>H_e</i>	0.727	0.728	0.742	0.707	0.726	0.675	0.664	0.756	0.693
		<i>F_{IS}</i>	0.027	-0.127	-0.084	0.000	-0.079	-0.068	0.050	-0.016	0.075
		<i>P-val</i>	0.926	0.797	0.997	0.997	0.058	0.070	0.803	0.945	0.546
		<i>A_e</i>	5.951	6.363	6.542	6.518	5.564	5.959	4.990	5.989	6.510

Locus	A (range)		Ben			Bru			Dam		
			2009	2010	2011	2009	2010	2011	2009	2010	2011
<i>Ap071</i>	8 (249-277)	<i>N</i>	64	61	61	59	59	49	56	54	52
		<i>N_a</i>	3	3	4	4	4	4	4	4	5
		<i>H_o</i>	0.406	0.426	0.410	0.492	0.339	0.408	0.518	0.407	0.346
		<i>H_e</i>	0.458	0.496	0.525	0.527	0.522	0.570	0.491	0.477	0.583
		<i>F_{IS}</i>	0.113	0.141	0.221	0.068	0.353	0.286	-0.056	0.147	0.409
		<i>P-val</i>	0.032	0.132	0.006	0.243	0.000	0.000	0.022	0.174	0.000
		<i>A_e</i>	2.998	2.994	3.984	3.822	3.997	3.908	3.599	3.864	4.308
<i>Aps1</i>	5 (79-103)	<i>N</i>	65	62	61	59	60	50	55	56	53
		<i>N_a</i>	2	2	2	2	2	2	2	2	2
		<i>H_o</i>	0.185	0.081	0.148	0.153	0.200	0.100	0.164	0.179	0.113
		<i>H_e</i>	0.194	0.078	0.165	0.171	0.182	0.096	0.152	0.164	0.108
		<i>F_{IS}</i>	0.047	-0.034	0.109	0.106	-0.103	-0.043	-0.080	-0.089	-0.051
		<i>P-val</i>	0.542	1.000	0.390	0.401	1.000	1.000	1.000	1.000	1.000
		<i>A_e</i>	2.000	1.983	2.000	2.000	2.000	1.997	2.000	2.000	1.998
<i>Aa046</i>	5 (190-210)	<i>N</i>	65	62	61	59	60	50	57	56	53
		<i>N_a</i>	3	2	2	2	2	2	3	3	3
		<i>H_o</i>	0.523	0.661	0.525	0.508	0.633	0.540	0.632	0.661	0.491
		<i>H_e</i>	0.533	0.498	0.504	0.487	0.495	0.489	0.612	0.513	0.486
		<i>F_{IS}</i>	0.019	-0.332	-0.041	-0.045	-0.282	-0.106	-0.032	-0.290	-0.009
		<i>P-val</i>	0.186	0.011	0.801	0.792	0.036	0.561	0.000	0.031	0.524
		<i>A_e</i>	2.951	2.000	2.000	2.000	2.000	2.000	3.000	2.607	2.642
<i>Ap058</i>	6 (235-247)	<i>N</i>	65	62	61	58	60	50	57	56	53
		<i>N_a</i>	2	3	2	2	2	3	3	2	2
		<i>H_o</i>	0.523	0.597	0.525	0.569	0.600	0.500	0.632	0.589	0.434
		<i>H_e</i>	0.498	0.528	0.493	0.501	0.504	0.506	0.513	0.491	0.504
		<i>F_{IS}</i>	-0.051	-0.133	-0.064	-0.138	-0.192	0.013	-0.233	-0.201	0.140
		<i>P-val</i>	0.801	0.146	0.793	0.425	0.195	0.753	0.085	0.172	0.409
		<i>A_e</i>	2.000	2.911	2.000	2.000	2.000	2.680	2.596	2.000	2.000
<i>Aa039</i>	10 (265-305)	<i>N</i>	65	61	61	59	59	50	57	56	53
		<i>N_a</i>	5	5	6	6	7	5	5	6	8
		<i>H_o</i>	0.231	0.344	0.344	0.373	0.339	0.280	0.386	0.286	0.302
		<i>H_e</i>	0.277	0.342	0.412	0.378	0.362	0.302	0.394	0.275	0.346
		<i>F_{IS}</i>	0.169	-0.008	0.166	0.013	0.064	0.075	0.020	-0.039	0.128
		<i>P-val</i>	0.080	0.260	0.238	0.059	0.723	0.240	0.877	0.375	0.084
		<i>A_e</i>	4.619	4.506	5.449	5.321	5.582	4.769	4.193	5.245	6.396

Locus	A (range)		Dre				Leo		Loc		Neq		
			2008	2009	2010	2011	2009	2011	2009	2011	2009	2010	2011
<i>Asa8</i>	6 (114-134)	<i>N</i>	49	19	56	53	58	49	63	35	57	59	45
		<i>N_a</i>	5	5	4	5	5	4	4	5	5	5	5
		<i>H_o</i>	0.449	0.421	0.429	0.415	0.621	0.510	0.476	0.429	0.579	0.492	0.356
		<i>H_e</i>	0.547	0.485	0.431	0.413	0.655	0.603	0.592	0.458	0.509	0.500	0.443
		<i>F_{IS}</i>	0.180	0.135	0.006	-0.005	0.053	0.155	0.197	0.064	-0.139	0.017	0.199
		<i>P-val</i>	0.205	0.286	0.054	0.501	0.249	0.048	0.086	0.880	0.872	0.458	0.050
		<i>A_e</i>	4.906	NA	3.941	4.627	4.830	3.999	4.000	4.971	4.815	4.924	4.941
<i>Aa082</i>	5 (148-164)	<i>N</i>	48	19	54	52	57	48	63	35	57	58	45
		<i>N_a</i>	3	3	4	4	3	3	3	3	2	2	2
		<i>H_o</i>	0.208	0.158	0.167	0.154	0.263	0.167	0.286	0.343	0.105	0.138	0.156
		<i>H_e</i>	0.243	0.317	0.304	0.180	0.391	0.362	0.405	0.386	0.243	0.159	0.250
		<i>F_{IS}</i>	0.145	0.509	0.453	0.146	0.329	0.542	0.297	0.112	0.570	0.133	0.380
		<i>P-val</i>	0.202	0.036	0.001	0.027	0.024	0.000	0.032	0.452	0.000	0.346	0.032
		<i>A_e</i>	2.977	NA	3.581	3.536	2.839	2.708	2.790	3.000	2.000	2.000	2.000
<i>AsaC249</i>	12 (193-237)	<i>N</i>	48	19	51	53	58	49	63	35	57	59	45
		<i>N_a</i>	8	8	7	9	10	9	8	8	8	8	6
		<i>H_o</i>	0.708	0.632	0.686	0.642	0.707	0.592	0.603	0.629	0.614	0.627	0.600
		<i>H_e</i>	0.699	0.640	0.684	0.701	0.670	0.633	0.586	0.620	0.628	0.643	0.675
		<i>F_{IS}</i>	-0.013	0.014	-0.003	0.086	-0.055	0.066	-0.030	-0.014	0.023	0.025	0.112
		<i>P-val</i>	0.910	0.731	0.240	0.010	0.369	0.577	0.553	0.880	0.514	0.094	0.400
		<i>A_e</i>	7.601	NA	6.625	8.455	8.987	8.349	7.322	7.943	7.795	7.561	5.987
<i>Aa093</i>	7 (249-281)	<i>N</i>	49	19	54	53	58	49	63	34	57	56	45
		<i>N_a</i>	4	4	4	3	3	3	4	3	3	3	3
		<i>H_o</i>	0.408	0.368	0.407	0.472	0.655	0.673	0.492	0.559	0.474	0.375	0.489
		<i>H_e</i>	0.506	0.437	0.411	0.522	0.490	0.528	0.551	0.520	0.528	0.546	0.536
		<i>F_{IS}</i>	0.196	0.160	0.010	0.097	-0.342	-0.279	0.107	-0.076	0.104	0.316	0.089
		<i>P-val</i>	0.240	0.469	0.715	0.613	0.012	0.079	0.145	0.555	0.259	0.006	0.297
		<i>A_e</i>	3.693	NA	3.624	2.998	2.586	2.973	3.531	3.000	2.937	2.997	2.987

Locus	A (range)		Dre				Leo		Loc		Neq		
			2008	2009	2010	2011	2009	2011	2009	2011	2009	2010	2011
<i>Aa070</i>	11 (188-260)	<i>N</i>	49	19	55	53	58	49	61	35	57	59	45
		<i>N_a</i>	6	4	7	5	6	4	5	5	6	5	5
		<i>H_o</i>	0.449	0.526	0.418	0.434	0.224	0.082	0.230	0.324	0.158	0.254	0.267
		<i>H_e</i>	0.472	0.467	0.414	0.427	0.210	0.118	0.228	0.358	0.151	0.235	0.279
		<i>F_{IS}</i>	0.049	-0.132	-0.010	-0.017	-0.068	0.311	-0.007	0.098	-0.045	-0.083	0.044
		<i>P-val</i>	0.742	1.000	0.215	0.517	1.000	0.150	0.510	0.567	1.000	1.000	0.703
		<i>A_e</i>	5.633	NA	6.037	4.941	5.025	3.380	4.452	5.000	4.604	4.486	4.640
<i>Aa081</i>	7 (134-158)	<i>N</i>	49	19	56	53	58	49	63	35	57	58	45
		<i>N_a</i>	5	5	5	6	5	5	5	5	5	6	5
		<i>H_o</i>	0.816	0.789	0.714	0.755	0.741	0.776	0.619	0.714	0.632	0.724	0.844
		<i>H_e</i>	0.753	0.687	0.736	0.699	0.716	0.724	0.727	0.695	0.743	0.723	0.756
		<i>F_{IS}</i>	-0.085	-0.154	0.030	-0.080	-0.035	-0.072	0.150	-0.028	0.151	-0.001	-0.118
		<i>P-val</i>	0.229	0.182	0.422	0.285	0.375	0.867	0.343	0.609	0.107	0.040	0.212
		<i>A_e</i>	4.998	NA	4.607	5.283	4.586	4.973	4.905	4.971	4.976	5.172	4.999
<i>Aps2A</i>	16 (68-140)	<i>N</i>	49	19	56	52	57	49	63	35	57	59	45
		<i>N_a</i>	8	8	9	11	9	7	6	7	6	8	6
		<i>H_o</i>	0.735	0.789	0.482	0.596	0.579	0.653	0.619	0.486	0.649	0.644	0.666
		<i>H_e</i>	0.671	0.691	0.557	0.618	0.679	0.652	0.595	0.565	0.647	0.611	0.685
		<i>F_{IS}</i>	-0.096	-0.194	0.135	0.035	0.148	-0.002	-0.040	0.142	-0.003	-0.055	0.027
		<i>P-val</i>	0.713	0.790	0.065	0.360	0.071	0.274	0.067	0.236	0.312	0.553	0.719
		<i>A_e</i>	7.573	NA	7.609	9.450	7.979	6.205	5.321	6.942	5.533	6.625	5.752
<i>AsaD042</i>	6 (150-170)	<i>N</i>	49	18	56	52	58	49	63	35	57	59	45
		<i>N_a</i>	5	6	6	6	5	6	5	6	5	5	5
		<i>H_o</i>	0.673	0.778	0.786	0.731	0.638	0.612	0.698	0.7428	0.579	0.593	0.7333
		<i>H_e</i>	0.744	0.765	0.741	0.729	0.645	0.689	0.722	0.7635	0.703	0.638	0.734
		<i>F_{IS}</i>	0.085	-0.017	-0.061	-0.0023	0.011	0.113	0.033	0.0275	0.177	0.071	0.0014
		<i>P-val</i>	0.607	0.896	0.578	0.8285	0.299	0.290	0.508	0.328	0.302	0.174	0.1797
		<i>A_e</i>	4.973	NA	5.933	5.536	4.969	5.882	4.992	5.971	4.999	4.922	4.999
<i>Ap010</i>	13 (203-243)	<i>N</i>	48	18	53	53	58	49	63	35	56	59	45
		<i>N_a</i>	9	7	9	10	6	6	8	7	6	6	6
		<i>H_o</i>	0.771	0.833	0.755	0.774	0.586	0.673	0.778	0.543	0.679	0.797	0.756
		<i>H_e</i>	0.717	0.748	0.708	0.780	0.662	0.646	0.761	0.731	0.695	0.736	0.750
		<i>F_{IS}</i>	-0.076	-0.118	-0.067	0.009	0.115	-0.042	-0.023	0.260	0.024	-0.083	-0.008
		<i>P-val</i>	0.113	1.000	0.607	0.837	0.103	0.641	0.899	0.025	0.206	0.308	0.978
		<i>A_e</i>	8.728	NA	7.798	9.736	5.586	5.693	7.037	6.971	5.839	5.823	5.987

Locus	A (range)		Dre				Leo		Loc		Neq		
			2008	2009	2010	2011	2009	2011	2009	2011	2009	2010	2011
<i>Ap071</i>	8 (249-277)	<i>N</i>	49	19	55	53	57	49	63	34	57	59	45
		<i>N_a</i>	5	4	4	4	5	3	6	4	4	4	4
		<i>H_o</i>	0.429	0.474	0.673	0.660	0.456	0.388	0.460	0.500	0.526	0.492	0.578
		<i>H_e</i>	0.621	0.656	0.594	0.588	0.603	0.512	0.489	0.528	0.619	0.577	0.570
		<i>F_{IS}</i>	0.312	0.283	-0.133	-0.124	0.245	0.245	0.059	0.054	0.151	0.149	-0.015
		<i>P-val</i>	0.000	0.047	0.350	0.254	0.006	0.054	0.902	0.889	0.497	0.125	0.128
		<i>A_e</i>	4.686	NA	3.856	3.641	4.436	3.000	4.851	4.000	3.976	3.988	3.942
<i>Aps1</i>	5 (79-103)	<i>N</i>	48	19	56	53	58	49	63	35	57	59	45
		<i>N_a</i>	3	2	2	2	2	2	2	2	2	2	2
		<i>H_o</i>	0.292	0.158	0.089	0.019	0.276	0.122	0.159	0.571	0.070	0.288	0.089
		<i>H_e</i>	0.272	0.149	0.086	0.019	0.240	0.116	0.174	0.056	0.068	0.249	0.126
		<i>F_{IS}</i>	0.073	-0.059	-0.038	NA	-0.152	-0.055	0.087	-0.015	-0.028	-0.160	0.296
		<i>P-val</i>	0.289	1.000	1.000	1.000	0.579	1.000	0.438	1.000	1.000	0.589	0.163
		<i>A_e</i>	2.917	NA	1.992	1.642	2.000	1.999	2.000	2.000	1.976	2.000	2.000
<i>Aa046</i>	5 (190-210)	<i>N</i>	49	19	56	53	58	49	63	35	57	59	45
		<i>N_a</i>	3	3	2	2	3	2	3	2	2	2	2
		<i>H_o</i>	0.531	0.316	0.607	0.585	0.517	0.429	0.508	0.429	0.579	0.644	0.644
		<i>H_e</i>	0.470	0.472	0.488	0.498	0.552	0.492	0.518	0.481	0.499	0.493	0.506
		<i>F_{IS}</i>	-0.130	0.337	-0.246	-0.176	0.064	0.130	0.020	0.110	-0.162	-0.311	-0.279
		<i>P-val</i>	0.704	0.159	0.098	0.266	0.185	0.394	0.439	0.720	0.287	0.031	0.078
		<i>A_e</i>	2.973	NA	2.000	2.000	2.996	2.000	2.905	2.000	2.000	2.000	2.000
<i>Ap058</i>	6 (235-247)	<i>N</i>	49	19	56	53	58	49	63	35	57	59	45
		<i>N_a</i>	3	3	4	3	3	3	3	3	2	3	2
		<i>H_o</i>	0.429	0.526	0.518	0.585	0.552	0.531	0.508	0.314	0.421	0.475	0.533
		<i>H_e</i>	0.490	0.536	0.475	0.523	0.516	0.496	0.485	0.446	0.503	0.497	0.449
		<i>F_{IS}</i>	0.127	0.019	-0.092	-0.121	-0.070	-0.070	-0.047	0.298	0.164	0.045	-0.189
		<i>P-val</i>	0.714	1.000	0.164	0.704	0.802	0.862	0.481	0.058	0.288	0.568	0.313
		<i>A_e</i>	2.973	NA	3.549	2.874	2.831	2.694	2.540	2.971	2.000	2.576	2.000
<i>Aa039</i>	10 (265-305)	<i>N</i>	43	19	54	52	58	47	63	34	56	58	45
		<i>N_a</i>	8	5	6	7	7	6	6	3	5	7	6
		<i>H_o</i>	0.605	0.421	0.574	0.538	0.534	0.553	0.333	0.235	0.464	0.397	0.355
		<i>H_e</i>	0.708	0.602	0.615	0.658	0.513	0.539	0.298	0.262	0.519	0.389	0.427
		<i>F_{IS}</i>	0.147	0.306	0.068	0.183	-0.042	-0.027	-0.119	0.102	0.106	-0.020	0.169
		<i>P-val</i>	0.110	0.085	0.180	0.081	0.755	0.578	1.000	0.538	0.082	0.939	0.133
		<i>A_e</i>	7.372	NA	5.969	6.535	6.485	5.974	5.101	3.000	4.844	6.280	5.453

Locus	A (range)		Orl		Sen			Sew		Sou		
			2010	2011	2008	2010	2011	2009	2011	2009	2010	2011
<i>Asa8</i>	6 (114-134)	<i>N</i>	59	52	47	62	49	62	51	57	59	60
		<i>N_a</i>	5	5	5	5	4	5	5	5	5	5
		<i>H_o</i>	0.492	0.538	0.574	0.548	0.490	0.355	0.471	0.509	0.661	0.617
		<i>H_e</i>	0.480	0.602	0.583	0.524	0.520	0.417	0.436	0.458	0.548	0.532
		<i>F_{IS}</i>	-0.025	0.106	0.016	-0.047	0.058	0.149	-0.080	-0.112	-0.209	-0.161
		<i>P-val</i>	0.879	0.332	0.008	0.387	0.198	0.037	0.534	0.591	0.011	0.890
		<i>A_e</i>	4.914	4.999	4.980	4.545	4.000	4.758	4.662	4.930	4.910	4.565
<i>Aa082</i>	5 (148-164)	<i>N</i>	53	52	47	62	48	60	51	57	57	57
		<i>N_a</i>	4	5	2	3	2	4	3	2	2	3
		<i>H_o</i>	0.226	0.192	0.298	0.210	0.146	0.117	0.078	0.123	0.228	0.070
		<i>H_e</i>	0.277	0.420	0.286	0.297	0.321	0.216	0.308	0.231	0.303	0.164
		<i>F_{IS}</i>	0.185	0.545	-0.044	0.296	0.548	0.462	0.747	0.470	0.250	0.574
		<i>P-val</i>	0.086	0.000	1.000	0.008	0.001	0.001	0.000	0.004	0.078	0.001
		<i>A_e</i>	3.283	4.419	2.000	2.548	2.000	3.842	2.999	2.000	2.000	2.839
<i>AsaC249</i>	12 (193-237)	<i>N</i>	58	52	47	61	49	62	51	56	57	60
		<i>N_a</i>	8	6	6	6	10	10	7	8	8	9
		<i>H_o</i>	0.466	0.538	0.617	0.492	0.633	0.613	0.647	0.554	0.351	0.700
		<i>H_e</i>	0.446	0.508	0.590	0.536	0.582	0.598	0.659	0.554	0.395	0.627
		<i>F_{IS}</i>	-0.044	-0.060	-0.047	0.083	-0.088	-0.025	0.019	0.000	0.114	-0.117
		<i>P-val</i>	0.572	0.659	0.838	0.009	0.299	0.882	0.498	0.260	0.079	0.431
		<i>A_e</i>	6.665	5.535	5.723	5.556	8.961	8.334	6.853	7.372	6.700	8.014
<i>Aa093</i>	7 (249-281)	<i>N</i>	52	52	46	57	48	61	51	57	38	57
		<i>N_a</i>	5	4	4	3	4	3	3	3	3	3
		<i>H_o</i>	0.519	0.615	0.587	0.386	0.542	0.361	0.490	0.509	0.316	0.333
		<i>H_e</i>	0.567	0.547	0.577	0.477	0.529	0.494	0.520	0.529	0.534	0.522
		<i>F_{IS}</i>	0.086	-0.126	-0.018	0.192	-0.024	0.271	0.059	0.039	0.412	0.363
		<i>P-val</i>	0.078	0.534	0.849	0.253	0.880	0.010	0.117	0.919	0.004	0.005
		<i>A_e</i>	4.523	3.650	3.980	2.839	3.625	3.000	3.000	2.937	2.999	2.839

Locus	A (range)		Orl		Sen			Sew		Sou		
			2010	2011	2008	2010	2011	2009	2011	2009	2010	2011
<i>Aa070</i>	11 (188-260)	<i>N</i>	59	52	47	61	49	62	50	57	59	60
		<i>N_a</i>	8	4	5	5	5	6	6	7	6	5
		<i>H_o</i>	0.305	0.231	0.298	0.197	0.327	0.290	0.260	0.228	0.271	0.267
		<i>H_e</i>	0.307	0.231	0.302	0.185	0.294	0.304	0.241	0.213	0.246	0.240
		<i>F_{IS}</i>	0.006	0.002	0.013	-0.066	-0.112	0.046	-0.081	-0.074	-0.105	-0.110
		<i>P-val</i>	0.397	0.362	0.084	1.000	1.000	0.302	1.000	1.000	1.000	1.000
		<i>A_e</i>	6.504	3.970	4.630	4.167	4.855	5.290	5.248	5.224	4.551	3.948
<i>Aa081</i>	7 (134-158)	<i>N</i>	59	52	47	62	49	62	51	56	59	60
		<i>N_a</i>	5	5	5	5	5	5	5	5	4	5
		<i>H_o</i>	0.695	0.846	0.702	0.661	0.496	0.790	0.824	0.679	0.678	0.650
		<i>H_e</i>	0.719	0.724	0.752	0.731	0.750	0.709	0.712	0.709	0.692	0.708
		<i>F_{IS}</i>	0.034	-0.170	0.067	0.097	-0.062	-0.116	-0.158	0.043	0.021	0.082
		<i>P-val</i>	0.339	0.751	0.138	0.805	0.835	0.076	0.375	0.217	0.905	0.652
		<i>A_e</i>	4.576	4.960	4.995	4.798	5.000	4.547	4.666	4.607	3.988	4.966
<i>Aps2A</i>	16 (68-140)	<i>N</i>	59	52	47	61	47	62	51	57	59	60
		<i>N_a</i>	6	6	7	7	6	8	5	8	5	7
		<i>H_o</i>	0.576	0.615	0.574	0.557	0.553	0.548	0.510	0.719	0.559	0.683
		<i>H_e</i>	0.568	0.626	0.655	0.515	0.656	0.582	0.507	0.656	0.516	0.645
		<i>F_{IS}</i>	-0.015	0.018	0.125	-0.083	0.158	0.058	-0.005	-0.097	-0.084	-0.061
		<i>P-val</i>	0.522	0.004	0.117	0.304	0.300	0.104	0.930	0.760	0.745	0.898
		<i>A_e</i>	5.791	5.304	6.447	6.042	5.906	7.104	4.888	6.323	4.816	6.041
<i>AsaD042</i>	6 (150-170)	<i>N</i>	59	52	47	62	49	62	51	57	59	60
		<i>N_a</i>	6	5	6	6	6	6	6	5	5	5
		<i>H_o</i>	0.678	0.692	0.830	0.758	0.755	0.548	0.765	0.596	0.661	0.700
		<i>H_e</i>	0.717	0.719	0.749	0.729	0.765	0.668	0.706	0.642	0.639	0.709
		<i>F_{IS}</i>	0.055	0.0377	-0.109	-0.040	0.013	0.180	-0.084	0.0721	-0.035	0.0124
		<i>P-val</i>	0.025	0.4875	0.369	0.829	0.897	0.361	0.305	0.7157	0.892	0.772
		<i>A_e</i>	5.570	4.996	5.723	5.548	5.694	5.820	5.656	4.986	4.958	4.994
<i>Ap010</i>	13 (203-243)	<i>N</i>	59	52	47	62	49	62	51	56	59	60
		<i>N_a</i>	7	9	7	9	7	8	8	9	7	8
		<i>H_o</i>	0.746	0.750	0.681	0.645	0.694	0.758	0.804	0.679	0.559	0.700
		<i>H_e</i>	0.676	0.715	0.686	0.709	0.684	0.775	0.804	0.675	0.613	0.678
		<i>F_{IS}</i>	-0.105	-0.050	0.007	0.091	-0.015	0.022	0.000	-0.006	0.089	-0.032
		<i>P-val</i>	0.183	0.422	0.754	0.258	0.248	0.038	0.285	0.175	0.464	0.381
		<i>A_e</i>	6.386	8.071	6.372	7.443	6.600	7.904	7.662	7.668	6.152	7.131

Locus	A (range)		Orl		Sen			Sew		Sou		
			2010	2011	2008	2010	2011	2009	2011	2009	2010	2011
<i>Ap071</i>	8 (249-277)	<i>N</i>	58	52	47	62	47	62	50	57	58	59
		<i>N_a</i>	4	4	4	4	3	4	4	4	4	4
		<i>H_o</i>	0.431	0.538	0.638	0.565	0.319	0.387	0.460	0.544	0.431	0.458
		<i>H_e</i>	0.573	0.613	0.620	0.612	0.554	0.652	0.649	0.652	0.597	0.580
		<i>F_{IS}</i>	0.249	0.122	-0.030	0.078	0.426	0.408	0.293	0.167	0.280	0.213
		<i>P-val</i>	0.088	0.673	0.345	0.166	0.001	0.000	0.018	0.068	0.013	0.338
		<i>A_e</i>	3.830	3.999	4.000	3.961	3.000	3.999	4.000	4.000	3.973	3.965
<i>Aps1</i>	5 (79-103)	<i>N</i>	59	52	47	62	49	62	51	57	59	60
		<i>N_a</i>	3	2	3	2	2	3	3	2	2	3
		<i>H_o</i>	0.068	0.058	0.191	0.097	0.143	0.145	0.275	0.070	0.051	0.183
		<i>H_e</i>	0.066	0.057	0.177	0.122	0.169	0.137	0.242	0.068	0.050	0.170
		<i>F_{IS}</i>	-0.020	-0.020	-0.084	0.206	0.154	-0.062	-0.137	-0.028	-0.018	-0.076
		<i>P-val</i>	1.000	1.000	1.000	0.214	0.332	1.000	0.634	1.000	1.000	1.000
		<i>A_e</i>	2.503	1.961	2.723	1.999	2.000	2.547	2.667	1.976	1.927	2.814
<i>Aa046</i>	5 (190-210)	<i>N</i>	59	52	46	62	49	62	51	57	59	60
		<i>N_a</i>	2	2	2	2	2	3	2	3	2	2
		<i>H_o</i>	0.475	0.500	0.565	0.597	0.531	0.242	0.353	0.509	0.559	0.483
		<i>H_e</i>	0.487	0.493	0.505	0.504	0.503	0.382	0.500	0.448	0.490	0.487
		<i>F_{IS}</i>	0.025	-0.015	-0.122	-0.186	-0.055	0.369	0.296	-0.137	-0.143	0.008
		<i>P-val</i>	1.000	1.000	0.553	0.203	0.778	0.003	0.047	0.569	0.296	1.000
		<i>A_e</i>	2.000	2.000	2.000	2.000	2.000	2.548	2.000	2.596	2.000	2.000
<i>Ap058</i>	6 (235-247)	<i>N</i>	59	52	44	62	49	61	51	57	58	60
		<i>N_a</i>	3	2	2	2	2	4	2	2	3	3
		<i>H_o</i>	0.525	0.519	0.432	0.516	0.449	0.525	0.510	0.474	0.638	0.617
		<i>H_e</i>	0.529	0.496	0.499	0.491	0.503	0.540	0.495	0.504	0.534	0.520
		<i>F_{IS}</i>	0.007	-0.048	0.136	-0.052	0.109	0.028	-0.029	0.060	-0.198	-0.188
		<i>P-val</i>	1.000	0.782	0.541	0.796	0.566	0.036	1.000	0.791	0.307	0.135
		<i>A_e</i>	2.927	2.000	2.000	2.000	2.000	3.722	2.000	2.000	2.973	2.814
<i>Aa039</i>	10 (265-305)	<i>N</i>	58	52	44	62	49	60	51	57	59	60
		<i>N_a</i>	5	5	5	6	6	7	6	7	6	8
		<i>H_o</i>	0.345	0.404	0.295	0.306	0.347	0.650	0.510	0.368	0.322	0.383
		<i>H_e</i>	0.367	0.418	0.301	0.302	0.400	0.597	0.614	0.355	0.318	0.397
		<i>F_{IS}</i>	0.060	0.033	0.019	-0.016	0.133	-0.089	0.171	-0.038	-0.013	0.035
		<i>P-val</i>	0.737	0.243	0.767	0.270	0.106	0.133	0.198	0.340	0.453	0.848
		<i>A_e</i>	4.735	4.843	4.673	5.168	5.633	6.162	5.821	6.285	5.356	6.448

Locus	A (range)		Vea			Web			Wig		Den	Mil
			2009	2010	2011	2009	2010	2011	2009	2011	2005	2005
<i>Asa8</i>	6 (114-134)	<i>N</i>	68	52	52	37	53	52	50	60	47	51
		<i>N_a</i>	5	5	5	5	5	5	4	4	5	5
		<i>H_o</i>	0.441	0.442	0.538	0.486	0.509	0.442	0.480	0.417	0.447	0.451
		<i>H_e</i>	0.459	0.460	0.603	0.497	0.415	0.564	0.526	0.498	0.517	0.544
		<i>F_{IS}</i>	0.039	0.039	0.108	0.022	-0.230	0.218	0.089	0.164	0.137	0.173
		<i>P-val</i>	0.070	0.152	0.053	0.034	0.464	0.003	0.305	0.047	0.210	0.022
		<i>A_e</i>	4.926	4.842	4.957	4.994	4.283	4.987	4.000	3.998	4.851	4.890
<i>Aa082</i>	5 (148-164)	<i>N</i>	67	51	49	37	53	51	48	59	43	44
		<i>N_a</i>	2	3	4	2	3	3	3	4	2	3
		<i>H_o</i>	0.164	0.235	0.265	0.243	0.396	0.255	0.250	0.220	0.163	0.114
		<i>H_e</i>	0.306	0.227	0.412	0.294	0.420	0.398	0.410	0.363	0.291	0.258
		<i>F_{IS}</i>	0.465	-0.037	0.358	0.174	0.057	0.362	0.392	0.396	0.444	0.562
		<i>P-val</i>	0.001	0.131	0.000	0.286	0.617	0.013	0.001	0.001	0.012	0.001
		<i>A_e</i>	2.000	2.667	3.693	2.000	2.874	2.667	2.917	3.153	2.000	2.773
<i>AsaC249</i>	12 (193-237)	<i>N</i>	68	48	52	37	53	52	50	60	47	51
		<i>N_a</i>	7	7	9	6	8	8	8	7	8	8
		<i>H_o</i>	0.529	0.479	0.673	0.514	0.491	0.519	0.600	0.533	0.574	0.569
		<i>H_e</i>	0.563	0.470	0.659	0.527	0.433	0.572	0.566	0.591	0.567	0.587
		<i>F_{IS}</i>	0.060	-0.019	-0.022	0.026	-0.183	0.093	-0.060	0.098	-0.013	0.032
		<i>P-val</i>	0.397	0.352	0.873	0.433	1.000	0.190	0.361	0.236	0.876	0.186
		<i>A_e</i>	6.433	6.601	7.960	5.994	7.015	7.185	7.229	6.485	7.165	7.640
<i>Aa093</i>	7 (249-281)	<i>N</i>	69	51	52	37	49	52	50	60	47	48
		<i>N_a</i>	4	3	4	4	4	4	3	4	2	3
		<i>H_o</i>	0.551	0.510	0.442	0.297	0.184	0.500	0.540	0.367	0.447	0.500
		<i>H_e</i>	0.574	0.524	0.533	0.505	0.526	0.534	0.557	0.533	0.431	0.516
		<i>F_{IS}</i>	0.041	0.028	0.172	0.415	0.653	0.064	0.031	0.314	-0.037	0.031
		<i>P-val</i>	0.971	0.576	0.019	0.009	0.000	0.642	0.290	0.006	1.000	1.000
		<i>A_e</i>	3.929	2.891	3.536	3.919	3.602	3.641	3.000	3.488	2.000	2.708

Locus	A (range)		Vea			Web			Wig		Den	Mil
			2009	2010	2011	2009	2010	2011	2009	2011	2005	2005
<i>Aa070</i>	11 (188-260)	<i>N</i>	69	52	52	37	53	52	50	60	47	51
		<i>N_a</i>	7	5	4	4	4	4	5	6	6	5
		<i>H_o</i>	0.290	0.231	0.154	0.243	0.151	0.269	0.220	0.417	0.277	0.176
		<i>H_e</i>	0.265	0.247	0.163	0.243	0.143	0.286	0.207	0.401	0.303	0.168
		<i>F_{IS}</i>	-0.095	0.068	0.059	0.000	-0.055	0.061	-0.064	-0.041	0.089	-0.050
		<i>P-val</i>	1.000	0.045	0.201	0.253	1.000	0.224	1.000	0.633	0.259	1.000
		<i>A_e</i>	5.336	4.725	3.535	3.838	3.281	3.615	4.808	5.474	5.165	4.488
		<i>N</i>	68	52	52	37	53	52	50	60	47	51
<i>Aa081</i>	7 (134-158)	<i>N_a</i>	5	5	7	5	6	5	5	5	4	5
		<i>H_o</i>	0.809	0.865	0.788	0.730	0.792	0.788	0.620	0.800	0.723	0.725
		<i>H_e</i>	0.726	0.712	0.745	0.740	0.705	0.718	0.731	0.732	0.648	0.742
		<i>F_{IS}</i>	-0.115	-0.217	-0.059	0.015	-0.125	-0.099	0.154	-0.094	-0.118	0.023
		<i>P-val</i>	0.816	0.207	0.595	0.249	0.025	0.413	0.332	0.041	0.703	0.957
		<i>A_e</i>	4.940	4.996	6.306	5.000	5.281	4.960	4.680	4.921	3.999	4.965
		<i>N</i>	66	52	52	37	53	52	50	60	47	51
		<i>N_a</i>	7	5	7	7	6	5	8	6	7	7
<i>Aps2A</i>	16 (68-140)	<i>H_o</i>	0.576	0.635	0.538	0.784	0.679	0.596	0.640	0.617	0.596	0.725
		<i>H_e</i>	0.612	0.635	0.605	0.640	0.575	0.566	0.627	0.587	0.649	0.628
		<i>F_{IS}</i>	0.059	0.001	0.111	-0.229	-0.184	-0.055	-0.021	-0.050	0.082	-0.157
		<i>P-val</i>	0.018	0.024	0.345	0.183	0.413	0.973	0.827	0.811	0.431	0.264
		<i>A_e</i>	6.118	4.843	6.484	6.832	5.554	4.986	7.320	5.367	6.408	6.586
		<i>N</i>	68	52	52	36	52	52	50	60	47	51
		<i>N_a</i>	6	6	5	5	5	6	6	5	5	6
		<i>H_o</i>	0.662	0.712	0.615	0.556	0.731	0.654	0.760	0.767	0.596	0.725
<i>AsaD042</i>	6 (150-170)	<i>H_e</i>	0.732	0.712	0.695	0.727	0.635	0.728	0.736	0.735	0.635	0.683
		<i>F_{IS}</i>	0.097	0.0008	0.116	0.238	-0.152	0.102	-0.034	-0.0436	0.063	-0.063
		<i>P-val</i>	0.169	0.0634	0.368	0.000	0.213	0.083	0.239	0.3063	0.043	0.475
		<i>A_e</i>	5.751	5.948	5.000	5.000	4.985	5.640	5.680	4.998	4.649	5.895
		<i>N</i>	68	52	52	37	50	52	50	60	47	51
		<i>N_a</i>	5	5	7	5	6	8	6	6	7	9
		<i>H_o</i>	0.588	0.654	0.750	0.649	0.540	0.654	0.760	0.783	0.660	0.667
		<i>H_e</i>	0.558	0.655	0.683	0.665	0.710	0.722	0.705	0.703	0.724	0.660
<i>Ap010</i>	13 (203-243)	<i>F_{IS}</i>	-0.056	0.002	-0.099	0.024	0.242	0.096	-0.079	-0.116	0.089	-0.007
		<i>P-val</i>	0.861	0.729	0.983	0.311	0.017	0.248	0.707	0.243	0.084	0.377
		<i>A_e</i>	4.993	5.000	6.308	4.994	5.679	6.961	5.969	5.553	6.718	8.670

Locus	A (range)		Vea			Web			Wig		Den	Mil
			2009	2010	2011	2009	2010	2011	2009	2011	2005	2005
<i>Ap071</i>	8 (249-277)	<i>N</i>	68	52	52	36	50	52	50	60	47	51
		<i>N_a</i>	4	4	4	5	4	4	4	4	4	4
		<i>H_o</i>	0.574	0.269	0.577	0.361	0.520	0.423	0.480	0.383	0.489	0.529
		<i>H_e</i>	0.590	0.547	0.629	0.680	0.541	0.509	0.563	0.586	0.557	0.544
		<i>F_{IS}</i>	0.028	0.511	0.084	0.472	0.040	0.170	0.149	0.347	0.123	0.027
		<i>P-val</i>	0.363	0.000	0.837	0.000	0.415	0.034	0.441	0.000	0.194	0.928
		<i>A_e</i>	3.971	3.997	3.987	4.889	3.680	3.882	3.680	3.567	3.723	3.891
<i>Aps1</i>	5 (79-103)	<i>N</i>	69	52	52	37	53	52	50	60	47	51
		<i>N_a</i>	2	2	2	2	2	2	2	2	2	2
		<i>H_o</i>	0.130	0.115	0.115	0.243	0.151	0.135	0.140	0.150	0.128	0.059
		<i>H_e</i>	0.123	0.110	0.110	0.294	0.141	0.127	0.132	0.140	0.121	0.058
		<i>F_{IS}</i>	-0.063	-0.052	-0.052	0.174	-0.072	-0.063	-0.065	-0.073	-0.058	-0.020
		<i>P-val</i>	1.000	1.000	1.000	0.289	1.000	1.000	1.000	1.000	1.000	1.000
		<i>A_e</i>	1.998	1.999	1.999	2.000	2.000	2.000	2.000	2.000	2.000	1.965
<i>Aa046</i>	5 (190-210)	<i>N</i>	69	52	52	37	53	52	50	60	47	51
		<i>N_a</i>	2	2	2	3	2	2	3	2	2	2
		<i>H_o</i>	0.493	0.481	0.385	0.459	0.604	0.442	0.440	0.500	0.511	0.471
		<i>H_e</i>	0.457	0.505	0.493	0.515	0.500	0.505	0.506	0.504	0.494	0.495
		<i>F_{IS}</i>	-0.079	0.048	0.221	0.109	-0.209	0.125	0.468	0.007	-0.034	0.051
		<i>P-val</i>	0.598	0.785	0.154	0.442	0.167	0.413	0.467	1.000	1.000	0.779
		<i>A_e</i>	2.000	2.000	2.000	2.994	2.000	2.000	2.680	2.000	2.000	2.000
<i>Ap058</i>	6 (235-247)	<i>N</i>	69	52	52	37	53	52	50	60	47	51
		<i>N_a</i>	2	2	3	2	2	2	2	3	3	4
		<i>H_o</i>	0.478	0.462	0.462	0.676	0.491	0.442	0.540	0.533	0.574	0.412
		<i>H_e</i>	0.500	0.493	0.514	0.494	0.490	0.496	0.481	0.494	0.474	0.486
		<i>F_{IS}</i>	0.044	0.064	0.103	-0.376	-0.001	0.109	-0.125	-0.080	-0.215	0.155
		<i>P-val</i>	0.809	0.777	0.487	0.040	1.000	0.573	0.551	0.405	0.242	0.290
		<i>A_e</i>	2.000	2.000	2.654	2.000	2.000	2.000	2.000	2.567	2.981	3.333
<i>Aa039</i>	10 (265-305)	<i>N</i>	66	52	52	35	49	52	50	60	47	51
		<i>N_a</i>	6	6	7	7	6	7	7	7	7	5
		<i>H_o</i>	0.379	0.462	0.327	0.143	0.367	0.385	0.620	0.583	0.340	0.353
		<i>H_e</i>	0.378	0.415	0.351	0.191	0.351	0.461	0.629	0.540	0.396	0.399
		<i>F_{IS}</i>	-0.003	-0.112	0.070	0.254	-0.047	0.167	-0.173	-0.080	0.142	0.116
		<i>P-val</i>	0.601	0.655	0.046	0.022	1.000	0.219	0.906	0.336	0.213	0.283
		<i>A_e</i>	5.470	5.686	5.883	6.857	5.270	6.410	6.160	6.689	6.131	4.877

Appendix 4: Genic differentiation for Maine collections where the bolded values indicate significant p-values after sequential Bonferroni correction. Full names are given in Table 3.1.

N	62	51	57	59	45	59	60	50	49	19	56	53
Locations	Sew'09	Sew'11	Neq'09	Neq'10	Neq'11	Bru'09	Bru'10	Bru'11	Dre'08	Dre'09	Dre'10	Dre'11
Sew'09
Sew'11	0.111
Neq'09	0.000	0.007
Neq'10	0.000	0.006	0.020
Neq'11	0.000	0.000	0.147	0.066
Bru'09	0.000	0.000	0.000	0.003	0.030
Bru'10	0.000	0.000	0.006	0.053	0.302	0.431
Bru'11	0.000	0.000	0.000	0.003	0.164	0.572	0.273
Dre'08	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dre'09	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.854	.	.	.
Dre'10	0.003	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.063	.	.
Dre'11	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.045	.
Web'09	0.000	0.000	0.000	0.030	0.002	0.003	0.041	0.016	0.000	0.000	0.000	0.000
Web'10	0.000	0.000	0.000	0.000	0.000	0.024	0.066	0.036	0.000	0.000	0.000	0.000
Web'11	0.000	0.001	0.002	0.018	0.175	0.534	0.324	0.529	0.000	0.000	0.000	0.000
Ben'09	0.000	0.000	0.000	0.000	0.019	0.048	0.385	0.301	0.000	0.000	0.000	0.000
Ben'10	0.000	0.000	0.000	0.000	0.025	0.161	0.230	0.382	0.000	0.000	0.000	0.000
Ben'11	0.000	0.001	0.000	0.044	0.172	0.372	0.707	0.270	0.000	0.000	0.000	0.000
Loc'09	0.000	0.000	0.000	0.448	0.061	0.207	0.347	0.281	0.000	0.000	0.000	0.000
Loc'11	0.000	0.000	0.000	0.000	0.045	0.240	0.048	0.140	0.000	0.000	0.000	0.000
Dam'09	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dam'10	0.000	0.000	0.000	0.005	0.090	0.012	0.492	0.664	0.000	0.000	0.000	0.000
Dam'11	0.000	0.000	0.000	0.002	0.010	0.730	0.176	0.699	0.000	0.000	0.000	0.000

N	62	51	57	59	45	59	60	50	49	19	56	53
Locations	Sew'09	Sew'11	Neq'09	Neq'10	Neq'11	Bru'09	Bru'10	Bru'11	Dre'08	Dre'09	Dre'10	Dre'11
Sen'08	0.000	0.000	0.000	0.001	0.403	0.138	0.604	0.567	0.000	0.000	0.000	0.000
Sen'10	0.000	0.000	0.003	0.005	0.124	0.149	0.174	0.108	0.000	0.000	0.000	0.000
Sen'11	0.000	0.000	0.005	0.000	0.117	0.178	0.936	0.297	0.000	0.000	0.000	0.000
Wig'09	0.000	0.001	0.104	0.099	0.070	0.004	0.029	0.003	0.000	0.000	0.000	0.000
Wig'11	0.000	0.000	0.008	0.055	0.298	0.002	0.017	0.001	0.000	0.000	0.000	0.000
Orl'10	0.000	0.000	0.312	0.000	0.000	0.000	0.011	0.001	0.000	0.001	0.000	0.000
Orl'11	0.000	0.000	0.077	0.002	0.007	0.000	0.007	0.001	0.000	0.000	0.000	0.000
Sou'09	0.000	0.000	0.044	0.003	0.029	0.000	0.020	0.001	0.000	0.000	0.000	0.000
Sou'10	0.000	0.000	0.003	0.002	0.000	0.000	0.009	0.005	0.000	0.000	0.000	0.000
Sou'11	0.000	0.000	0.077	0.024	0.169	0.062	0.144	0.118	0.000	0.000	0.000	0.000
Vea'09	0.000	0.000	0.008	0.002	0.047	0.000	0.108	0.003	0.000	0.000	0.000	0.000
Vea'10	0.000	0.000	0.013	0.001	0.318	0.147	0.275	0.388	0.000	0.000	0.000	0.000
Vea'11	0.000	0.000	0.087	0.008	0.147	0.135	0.541	0.508	0.000	0.000	0.000	0.000
Leo'09	0.000	0.000	0.000	0.002	0.000	0.002	0.002	0.000	0.000	0.000	0.000	0.000
Leo'11	0.000	0.000	0.000	0.001	0.009	0.037	0.303	0.060	0.000	0.000	0.000	0.000
Den'05	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mil'05	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000

N Locations	37 Web'09	53 Web'10	52 Web'11	65 Ben'09	62 Ben'10	61 Ben'11	63 Loc'09	45 Loc'11	57 Dam'09	56 Dam'10	53 Dam'11
Sew'09											
Sew'11											
Neq'09											
Neq'10											
Neq'11											
Bru'09											
Bru'10											
Bru'11											
Dre'08											
Dre'09											
Dre'10											
Dre'11											
Web'09											
Web'10	0.001										
Web'11	0.015	0.001									
Ben'09	0.038	0.004	0.034								
Ben'10	0.000	0.003	0.048	0.154							
Ben'11	0.103	0.027	0.611	0.076	0.115						
Loc'09	0.189	0.003	0.186	0.509	0.077	0.819					
Loc'11	0.000	0.032	0.039	0.075	0.076	0.028	0.023				
Dam'09	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000			
Dam'10	0.026	0.355	0.017	0.412	0.092	0.139	0.372	0.003	0.000		
Dam'11	0.001	0.048	0.391	0.113	0.527	0.169	0.186	0.228	0.000	0.149	

N Locations	37 Web'09	53 Web'10	52 Web'11	65 Ben'09	62 Ben'10	61 Ben'11	63 Loc'09	45 Loc'11	57 Dam'09	56 Dam'10	53 Dam'11
Sen'08	0.599	0.001	0.742	0.391	0.075	0.612	0.355	0.194	0.000	0.022	0.259
Sen'10	0.168	0.214	0.013	0.013	0.141	0.112	0.467	0.028	0.000	0.335	0.087
Sen'11	0.010	0.006	0.173	0.775	0.503	0.657	0.217	0.117	0.000	0.296	0.104
Wig'09	0.001	0.000	0.154	0.000	0.002	0.012	0.105	0.000	0.000	0.000	0.017
Wig'11	0.011	0.000	0.117	0.000	0.005	0.078	0.040	0.002	0.000	0.000	0.001
Orl'10	0.000	0.000	0.003	0.000	0.000	0.000	0.001	0.001	0.000	0.000	0.000
Orl'11	0.000	0.000	0.048	0.000	0.000	0.005	0.001	0.000	0.000	0.000	0.000
Sou'09	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sou'10	0.000	0.000	0.007	0.000	0.001	0.000	0.003	0.000	0.000	0.000	0.006
Sou'11	0.011	0.000	0.203	0.004	0.047	0.044	0.393	0.003	0.000	0.032	0.063
Vea'09	0.003	0.000	0.004	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000
Vea'10	0.000	0.023	0.056	0.006	0.332	0.086	0.019	0.045	0.000	0.014	0.048
Vea'11	0.005	0.001	0.079	0.057	0.432	0.074	0.235	0.005	0.000	0.069	0.084
Leo'09	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.001
Leo'11	0.000	0.004	0.010	0.001	0.151	0.071	0.144	0.008	0.000	0.016	0.002
Den'05	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mil'05	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.004

N Locations	47 Sen'08	62 Sen'10	49 Sen'11	50 Wig'09	60 Wig'11	59 Orl'10	52 Orl'11	57 Sou'09	59 Sou'10	60 Sou'11
Sen'08	.									
Sen'10	0.568	.								
Sen'11	0.751	0.073	.							
Wig'09	0.053	0.010	0.040	.						
Wig'11	0.147	0.043	0.387	0.564	.					
Orl'10	0.001	0.007	0.010	0.024	0.002	.				
Orl'11	0.002	0.001	0.007	0.000	0.000	0.207	.			
Sou'09	0.000	0.005	0.011	0.000	0.001	0.022	0.106	.		
Sou'10	0.000	0.009	0.001	0.000	0.000	0.419	0.017	0.123	.	
Sou'11	0.166	0.178	0.572	0.048	0.068	0.011	0.061	0.419	0.164	.
Vea'09	0.016	0.014	0.140	0.006	0.030	0.001	0.013	0.150	0.008	0.043
Vea'10	0.098	0.114	0.510	0.006	0.125	0.012	0.029	0.076	0.014	0.342
Vea'11	0.229	0.090	0.927	0.027	0.031	0.006	0.150	0.351	0.084	0.870
Leo'09	0.000	0.000	0.002	0.001	0.000	0.000	0.000	0.001	0.000	0.008
Leo'11	0.030	0.017	0.355	0.017	0.005	0.000	0.000	0.020	0.007	0.480
Den'05	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.018	0.011	0.005
Mil'05	0.000	0.000	0.009	0.000	0.000	0.007	0.024	0.002	0.003	0.006

N	69	52	52	58	49	47	51
Locations	Vea'09	Vea'10	Vea'11	Leo'09	Leo'11	Den'05	Mil'05
Sew'09							
Sew'11							
Neq'09							
Neq'10							
Neq'11							
Bru'09							
Bru'10							
Bru'11							
Dre'08							
Dre'09							
Dre'10							
Dre'11							
Web'09							
Web'10							
Web'11							
Ben'09							
Ben'10							
Ben'11							
Loc'09							
Loc'11							
Dam'09							
Dam'10							
Dam'11							

N Locations	69 Vea'09	52 Vea'10	52 Vea'11	58 Leo'09	49 Leo'11	47 Den'05	51 Mil'05
Sen'08							
Sen'10							
Sen'11							
Wig'09							
Wig'11							
Orl'10							
Orl'11							
Sou'09							
Sou'10							
Sou'11							
Vea'09	.						
Vea'10	0.441	.					
Vea'11	0.633	0.861	.				
Leo'09	0.000	0.004	0.073	.			
Leo'11	0.005	0.609	0.919	0.093	.		
Den'05	0.000	0.000	0.002	0.001	0.000	.	
Mil'05	0.000	0.011	0.055	0.000	0.000	0.000	.

Appendix 5: Pairwise F_{ST} (below diagonal) and their associated p-values (above diagonal) for Maine collections. The highly significant ($p < 0.001$) global F_{ST} was 0.012. The bolded values indicate significant F_{ST} values after sequential Bonferroni correction. Full names are given in Table 3.1.

N	62	51	57	59	45	59	60	50	49	19	56	53
Locations	Sew'09	Sew'11	Neq'09	Neq'10	Neq'11	Bru'09	Bru'10	Bru'11	Dre'08	Dre'09	Dre'10	Dre'11
Sew'09	.	0.039	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.001	0.000
Sew'11	0.005	.	0.014	0.004	0.000	0.000	0.000	0.000	0.000	0.028	0.063	0.104
Neq'09	0.016	0.007	.	0.221	0.172	0.006	0.058	0.005	0.000	0.004	0.000	0.006
Neq'10	0.020	0.009	0.002	.	0.141	0.004	0.122	0.014	0.000	0.001	0.000	0.000
Neq'11	0.030	0.015	0.003	0.003	.	0.142	0.139	0.054	0.000	0.000	0.000	0.000
Bru'09	0.039	0.018	0.008	0.008	0.003	.	0.242	0.878	0.000	0.000	0.000	0.000
Bru'10	0.026	0.015	0.004	0.003	0.004	0.001	.	0.222	0.000	0.000	0.000	0.000
Bru'11	0.045	0.026	0.010	0.008	0.006	-0.003	0.003	.	0.000	0.000	0.000	0.000
Dre'08	0.015	0.013	0.021	0.028	0.025	0.039	0.033	0.050	.	0.713	0.137	0.007
Dre'09	0.004	0.011	0.018	0.023	0.029	0.037	0.031	0.050	-0.004	.	0.345	0.543
Dre'10	0.011	0.004	0.018	0.018	0.019	0.031	0.028	0.038	0.003	0.001	.	0.020
Dre'11	0.012	0.004	0.009	0.016	0.020	0.020	0.019	0.030	0.009	-0.001	0.006	.
Web'09	0.030	0.023	0.015	0.002	0.014	0.014	0.010	0.012	0.036	0.022	0.026	0.028
Web'10	0.032	0.019	0.016	0.012	0.014	0.006	0.008	0.005	0.049	0.042	0.033	0.028
Web'11	0.032	0.015	0.008	0.007	0.004	-0.002	0.000	0.001	0.028	0.029	0.026	0.016
Ben'09	0.033	0.021	0.009	0.007	0.003	0.000	-0.001	0.000	0.035	0.035	0.029	0.024
Ben'10	0.036	0.022	0.006	0.009	0.006	-0.001	0.001	-0.002	0.041	0.041	0.033	0.024
Ben'11	0.028	0.014	0.003	0.001	0.001	0.001	0.001	0.003	0.026	0.021	0.022	0.014
Loc'09	0.023	0.014	0.005	-0.002	0.003	0.004	0.001	0.003	0.029	0.024	0.020	0.021
Loc'11	0.049	0.029	0.019	0.018	0.002	0.001	0.009	0.003	0.046	0.054	0.037	0.035
Dam'09	0.036	0.024	0.008	0.009	0.013	0.005	0.001	0.006	0.042	0.034	0.038	0.022
Dam'10	0.035	0.021	0.009	0.003	0.005	0.003	-0.001	-0.001	0.047	0.045	0.035	0.030
Dam'11	0.044	0.023	0.008	0.010	0.006	-0.004	0.002	-0.005	0.044	0.046	0.035	0.025

N Locations	62 Sew'09	51 Sew'11	57 Neq'09	59 Neq'10	45 Neq'11	59 Bru'09	60 Bru'10	50 Bru'11	49 Dre'08	19 Dre'09	56 Dre'10	53 Dre'11
Sen'08	0.030	0.020	0.008	0.006	0.001	0.000	-0.001	-0.001	0.030	0.031	0.027	0.023
Sen'10	0.022	0.013	0.009	0.005	0.008	0.006	0.004	0.005	0.033	0.027	0.017	0.021
Sen'11	0.029	0.020	0.004	0.009	0.004	0.000	-0.003	0.002	0.033	0.034	0.032	0.021
Wig'09	0.026	0.014	0.002	0.004	0.003	0.004	0.003	0.004	0.025	0.027	0.023	0.016
Wig'11	0.020	0.010	0.002	0.002	0.000	0.005	0.005	0.007	0.019	0.019	0.014	0.011
Orl'10	0.019	0.017	0.002	0.007	0.011	0.007	0.000	0.007	0.031	0.023	0.027	0.015
Orl'11	0.018	0.014	0.002	0.007	0.011	0.008	0.002	0.010	0.028	0.017	0.024	0.012
Sou'09	0.020	0.027	0.007	0.010	0.012	0.015	0.005	0.016	0.040	0.034	0.038	0.028
Sou'10	0.035	0.032	0.012	0.013	0.021	0.013	0.005	0.008	0.051	0.049	0.044	0.034
Sou'11	0.022	0.017	0.003	0.004	0.002	0.004	0.002	0.006	0.028	0.028	0.025	0.020
Vea'09	0.019	0.020	0.006	0.007	0.009	0.011	0.005	0.011	0.028	0.023	0.022	0.020
Vea'10	0.027	0.019	0.005	0.009	0.003	0.002	0.004	0.002	0.034	0.033	0.026	0.022
Vea'11	0.023	0.019	0.005	0.009	0.007	0.004	0.002	0.006	0.033	0.031	0.028	0.022
Leo'09	0.038	0.026	0.011	0.015	0.009	0.008	0.007	0.010	0.036	0.045	0.039	0.031
Leo'11	0.030	0.022	0.006	0.010	0.006	0.008	0.005	0.008	0.032	0.037	0.030	0.027
Den'05	0.047	0.045	0.023	0.030	0.028	0.022	0.013	0.025	0.062	0.060	0.067	0.041
Mil'05	0.040	0.025	0.006	0.012	0.017	0.008	0.008	0.005	0.048	0.042	0.040	0.021

N Locations	37 Web'09	53 Web'10	52 Web'11	65 Ben'09	62 Ben'10	61 Ben'11	63 Loc'09	35 Loc'11	57 Dam'09	56 Dam'10	53 Dam'11
Sew'09	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sew'11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Neq'09	0.000	0.000	0.010	0.003	0.021	0.163	0.048	0.000	0.007	0.007	0.011
Neq'10	0.203	0.000	0.012	0.012	0.003	0.293	0.784	0.000	0.003	0.101	0.003
Neq'11	0.002	0.001	0.108	0.172	0.040	0.330	0.131	0.284	0.001	0.065	0.063
Bru'09	0.001	0.034	0.797	0.530	0.528	0.291	0.079	0.291	0.044	0.137	0.948
Bru'10	0.006	0.009	0.411	0.548	0.332	0.365	0.273	0.013	0.259	0.570	0.197
Bru'11	0.005	0.087	0.421	0.577	0.720	0.178	0.185	0.193	0.063	0.649	0.980
Dre'08	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dre'09	0.002	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Dre'10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dre'11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Web'09	.	0.002	0.003	0.038	0.001	0.206	0.444	0.000	0.002	0.040	0.001
Web'10	0.014	.	0.011	0.033	0.025	0.022	0.016	0.004	0.001	0.319	0.032
Web'11	0.013	0.008	.	0.313	0.272	0.505	0.254	0.172	0.077	0.067	0.556
Ben'09	0.006	0.006	0.001	.	0.819	0.380	0.529	0.220	0.014	0.558	0.509
Ben'10	0.014	0.006	0.001	-0.002	.	0.274	0.271	0.067	0.003	0.222	0.942
Ben'11	0.003	0.006	0.000	0.001	0.001	.	0.745	0.013	0.053	0.270	0.233
Loc'09	0.000	0.007	0.002	-0.001	0.001	-0.001	.	0.019	0.010	0.570	0.074
Loc'11	0.022	0.012	0.003	0.002	0.005	0.009	0.008	.	0.000	0.047	0.333
Dam'09	0.013	0.012	0.004	0.006	0.009	0.005	0.007	0.018	.	0.053	0.018
Dam'10	0.007	0.001	0.004	-0.001	0.002	0.001	-0.001	0.007	0.005	.	0.223
Dam'11	0.016	0.006	-0.001	0.000	-0.004	0.002	0.004	0.001	0.007	0.002	.

N	37	53	52	65	62	61	63	35	57	56	53
Locations	Web'09	Web'10	Web'11	Ben'09	Ben'10	Ben'11	Loc'09	Loc'11	Dam'09	Dam'10	Dam'11
Sen'08	0.003	0.009	-0.002	-0.004	-0.002	0.000	-0.002	0.001	0.007	0.003	-0.002
Sen'10	0.005	0.005	0.010	0.004	0.004	0.007	0.000	0.010	0.015	0.002	0.007
Sen'11	0.012	0.012	0.001	-0.003	-0.002	0.002	0.003	0.006	0.005	0.003	-0.001
Wig'09	0.014	0.015	-0.001	0.005	0.006	0.004	0.003	0.010	0.004	0.008	0.005
Wig'11	0.008	0.013	0.003	0.004	0.005	0.002	0.002	0.007	0.012	0.008	0.006
Orl'10	0.015	0.014	0.004	0.006	0.006	0.007	0.006	0.015	0.005	0.008	0.007
Orl'11	0.013	0.015	0.007	0.007	0.003	0.005	0.005	0.025	0.011	0.010	0.010
Sou'09	0.020	0.021	0.015	0.011	0.011	0.014	0.011	0.024	0.012	0.014	0.017
Sou'10	0.024	0.020	0.010	0.012	0.007	0.016	0.011	0.022	0.012	0.011	0.008
Sou'11	0.011	0.014	0.004	0.002	0.002	0.004	0.002	0.012	0.008	0.006	0.004
Vea'09	0.011	0.018	0.010	0.009	0.012	0.010	0.006	0.021	0.010	0.011	0.013
Vea'10	0.017	0.007	0.006	0.004	0.002	0.004	0.005	0.007	0.010	0.005	0.001
Vea'11	0.016	0.013	0.005	0.002	0.000	0.009	0.002	0.014	0.011	0.008	0.003
Leo'09	0.032	0.022	0.009	0.009	0.006	0.013	0.011	0.017	0.015	0.017	0.007
Leo'11	0.021	0.014	0.006	0.007	0.003	0.007	0.004	0.015	0.010	0.010	0.007
Den'05	0.049	0.039	0.021	0.023	0.017	0.028	0.030	0.032	0.022	0.030	0.019
Mil'05	0.022	0.021	0.012	0.011	0.004	0.012	0.013	0.022	0.013	0.014	0.002

N	47	62	49	50	60	59	52	57	59	60
Locations	Sen'08	Sen'10	Sen'11	Wig'09	Wig'11	Orl'10	Orl'11	Sou'09	Sou'10	Sou'11
Sew'09	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sew'11	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Neq'09	0.014	0.003	0.083	0.211	0.156	0.164	0.169	0.028	0.001	0.161
Neq'10	0.035	0.043	0.007	0.070	0.169	0.012	0.011	0.003	0.001	0.062
Neq'11	0.300	0.011	0.148	0.206	0.535	0.005	0.003	0.007	0.000	0.197
Bru'09	0.376	0.026	0.484	0.090	0.046	0.015	0.005	0.000	0.000	0.048
Bru'10	0.513	0.054	0.870	0.126	0.040	0.362	0.155	0.047	0.046	0.222
Bru'11	0.625	0.068	0.327	0.130	0.031	0.022	0.005	0.000	0.016	0.027
Dre'08	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dre'09	0.000	0.000	0.000	0.000	0.002	0.000	0.007	0.000	0.000	0.000
Dre'10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dre'11	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.000	0.000	0.000
Web'09	0.178	0.076	0.005	0.001	0.012	0.001	0.001	0.000	0.000	0.006
Web'10	0.011	0.050	0.002	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Web'11	0.733	0.004	0.347	0.568	0.208	0.100	0.013	0.000	0.004	0.064
Ben'09	0.974	0.069	0.883	0.039	0.066	0.015	0.006	0.001	0.001	0.147
Ben'10	0.699	0.060	0.731	0.028	0.049	0.021	0.100	0.001	0.015	0.213
Ben'11	0.583	0.010	0.280	0.096	0.237	0.016	0.055	0.000	0.000	0.106
Loc'09	0.728	0.379	0.105	0.153	0.191	0.020	0.030	0.000	0.001	0.250
Loc'11	0.325	0.006	0.071	0.008	0.040	0.001	0.000	0.000	0.000	0.002
Dam'09	0.017	0.000	0.065	0.077	0.001	0.040	0.001	0.001	0.001	0.007
Dam'10	0.138	0.146	0.131	0.010	0.010	0.008	0.002	0.001	0.002	0.019
Dam'11	0.664	0.020	0.599	0.053	0.037	0.017	0.003	0.000	0.011	0.066

N Locations	47 Sen'08	62 Sen'10	49 Sen'11	50 Wig'09	60 Wig'11	59 Orl'10	52 Orl'11	57 Sou'09	59 Sou'10	60 Sou'11
Sen'08	.	0.177	0.931	0.323	0.477	0.072	0.039	0.006	0.007	0.579
Sen'10	0.002	.	0.022	0.002	0.041	0.025	0.036	0.000	0.001	0.034
Sen'11	-0.004	0.007	.	0.183	0.298	0.249	0.097	0.121	0.045	0.894
Wig'09	0.001	0.010	0.002	.	0.640	0.164	0.003	0.002	0.003	0.093
Wig'11	0.000	0.005	0.001	-0.001	.	0.058	0.011	0.002	0.000	0.247
Orl'10	0.004	0.006	0.001	0.002	0.004	.	0.380	0.166	0.348	0.137
Orl'11	0.005	0.005	0.003	0.010	0.007	0.000	.	0.082	0.015	0.199
Sou'09	0.009	0.014	0.003	0.011	0.010	0.002	0.003	.	0.066	0.633
Sou'10	0.009	0.012	0.005	0.011	0.013	0.000	0.006	0.004	.	0.059
Sou'11	-0.001	0.005	-0.003	0.004	0.001	0.003	0.003	0.000	0.005	.
Vea'09	0.005	0.007	0.002	0.006	0.004	0.002	0.005	0.001	0.006	-0.001
Vea'10	0.002	0.005	-0.001	0.004	0.002	0.003	0.005	0.005	0.007	-0.002
Vea'11	0.000	0.005	-0.003	0.005	0.006	0.004	0.002	0.002	0.004	-0.003
Leo'09	0.008	0.019	0.004	0.008	0.012	0.014	0.011	0.012	0.014	0.005
Leo'11	0.004	0.012	0.002	0.002	0.007	0.010	0.008	0.007	0.011	0.000
Den'05	0.020	0.036	0.008	0.022	0.025	0.013	0.017	0.007	0.011	0.011
Mil'05	0.007	0.014	0.003	0.012	0.010	0.007	0.005	0.013	0.007	0.007

N Locations	69 Vea'09	52 Vea'10	52 Vea'11	58 Leo'09	49 Leo'11	47 Den'05	51 Mil'05
Sew'09	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sew'11	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Neq'09	0.014	0.058	0.064	0.001	0.028	0.000	0.042
Neq'10	0.009	0.006	0.007	0.000	0.004	0.000	0.001
Neq'11	0.011	0.277	0.032	0.009	0.062	0.000	0.000
Bru'09	0.001	0.223	0.072	0.008	0.010	0.000	0.011
Bru'10	0.024	0.104	0.207	0.010	0.060	0.001	0.011
Bru'11	0.001	0.352	0.071	0.004	0.023	0.000	0.089
Dre'08	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dre'09	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dre'10	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Dre'11	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Web'09	0.003	0.000	0.001	0.000	0.000	0.000	0.000
Web'10	0.000	0.023	0.001	0.000	0.001	0.000	0.000
Web'11	0.002	0.046	0.045	0.004	0.035	0.000	0.002
Ben'09	0.001	0.115	0.237	0.002	0.016	0.000	0.001
Ben'10	0.000	0.225	0.450	0.022	0.117	0.000	0.085
Ben'11	0.001	0.085	0.009	0.001	0.027	0.000	0.001
Loc'09	0.012	0.045	0.181	0.001	0.090	0.000	0.000
Loc'11	0.000	0.043	0.002	0.000	0.001	0.000	0.000
Dam'09	0.001	0.004	0.001	0.000	0.002	0.000	0.001
Dam'10	0.001	0.075	0.011	0.000	0.006	0.000	0.001
Dam'11	0.000	0.319	0.122	0.016	0.020	0.000	0.252

N Locations	69 Vea'09	52 Vea'10	52 Vea'11	58 Leo'09	49 Leo'11	47 Den'05	51 Mil'05
Sen'08	0.044	0.221	0.502	0.010	0.098	0.000	0.018
Sen'10	0.009	0.042	0.047	0.000	0.001	0.000	0.000
Sen'11	0.180	0.633	0.884	0.065	0.208	0.013	0.118
Wig'09	0.028	0.109	0.061	0.009	0.197	0.000	0.002
Wig'11	0.044	0.295	0.036	0.001	0.023	0.000	0.004
Orl'10	0.131	0.168	0.069	0.000	0.002	0.001	0.022
Orl'11	0.032	0.043	0.222	0.001	0.010	0.000	0.036
Sou'09	0.344	0.086	0.259	0.000	0.028	0.013	0.001
Sou'10	0.013	0.026	0.074	0.000	0.004	0.005	0.022
Sou'11	0.696	0.635	0.971	0.054	0.577	0.003	0.015
Vea'09	.	0.270	0.311	0.000	0.019	0.000	0.000
Vea'10	0.001	.	0.425	0.052	0.276	0.000	0.020
Vea'11	0.001	0.000	.	0.300	0.635	0.002	0.043
Leo'09	0.014	0.005	0.001	.	0.674	0.000	0.000
Leo'11	0.007	0.002	-0.001	-0.002	.	0.000	0.000
Den'05	0.022	0.017	0.012	0.014	0.019	.	0.002
Mil'05	0.013	0.007	0.006	0.014	0.015	0.012	.