Genetic variation between outer-coastal and fjord populations of Pacific herring (*Clupea pallasii*) in the eastern Gulf of Alaska

Sharon L. Wildes (contact author)
Johanna J. Vollenweider
Hanhvan T. Nguyen
Jeffrey R. Guyon

Email address for contact author: Sharon.Wildes@noaa.gov

Auke Bay Laboratories
Alaska Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
17109 Point Lena Loop Rd.
Juneau, Alaska, 99801-8344

Abstract—Pacific herring (*Clupea pallasii*) from the Gulf of Alaska were screened for temporal and spatial genetic variation with 15 microsatellite loci. Thirteen collections were examined in this study: 11 from Southeast Alaska and 2 from Prince William Sound, Alaska. Although F_{ST} values were low, a neighbor-joining tree based on genetic distance, homogeneity, and F_{ST} values revealed that collectively, the Berners Bay and Lynn Canal (interior) collections were genetically distinct from Sitka Sound and Prince of Wales Island (outer-coastal) collections. Temporal genetic variation within regions (among three years of Berners Bay spawners and between the two Sitka Sound spawners) was zero, whereas 0.05% was attributable to genetic variation between Berners Bay and Sitka Sound. This divergence may be attributable to environmental differences between interior archipelago waters and outer-coast habitats, such as differences in temperature and salinity. Early spring collections of nonspawning Lynn Canal herring were nearly genetically identical to collections of spawning herring in Berners Bay two months later—an indication that Berners Bay spawners over-winter in Lynn Canal. Southeast Alaskan herring (collectively) were significantly different from those in Prince William Sound. This study illustrates that adequate sample size is needed to detect variation in pelagic fish species with a large effective population size, and microsatellite markers may be useful in detecting low-level genetic divergence in Pacific herring in the Gulf of Alaska.

Manuscript submitted 31 August 2010.
Manuscript accepted 22 June 2011.
Fish. Bull. 109:382–393 (2011).

Defining the population structure of a species is fundamental for fishery management and resource conservation. Sustainable management of commercially harvested stocks depends on a clear understanding of the extent of fish movements and migratory behavior, spawning-site fidelity, and degree of immigration and emigration. One means to assess population structure is through genetic analysis. Armed with the knowledge of genetic stock structure, managers can use the appropriate spatial scale to understand life history, essential habitat, migration patterns, distribution, connectivity and isolation of stocks, recruitment, and spawning behavior. Understanding genetic diversity, which allows for adaptation to changing environmental conditions, is vital information for conserving a species. Detecting genetic divergence within pelagic fish species, however, is often difficult because of large population sizes that retard genetic drift and gene flow among cohorts through dispersal and migration (Shaklee and Bentzen 1998; Waples, 1998). Even small numbers of migrants or episodic straying events can lead to increased genetic connectivity among otherwise isolated stocks. Genetic divergence may occur if gene flow is interrupted by a single factor or combination of factors such as physical barriers, temporal variation (time of spawning), and spawning-site and natal-site fidelity.

A particularly large void of genetic information exists for forage fishes in the Gulf of Alaska. These species play a role of great consequence in marine ecosystems, as prey for most commercially important fish species. Without these nutritionally rich fish, many higher trophic level species might lack the resources to overwinter. Yet the amount of genetic information available for forage species is minimal at best. One example of a forage species is the Pacific herring (*Clupea pallasii*), which provides a critical link between lower and higher trophic levels. Herring typically eat crustaceans and small fish, and serve as forage for whales, sea lions, birds, larger fish, (Bakun, 2006; Hart, 1973; Hourston and Haegele, 1980), and humans.

There are few genetic studies of Pacific herring in Alaska, particularly in regions that have experienced a recent decline in stocks, such as Prince William Sound in the central Gulf of Alaska (GOA), and Lynn Canal in southeast Alaska. Herring abundance in Lynn Canal has declined since the late 1970s and has not recovered to pre-1980 levels, despite the closure of the fishery in 1981. One criterion for listing a stock

Table 1

Location and dates for Pacific herring (*Clupea pallasii*) collections from Southeast and Prince William Sound (PWS), Alaska. Sample size (*n*) reflects the number of individuals successfully genotyped and used in analyses. The two Lynn Canal collections in the early spring of 2008 are noted as Lynn Canal 08, a and b.

Sample	Latitute (N)	Longitude (W)	Sampling date	*n*
Spawning fish				
Berners Bay 07	58°40.9′	134°59.1′	4/5/2007	52
Berners Bay 08	58°40.9′	134°59.1′	5/3/2008	126
Berners Bay 09	58°39.3′	134°58.5′	5/5/2009	148
Hobart Bay 08	57°27.6′	133°21.1′	5/9/2008	128
Hoonah Sound 08	57°36.6′	135°21.5′	4/23/2008	100
Sitka Sound 07	57°05.1′	135°30.4′	3/29/2007	75
Sitka Sound 08	57°08.9′	135°28.7′	4/4/2008	131
Nonspawning fish				
Lynn Canal 07	58°27.2′	134°47.0′	11/10/2007	97
Lynn Canal 08a	58°27.2′	134°49.0′	2/23/2008	98
Lynn Canal 08b	58°29.6′	134°49.2′	2/25/2008	98
Nichols Bay 07	54°43.8′	132°08.3′	6/14/2007	97
Western PWS07	60°13.6′	148°11.0′	7/15/2007	99
Eastern PWS07	60°39.2′	134°49.2′	12/2/2007	92

as threatened or endangered under the Endangered Species Act is a stock's discreteness or uniqueness. To date, four genetic studies have been completed in the eastern GOA. One study of allozymes indicated that, in general, GOA populations are genetically distinct from those to the south in British Columbia, Canada and west of Kodiak Island, (Grant and Utter, 1984): one locus in that study indicated heterogeneity among populations within the GOA. In more recent studies of microsatellite DNA variation, genetically discrete stocks of herring were detected in British Columbia (Beacham et al., 2008) and in Puget Sound in Washington State (Small et al., 2005). In both studies, genetic divergence among these discrete stocks was attributed to different spawning times, geographic isolation, or both. In the fourth study, O'Connell et al. (1998a) confirmed genetic differentiation between Prince William Sound and western Alaska Pacific herring populations, using microsatellites.

This study was conducted to determine whether Lynn Canal Pacific herring (hereafter, herring) in Southeast Alaska are genetically distinct from other eastern Gulf of Alaska herring and whether overwintering Lynn Canal herring spawn in Berners Bay. We evaluated 22 existing microsatellite loci developed for Pacific and Atlantic herring (*Clupea harengus*) (Miller et al., 2001; McPherson et al., 2001; Olsen et al., 2002; O'Connell et al., 1998b) for their ability to distinguish herring populations in the eastern Gulf of Alaska. Our results indicate that this class of highly polymorphic nuclear DNA markers, combined with adequate sample sizes, can resolve spatial patterns of genetic heterogeneity consistent with discrete stocks of Pacific herring.

Materials and methods

Sample collections

Thirteen collections of herring were made in seven locations in Southeast Alaska from 2007 to 2009 (Table 1, Fig. 1). These samples included three collections of nonspawning, overwintering herring in Lynn Canal and three collections of spawning herring in associated Berners Bay, which is located on the eastern side of Lynn Canal and hosts a high concentration of spring spawning herring. Three consecutive years of spawning fish were sampled in Berners Bay: about two weeks before spawning in 2007 (Berners07) and during the spawning season in 2008 and 2009 (Berners08 and Berners09, respectively). Three samples of overwintering fish were collected in Lynn Canal during the winter of 2007–08. The Lynn07 collection was made in early winter (November), whereas collections from along the shoreline (Lynn08a) and from a deep trench offshore (Lynn08b) were made several days apart, approximately two months (late February) before the spawning season. In 2008, samples of spawning fish were also collected in Hobart Bay, approximately 200 km south of Berners Bay on the mainland in central Southeast Alaska, and in Hoonah Sound, on northern Chichagof Island. Two collections were made in Sitka Sound, on the outer coast of Baranof Island, during the spawning season in 2007 and 2008, and one collection of immature fish was obtained from Nichols Bay located at southern Prince of Wales Island in 2007. Two additional collections were made in Prince William Sound: one from a postspawning group in Whale Bay in 2007, located on the western side of the sound (wPWS), and one from an

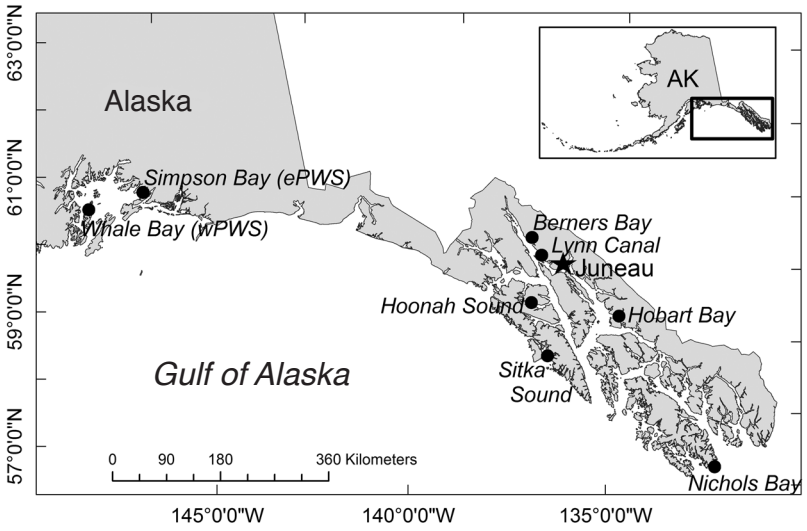

Figure 1

Sampling locations for Pacific herring (*Clupea pallasii*) collected in the Gulf of Alaska for genetic analysis, 2007–09.

overwintering aggregate from Simpson Bay, in eastern Prince William Sound (ePWS) in December, 2007.

All samples were collected by trawl, seine, or castnet. Lynn Cana and Hobart Bay samples were collected with a midwater trawl. Spawning herring in Berners Bay were collected along the shore with a castnet. Hoonah Sound samples were hand netted from the commercial roe-on-kelp fishery. In Sitka Sound, herring were collected from a purse seiner chartered by the Alaska Department of Fish and Game (ADFG) during the test fishery for the Sitka Sound sac roe fishery. Samples from Nichols Bay and Prince William Sound were collected with a beach seine. All samples, except those from Nichols Bay, were mature fish. All collections were shipped as whole frozen fish to the Auke Bay Laboratories in Juneau, AK.

Laboratory procedures

DNA was extracted from tissue samples (heart, muscle, or fin) by following DNeasy genomic DNA extraction methods (Qiagen, Valencia, CA). A master mix of 1 µL 10× PCR buffer, 4.54 µL deionized water, 0.6 µL $MgCl_2$ (25 mM), 0.8 µL dNTPs (10 mM), 0.5 µL forward primer (10 mM), 0.4 µL reverse primer (10 mM), 1.0 µL of fluorescent labeled primer (1 mM), and 0.16 µL of TAQ DNA polymerase was combined with 1 µL of DNA. Initially, 22 microsatellite loci were amplified by polymerase chain reaction (PCR), by using a Gene Amp 9700 thermocycler (Applied Biosystems, Foster City, CA). The resulting PCR products were size fractionated on a DNA sequencer (Licor 4200 and 4300) by using known molecular size standards, and genotypes were scored by using SAGA software (Licor, inc., Lincoln, NE). Loci were double-scored and those with a 2-bp repeat were aligned by allele size and analyzed a second time to ensure data integrity. Individuals for which there were missing data for three or more of the 15 loci were dropped from further analyses. The numbers of individuals used in analyses (n) are given in Table 1.

Genetic analysis

Initial data from 22 loci were examined for the possibility of scoring errors, null alleles, or large allele drop out with MICROCHECKER software (van Oosterhout et al., 2004). Deviation from Hardy-Weinberg expectation (HWE) was tested for each locus for each sample collection by using Fisher's exact test with GENEPOP software, vers. 4.0 (Raymond and Rousset, 1995). Linkage disequilibrium was tested with Slatkin's (1994) method implemented in GENEPOP to confirm that loci were segregating independently. Markov chain parameters of 50,000 dememorizations, 500 batches, and 25,000 iterations were used to calculate an accurate and reliable test. The methods of Weir and Cockerham (1984) implemented in FSTAT, vers. 2.9.3.2 (Goudet, 1995) were used to calculate F_{IS} for each collection by locus, and each locus over all collections. A gene diversity analysis was conducted with GENEPOP software to examine observed and expected heterozygosities per locus and per collection. Allelic richness by collection was calculated in FSTAT. Effective number of alleles was calculated as $1/(1–H_e)$. Chord distances (Cavalli-Sforza and Edwards, 1967) were calculated among all pairs of collections with PHYLIP software, vers. 3.69 (Felsenstein, 1989), and a neighbor-joining tree was constructed to examine the relationships among collections. Data were bootstrapped (1000 replicates) by using allele replacement and loci replacement in PHYLIP, and a summary of the replicates (consensus tree) was constructed to examine the consistency of putative genetic partitions.

Homogeneity tests of allelic frequencies were conducted for all pairs of collections by using χ^2 probabilities (Markov chain algorithm, GENEPOP, vers. 4.0). *P*-values were corrected for multiple testing with the false discovery rate test (Benjamini and Yekutieli, 2001). Pairwise F_{ST} values, a measure of genetic divergence, were calculated with the Weir and Cockerham (1984) algorithm in FSTAT and evaluated with a permutation test.

The amount of molecular diversity was measured within sites at locations for which multiple years of data from the same location were available (Berners Bay and Sitka Sound). The diversity among years was compared with the diversity between locations, by using a hierarchical AMOVA (analysis of molecular variance) with 1000 permutations in ARLEQUIN, vers. 3.5 (Excoffier and Schneider, 2005). The amount of variation was partitioned in the following categories: within the individual sample (Fsc), among collections from different years at the same location (Fct), and between locations (Fst).

Results

Seven of the 22 loci were dropped from further analyses because of either large allele drop out (*Cpa102*), suspected null alleles (*Cpa8*), stutter bands (*Cpa104, Cha123*), one-bp shifts (*Cpa100*), or because of our inability to resolve the loci for all data sets (*Cpa101, Cpa107a*), or because of one-bp shifts (*Cpa100*); however, further optimization in the laboratory may prove these loci useful for future studies. The remaining 15 loci were *Cpa103, Cpa108, Cpa111, Cpa112, Cpa113, Cpa114*, (Olsen et al., 2002) *Cpa4, Cpa6, Cpa27, Cpa107, Cpa125, Cpa134*, (Miller et al., 2001) *Cha1017* and *Cha1020*, (McPherson et al., 2001) and *Cha63* (O'Connell et al., 1998b) (Appendix). These loci were highly polymorphic in general; the average number of alleles was 30.8, ranging from 7 at *Cha1017* to 64 at *Cpa112* (Appendix). Several loci with a large number of unique alleles, such as *Cpa134* (58 alleles), and *Cpa112* (64 alleles), exceeded the number of individuals (n=52) in the Berners07 collection. This finding illustrates the need for ample sample sizes for use with highly polymorphic markers. Average observed heterozygosity across all populations for each locus varied from 0.45 to 0.99. The number of alleles (n_a) found in each collection was similar overall, with the exception of those from the Berners07 collection, which were typically lower. The allelic richness (a) and effective number of alleles (n_{eff}) were also similar overall (Appendix).

Observed and expected heterozygosities were overall in close agreement for all loci. Nine of the 195 tests for Hardy Weinberg equilibrium had an excess of homozygotes—an amount expected by chance alone—and importantly, no excesses of homozygotes at any one locus were found in more than two collections—evidence that null alleles did not contribute significant bias to estimates derived from these 15 microsatellites. Low F_{IS} values for most loci indicate random mating within each collection. *Cha1017* and *Cpa27* had a slightly high overall F_{IS} value of 0.167 and 0.114, respectively. Nine out of 105 linkage disequilibrium tests in GENEPOP were significant. No locus had a significant value at more than two collections, indicating that all loci were likely inherited independently.

Global AMOVA results for the three Berners Bay collections and two Sitka Sound collections revealed that nearly all the variation was within the individuals (99.6%) and within the collection (0.35%). The remainder of the genetic variation was attributable to regional differences between the two locations (0.05%; P=0.058). No variation was attributable to temporal differences among collection years within a region.

Pairwise homogeneity tests of allelic frequency revealed statistically significant genetic differentiation at 10 (7 after correction for multiple testing) of the 21 sample pairs of spawning fish collections (below diagonal of boxed area in Table 2). The most notable result was that Berners08 and 09 spawners were significantly different from the Sitka Sound and Hoonah Sound spawners in all pairwise homogeneity tests of allele frequencies. The Berners07 collection of prespawning fish was generally homogeneous with all other collections, possibly because of small sample size. Berners Bay spawning herring and those collected in the winter from nearby Lynn Canal, were homogeneous, with the exception of the early winter collection (Lynn07). The two Lynn Canal collections in the early spring 2008 (Lynn08, a and b) were nearly identical to each other and were also highly similar to those collections of spawning herring in the nearby Berners08 sample taken several months later.

Among the collections of spawning fish, seven of the 21 F_{ST} estimates were significant before correction. Only one pairwise F_{ST} value was significant after correction: namely the F_{ST} value for Hoonah Sound and Berners08. Berners Bay and Lynn Canal collections, with the exception of Lynn07, exhibited an F_{ST} of 0, indicating a high level of genetic homogeneity in this region over several years of collection.

Significant F_{ST} values and differences in allele frequencies among regional groups were more evident after spatially homogeneous and temporal collections were pooled (Table 3). Collections of herring from Prince William Sound were significantly different (P=0.001) from both outer-coastal and interior collections in Southeast Alaska (except for the single collection of Hobart Bay). Herring samples from the two outer-coastal locations, Sitka Sound and Nichols Bay, were homogeneous (P=0.28), but were collectively divergent from interior Berners Bay and Lynn Canal collections.

Genetic distances and the resulting neighbor-joining tree generally mirrored the results in Tables 2 and 3, however, bootstrap support for the tree was weak. PWS grouped together in 60% of the resamplings, Berners08, Lynn08a, and Lynn08b grouped together 50% of the time, and all other branches grouped less than 50%.

Table 2

F_{ST} values estimated between sample pairs of Pacific herring (*Clupea pallasii*) in the eastern Gulf of Alaska (above diagonal). Below the diagonal are *P*-values from pseudo-exact homogeneity tests between collections. Significances based on 95% confidence intervals from permutation tests (bold) and after correction for multiple testing (asterisk) are indicated. Estimates within shaded area are comparisons between groups of spawning fish; individuals from all other sites were in nonspawning condition. *n*=the number of individuals in the sample. PWS=Prince William Sound.

	Berners Bay 2007 *n*=52	Berners Bay 2008 *n*=126	Berners Bay 2009 *n*=148	Hobart Bay 2008 *n*=128	Hoonah Sound 2008 *n*=100	Sitka Sound 2007 *n*=75	Sitka Sound 2008 *n*=131	Lynn Canal 2007 *n*=97	Lynn Canal 2008a *n*=98	Lynn Canal 2008b *n*=98	Nichols Bay 2007 *n*=97	Western PWS 2007 *n*=99	Eastern PWS 2007 *n*=92
Berners Bay 2007	—	0	0	0.004	0.0009	0.0002	0.0013	0.0008	0.0001	0	0.0018	0	0.0004
Berners Bay 2008	0.5186	—	0	0	**0.0009***	**0.0008**	**0.0004**	**0.0007**	0	0	0.0007	**0.0013**	**0.0003**
Berners Bay 2009	0.2853	0.1045	—	0.0007	**0.0004**	0.0002	**0**	0.0004	0	0	**0.0017***	0.0006	**0.0005**
Hobart Bay 2008	0.2276	**0.0287**	0.1564	—	**0.0009**	0.0002	0.0001	0.0006	0.0007	0	0	0.0015	0.0005
Hoonah Sound 2008	0.1056	**<0.0001***	**0.0027***	**0.0073***	—	0.0002	0.0006	**0.0014**	**0.0011**	**0.0019***	**0.0025**	**0.0010**	**0.0013***
Sitka Sound 2007	0.0567	**<0.0001***	**0.0044***	**0.0318**	0.0558	—	0.0001	0.0021	0.0014	0.0004	0.0012	0.0015	**0.0020**
Sitka Sound 2008	0.5448	**0.0052***	**0.0045***	0.3463	**0.0260**	0.4273	—	0	0.0009	0.0008	0.0011	**0.0012**	**0.0013**
Lynn Canal 2007	0.5566	**0.0063***	0.2992	0.2443	**0.0069***	**0.0050***	0.1494	—	0	0	**0.0015**	**0.0013**	**0.0001**
Lynn Canal 2008a	0.4297	0.5911	0.2555	0.3345	**0.0039***	**0.0370**	**0.0500**	0.4221	—	0	0.0009	0.0007	**0.0012**
Lynn Canal 2008b	0.7783	0.9922	0.2448	0.4537	**0.0004***	0.2216	0.3269	0.5837	0.9988	—	0.0006	0.0003	**0.0002**
Nichols Bay 2007	0.4599	0.1101	**<0.0001***	0.0889	**0.0001***	0.1423	0.2881	**0.0324**	0.2490	0.6419	—	0.0021	**0.0019**
Western PWS	0.5421	**0.0472**	0.1814	0.0536	**0.0076***	0.0859	**0.0466**	0.0742	0.5353	0.9099	0.1254	—	0.0012
Eastern PWS	0.3883	**0.0448**	0.1080	0.0663	**0.0006***	**0.0057***	**0.0151**	**0.0230**	**0.0058***	0.0896	**0.0008***	0.4830	—

Discussion

Genetic structure was evident among herring populations in the eastern Gulf of Alaska. Collections from the fjord system of Berners Bay and Lynn Canal were significantly differentiated from the outer coast collections (Sitka Sound). The level of differentiation was surprising given the small geographical separation of approximately 235 km between the two areas and the higher genetic connectivity typical among marine pelagic species such as herring. Spawning fish from three years of collection in Berners Bay and overwintering fish from Lynn Canal consistently grouped together, indicating these fish may be overwintering in Lynn Canal and spawning in Berners Bay. Early spring collections in Lynn Canal in 2008 (Lynn08a and Lynn08b) were particularly homogeneous with the spring spawning group in Berners Bay (Berners08). These three collections grouped together in the neighbor-joining tree and remained together more than 50% of the time in the consensus tree with both loci replacement and allele replacement bootstrapping methods. Hobart Bay, located several hundred km to the south of Berners Bay in the interior waters of Southeast Alaska, was not genetically distinct from either the fjord group of Berners Bay and Lynn Canal or outer-coastal Sitka Sound, indicating that either there is more extensive gene flow between these regions—Hobart Bay is an interior water body but located along the main waterway that bisects southeast Alaska—or that the sample size of the single collection at Hobart Bay (*n*=100) may not be large enough for detection of differentiation as statistical power decreases considerably if sample sizes are unbalanced (Goudet, 1996; Waples and Gaggiotti, 2006). Pacific herring from Hoonah Sound, however, were genetically distinct from herring in Berners Bay and Lynn Canal interior collections. Herring from this region also had a unique fatty acid signature differing from those at all other locations tested in Southeast Alaska (Otis[1]).

Results of our genetic study tend to verify previous morphological, tagging, and genetic studies that have indicated reduced gene flow among regions within Southeast Alas-

[1] Otis, T., R. Heintz, and J. Maselko. 2010. Investigation of Pacific herring (*Clupea pallasii*) stock structure in Alaska using otolith microchemistry and heart tissue fatty acid composition. Final Rept. submitted to EVOS-TC (*Exxon Valdez* Oil Spill Trustee Council). [Available at http://www/evostc.state.ak.us/Files.cfm?doc=/Store/FinalReports/2007-07769-Final.pdf]

Table 3

F_{ST} values for Pacific herring estimated between sample pairs from geographic regions in the eastern Gulf of Alaska (above diagonal). Below the diagonal are *P*-values from pseudo-exact homogeneity tests of allele frequencies between collections. Significance of tests, based on 95% confidence intervals from permutation tests before (bold) and after (asterisk) corrections for multiple tests are indicated. *n*=the number of individuals in the collection.

	Berners Bay–Lynn Canal *n*=619	Hobart Bay *n*=128	Hoonah Sound *n*=100	Sitka Sound *n*=206	Nichols Bay *n*=97	Prince William Sound *n*=191
Berners Bay–Lynn Canal	—	0.0004	**0.0011***	**0.0008***	**0.0013**	**0.0004***
Hobart Bay	0.1000	—	**0.0009**	0.0001	0	0.0007
Hoonah Sound	**<0.0001***	**0.0070**	—	0.0005	**0.0025**	**0.0009***
Sitka Sound	**<0.0001***	0.1600	**0.0100**	—	0.0011	**0.0011***
Nichols Bay	**0.0020***	0.0890	**0.0001***	0.2750	—	**0.0017**
Prince William Sound	**0.0010***	0.0500	**0.0001***	**0.0010***	**0.0020***	—

ka. McHugh (1954) points out that the expected broad latitudinal clines of morphological characters, such as vertebral counts and growth rates, differ sharply in some geographically adjacent areas within Southeast Alaska, despite similar environmental conditions affecting these phenotypes, and suggests that a degree of isolation may be responsible. Tagging studies corroborate these findings. Herring tagged in the Juneau area were not recovered in the Cape Ommaney reduction fishery on South Baranof Island (Rounsefell and Dahlgren, 1935); however, fish tagged in Sitka Sound and Craig (southern Prince of Wales Island near Nichols Bay) were both detected in this fishery and were interpreted as evidence of an extensive movement and intermingling between these two regions (Skud, 1963). Previous genetic studies indicate that outer-coastal herring from Southeast Alaska were not significantly different from the majority of spawning herring in British Columbia. That study indicated that herring spawning at heads of inlets or ends of fjords migrate relatively short distances to feed in the summer, whereas fish that spawn in exposed, coastal locations may migrate to the continental shelf to feed (Beacham et al., 2008).

Recent genetic studies of herring in the Northeast Pacific Ocean, have indicated that discrete populations exist in British Columbia (Beacham et al., 2008), and Puget Sound, Washington (Small et al., 2005), where some degree of geographic isolation or differences in spawning timing exist. The environments of outer-coastal areas in Southeast Alaska differ from those in interior waterways. This contrast may induce selective pressures on fjord/inland populations owing to selection effects of salinity and may effectively isolate these groups and lead to differentiation at neutral microsatellite loci through drift. A salinity of 21 to 22 ppt (parts per thousand) was reported for the interior waters of Berners Bay (Harris et al.[2]), compared to salinities of outer-coastal seawater. Outer-coastal water salinity is higher and directly affects vertebral counts (Schmidt, 1917). Isolating mechanisms have been associated with specific salinity conditions on spawning locations (Bekkevold et al., 2005), and one microsatellite locus, *Cpa112*, is known to be influenced by divergent selection associated with salinity in Atlantic herring (Andre et al., 2010). *Cpa112* does not appear to be under selection in Pacific herring in the Gulf of Alaska because our study showed this locus to be relatively homogeneous, significantly differentiating only Prince William from Hoonah Sound.

Spawning time for herring varies annually in response to temperature. In 2009, initial spawning occurred 15–16 April for Nichols Bay, directly followed by Sitka Sound, 18–20 April, and three weeks later, 11–12 May for Berners Bay (Pritchett and Hebert[3]). Spawning usually occurs in Berners Bay approximately three weeks later than in Sitka Sound because interior waters remain colder later into the season.

Spawn timing and age of fish can be important considerations for collection of samples for genetic analyses. Spawning waves, where older fish spawn first, followed by younger year classes in the ensuing weeks, have been identified in Atlantic herring (McPherson et al., 2003). In age-structured populations with overlapping generations, allele frequencies are predicted to differ among age classes because of "sweepstakes" recruitment, where a small number of spawners are disproportionately successful in reproducing offspring, compared with the massive number of spawners who fail to leave offspring (Jorde and Ryman, 1996). Age analysis conducted by Pritchett and Hebert[3] revealed that the largest age class of herring in Lynn Canal in 2008 was 6 years, and 8+ years in Sitka Sound. Fish in

[2] Harris, P. M., S. W. Johnson, L. G. Holland, A. D. Neff, J. F. Thedinga, and S. D. Rice. 2005. Hydrocarbons and fisheries habitat in Berners Bay, Alaska: Baseline monitoring associated with the Kensington Gold Mine. Alaska Fisheries Science Center Processed Report 2005–06, 44 p. Alaska Fisheries Science Center, NMFS, NOAA, Juneau, AK.

[3] Pritchett, M., and K. Hebert. 2008. 2009 report to the Alaska Board of Fisheries: Southeast Alaska–Yakutat herring fisheries. Fishery Management Report 08-65, 25 p. Alaska Department of Fish and Game, Anchorage, AK.

our collections from 2008 were about two years younger (on average 4.6 years for Berners Bay herring and 6 years for Sitka Sound herring) than those in the ADFG database for that year. Analysis by year class may be beneficial in future studies in order to examine allele frequencies by age and possible heterogeneity among year classes or spawning waves.

Genetic differences were significant between Southeast Alaska (collectively) and the two collections from Prince William Sound, although no inference about population structure within Prince William Sound is made here, because both of the collections comprised nonspawning fish obtained from a single year. Larger sample sizes and multiyear collections and the use of microsatellite markers may be useful for further genetic studies within Prince William Sound.

Large numbers of alleles at some of the microsatellites and the large effective population size of herring necessitate analysis of adequate numbers of samples to detect population structure. Accordingly, samples sizes were increased during the course of the present study, effectively increasing the power of the analyses. Results of earlier analyses after one and two years of sampling did not reveal significant genetic differences among collections in our study, and notably the number of alleles at several loci exceeded the number of individuals in a collection—an important consideration when using highly polymorphic markers. Low F_{ST} values might be expected because highly polymorphic loci negatively correlate with F_{ST} values (O'Reilly et al., 2004). Berners07 was generally homogeneous with all other collections in pairwise tests of differentiation (homogeneity and F_{ST}), possibly owing to small sample size, which reduces the reliability of the estimate.

Low F_{ST} values, weak bootstrap support for some of the branches of the neighbor-joining tree, and an AMOVA illustrating high genetic variation within individual samples, indicate that population structure among regional groups of herring in Southeast Alaska is detectable but weak. This inference would further indicate low-level or episodic gene flow among these regions.

Conclusion

In conclusion, Pacific herring from Berners Bay and over-wintering fish in Lynn Canal, in the archipelago of Southeast Alaska, were genetically divergent from the spawners along the outer coast of the eastern Gulf of Alaska. Lack of recovery of the Berners Bay population despite closure of the fisheries, may be due to spatial isolation and adaptation to local environmental conditions. Other potential causes for the lack of recovery may be increased predation from expanding populations of sea lions and humpback whales (Rice et al.[4]) or water disturbance in spawning areas and water pollution. Additional pressures on this stock could lead to substantial declines in Lynn Canal herring abundance and in the abundances of fish and marine mammals that forage on them.

Acknowledgments

We thank all who contributed samples: R. Brenner, D. Harris, S. Moffitt (ADFG); C. Gabriele (Glacier Bay National Park); S. Johnson, J. Thedinga, F. Sewall, A. Eller, and K. Cox (Alaska Fisheries Science Center-[AFSC]). We thank C. Marvin (AFSC), N. Rutecki, and L. Miller for their assistance in the laboratory. M. Canino, M. Carls, C. Kondzela, A. Moles, T. McCraney, J. Rice (AFSC), D. Tallmon (University of Alaska Fairbanks), Stew Grant (ADFG) and Jeff Olsen (United States Fish and Wildlife Service [USFWS]) for early reviews of the manuscript, R. Waples (Northwest Fisheries Science Center) and J. Maselko (AFSC) for technical assistance, and J. Hudson (USFWS) for aging fish.

Literature cited

Andre, C., L. C. Larsson, L. Laikre, D. Bekkevold, J. Brigham, G. R. Carvalho, T. G. Dahlgren, W. F. Hutchinson, S. Mariani, K. Mudde, D. E. Ruzzante, and N. Ryman.
2010. Detecting population structure in a high gene-flow species, Atlantic herring (*Clupea harengus*): direct, simultaneous evaluation of neutral vs. putatively selected loci. Heredity, doi:10.1038, 11 p.

Bakun, A.
2006. Wasp-waist populations and marine ecosystem dynamics: navigating the "predator pit" topographies. Prog. Oceanogr. 68:271–288.

Beacham, T. D., J. F. Schweigert, C. MacConnachie, K. D. Le, and L. Flostrand.
2008. Use of microsatellites to determine population structure and migration of Pacific herring in British Columbia and adjacent regions. Trans. Am. Fish. Soc. 137:1795–1811.

Bekkevold, D., C. Andre, T. G. Dahlgren, L. A. W. Clausen, E. Torstensen, H. Mosegaard, G. R. Carvalho, T. B. Christensen, E. Norlinder, and D. E. Ruzzante.
2005. Environmental correlates of population differentiation in Atlantic herring. Evolution 59:2656–2668.

Benjamini, Y., and D. Yekutieli.
2001. The control of the false discovery rate in multiple testing under dependency. Ann. Stat. 29:1165–1188.

Cavalli-Sforza, L. L., and A. W. Edwards.
1967. Phylogenetic analysis. Models and estimation procedures. Am. J. Human Genet. 19:233–257.

Excoffier, L. G. L., and S. Schneider.
2005. Arlequin version 3.5: An integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1:47–50.

Felsenstein, J.
1989. PHYLIP—phylogeny inference package (vers. 3.2). Cladistics 5:164–166.

[4] Rice, S., R. Heintz, J. Moran, T. Quinn, and J. Straley. 2008. Significance of whale predation on natural mortality rate of Pacific herring in Prince William Sound. Annual Progress Report to the Exxon Valdez Oil Spill Trustee Council for Restoration Project 080804, 9 p. Alaska Fisheries Science Center, NMFS, NOAA, Juneau, AK. [Available at: http://www.evostc.state.ak.us/Projects/, accessed June 2011.]

Goudet, J.
1995. FSTAT: A computer program to calculate F-statistics. J. Heredity 86:485–486.
Goudet, J., M. Raymond, T. de Meeüs, and F. Rousset.
1996. Testing differentiation in diploid populations. Genetics 144:1933–1940.
Grant, W. S., and F. M. Utter.
1984. Biochemical population genetics of Pacific herring. Can. J. Fish. Aquat. Sci. 41:856–864.
Hart, J. L.
1973. Pacific fishes of Canada, 180 p. Fish. Res. Board Canada, Ottawa, Ontario.
Hourston, A. S. and C. W. Haegele.
1980. Herring on Canada's Pacific coast. Can. Spec. Publ. Fish. Aquat. Sci. 48:734–750.
Jorde, P. E., and N. Ryman
1996. Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. Genetics 143:1369–1381.
McHugh, J. L.
1954. Geographic variation in the Pacific herring. Copeia 1954:139–151.
McPherson, A. A., P. T. O'Reilly, T. L. McParland, M. W. Jones, and P. Bentzen.
2001. Isolation of nine novel tetranucleotide microsatellites in Atlantic herring (*Clupea harengus*). Mol. Ecol. Notes 1:31–32.
McPherson, A. A., R. L. Stephenson, and C. T. Taggart.
2003. Genetically different Atlantic herring (*Clupea harengus*) spawning waves. Mar. Ecol. Prog. Ser. 247:303–309.
Miller, K. M., K. Laberee, A. D. Schulze, and K. H. Kaukinen.
2001. Development of microsatellite loci in Pacific herring (*Clupea pallasi)*. Mol. Ecol. Notes 1:131–132.
O'Connell, M., M. C. Dillon, and J. M. Wright
1998b. Development of primers for polymorphic microsatellite loci in the Pacific herring (*Clupea harengus pallasi*). Mol. Ecol. 7:358–360.
O'Connell, M., M. C. Dillon, J. M. Wright, P. Bentzen, S. Merkouris, and J. Seeb.
1998a. Genetic structuring among Alaskan Pacific herring populations identified using microsatellite variation. J. Fish Biol. 53:150–163.
Olsen, J. B., C. J. Lewis, E. J. Kretschmer, S. L. Wilson, and J. E. Seeb.
2002. Characterization of 14 tetranucleotide microsatellite loci derived from Pacific herring. Mol. Ecol. Notes 2:101–103.
O'Reilly, P. T., M. F. Canino, and K. M. Bailey.
2004. Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. Mol. Ecol. 13:1799–1814.
Raymond, M., and F. Rousset.
1995. GENEPOP: population genetics software for exact tests and ecumenicism, vers. 1.2. J. Heredity 86:248–249.
Rounsefell, G., and H. Dahlgren.
1935. Races of herring, *Clupea pallasii*, in southeastern Alaska. U.S. Dept. Comm. Bull. Bureau of Fish. 17:119–141.
Schmidt, J.
1917. Racial investigations. I. *Zoarces viviparus* L. and local races of the same. Compt.-Rend. Lab, Carlsberg 13:279–397.
Shaklee, J. B., and P. Bentzen.
1998. Genetic identification of stocks of marine fish and shellfish. Bull. Mar. Sci. 62:589–621.
Skud, B. E.
1963. Herring tagging experiments in Southeastern Alaska. Fish. Bull. 63:19–32.
Slatkin, M.
1994. Linkage disequilibrium in growing and stable populations. Genetics 137:331–336.
Small, M. P., J. L. Loxterman, A. E. Frye, J. F. Von Bargen, C. Bowman, and S. F. Young.
2005. Temporal and spatial genetic structure among some Pacific herring populations in Puget Sound and the southern Strait of Georgia. Trans. Am. Fish. Soc. 134:1329–1341.
Van Oosterhout, C. H., W. F. Hutchinson, D. P. M. Wills, and P. F. Shipley.
2004. MICROCHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4: 535–538.
Waples, R. S.
1998. Separating the wheat from the chaff: patterns of genetic differentiation in high-gene flow species. Genetics 89:438–450.
Waples, R. S., and O. Gaggiotti.
2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol. Ecol. 15: 1419–1439.
Weir, B. S., and C. C. Cockerham.
1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Appendix

Statistical data on Gulf of Alaska Pacific herring (*Clupea pallasii*) by loci, including name of locus, GENEBANK accession number, allele size range and number of base pair (bp) repeats. Collection: 1=Berners Bay 07, 2=Berners Bay 08, 3=Berners Bay 09, 4=Lynn Canal 07, 5=Lynn Canal 08a, 6=Lynn Canal 08b, 7=Hobart Bay 07, 8=Hoonah Sound 08, 9=Sitka Sound 07, 10=Sitka Sound 08, 11=Nichols Bay 08, 12=west Prince William Sound, and 13=east Prince William Sound. Collection sizes (n), number of alleles (n_a), allele richness (a), observed heterozygosity (H_o), expected heterozygosity (H_e), effective number of alleles (n_{eff}), and estimated inbreeding coefficient (F_{is}). Total is n across all populations. All other values in the total column are an average across all collections.

Cha63	AF019987, allele size range 130–187, 2 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	49	126	136	98	95	97	128	100	72	131	97	99	92	1320
n_a	19	24	23	21	24	24	24	24	21	25	23	21	23	30
a	17.77	19.22	17.29	17.22	18.08	19.31	18.69	18.88	18.14	18.88	18.29	17.91	19.69	18.42
H_o	0.94	0.92	0.94	0.86	0.86	0.91	0.92	0.91	0.88	0.95	0.84	0.92	0.97	0.91
H_e	0.92	0.91	0.91	0.88	0.90	0.91	0.91	0.92	0.91	0.92	0.89	0.92	0.93	0.91
n_{eff}	12.50	11.11	11.11	8.33	10.00	11.11	11.11	12.50	11.11	12.50	9.09	12.50	14.29	11.33
F_{is}	−0.023	−0.009	−0.040	0.029	0.036	−0.002	−0.003	0.009	0.041	−0.029	−0.029	0.064	−0.004	−0.038

Cha1017	AF289096, allele size range 158–208, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	52	126	148	98	96	96	128	100	75	131	97	99	91	1337
n_a	6	6	7	5	6	6	6	5	6	5	5	6	6	7
a	5.49	5.31	5.63	4.93	5.45	5.52	4.84	4.74	5.24	4.87	4.76	5.24	5.22	5.16
H_o	0.58	0.57	0.57	0.48	0.54	0.52	0.49	0.54	0.49	0.53	0.49	0.47	0.45	0.52
H_e	0.56	0.55	0.58	0.54	0.58	0.55	0.55	0.58	0.60	0.54	0.53	0.50	0.54	0.55
n_{eff}	2.27	2.22	2.38	2.17	2.38	2.22	2.22	2.38	2.50	2.17	2.13	2.00	2.17	2.25
F_{is}	−0.023	−0.031	0.015	0.109	0.065	0.060	0.101	0.063	0.174	0.005	0.005	0.076	0.046	0.167

Cha1020	AF289095, allele size range 130–239, 2 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	52	113	148	91	95	98	123	99	70	117	93	98	49	1246
n_a	17	28	33	28	27	26	30	22	23	28	23	28	18	43
a	15.29	18.57	19.03	17.98	18.36	18.05	18.38	15.63	17.16	17.74	15.25	19.15	16.37	17.80
H_o	0.73	0.84	0.83	0.85	0.88	0.82	0.84	0.86	0.87	0.80	0.75	0.88	0.90	0.83
H_e	0.79	0.83	0.81	0.81	0.84	0.85	0.83	0.82	0.83	0.81	0.79	0.85	0.84	0.82
n_{eff}	4.76	5.88	5.26	5.26	6.25	6.67	5.88	5.56	5.88	5.26	4.76	6.67	6.25	5.72
F_{is}	0.074	−0.016	−0.023	−0.041	−0.054	0.035	−0.011	−0.049	−0.050	0.012	0.012	0.048	−0.032	−0.074

Cpa4	AF309800, allele size range 111–195, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	51	125	144	94	96	95	128	98	73	129	92	97	87	1309
n_a	15	16	18	17	18	17	17	18	17	18	16	18	16	22
a	13.99	13.66	14.37	14.58	14.26	14.53	14.15	14.57	14.62	15.33	14.65	15.23	14.25	14.48
H_o	0.90	0.88	0.92	0.95	0.91	0.88	0.91	0.87	0.95	0.89	0.90	0.96	0.94	0.91
H_e	0.91	0.89	0.90	0.91	0.90	0.90	0.91	0.90	0.90	0.92	0.92	0.91	0.91	0.90
n_{eff}	11.11	9.09	10.00	11.11	10.00	10.00	11.11	9.80	9.62	12.20	12.50	11.11	11.11	10.67
F_{is}	0.009	0.016	−0.021	−0.044	−0.008	0.018	−0.008	0.035	−0.055	0.029	0.029	0.016	−0.052	0.001

continued

Appendix (continued)

Cpa6	AF309801, allele size range 154–250, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	50	124	145	94	91	98	123	99	75	126	81	98	92	1296
n_a	14	16	17	18	18	17	19	17	17	17	15	16	16	22
a	12.97	12.53	13.00	13.34	13.89	12.87	13.08	13.2	13.59	12.21	12.54	12.17	12.32	12.87
H_o	0.76	0.73	0.82	0.79	0.71	0.80	0.84	0.65	0.73	0.75	0.68	0.79	0.72	0.75
H_e	0.81	0.74	0.78	0.76	0.78	0.80	0.76	0.74	0.75	0.72	0.75	0.74	0.76	0.76
n_{eff}	5.26	3.85	4.76	3.85	4.76	5.00	4.17	3.82	4.07	3.60	4.00	3.82	4.17	4.24
F_{is}	0.009	0.008	−0.054	0.006	0.087	−0.001	−0.096	0.124	0.027	−0.033	−0.033	0.098	−0.058	0.054

Cpa27	AF309799, allele size range 89–199, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	52	126	147	96	96	97	119	97	73	130	97	98	92	1320
n_a	12	13	13	13	15	14	13	14	15	16	16	11	13	26
a	10.75	10.08	10.86	10.19	11.58	10.32	9.46	10.94	10.85	11.14	11.72	9.90	10.58	10.75
H_o	0.71	0.84	0.84	0.79	0.78	0.74	0.74	0.85	0.85	0.74	0.70	0.81	0.71	0.78
H_e	0.81	0.82	0.83	0.81	0.84	0.79	0.76	0.83	0.81	0.81	0.76	0.83	0.80	0.81
n_{eff}	5.26	5.56	5.88	5.26	6.25	4.76	4.17	5.88	5.26	5.26	4.17	5.88	5.00	5.28
F_{is}	0.124	−0.022	−0.018	0.019	0.070	0.066	0.027	−0.013	−0.052	0.082	0.082	0.072	0.033	0.114

Cpa103	AF406939, allele size range 176–280, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	51	124	147	98	97	98	127	100	74	131	97	98	91	1333
n_a	13	19	19	18	17	18	15	17	18	17	17	19	17	26
a	12.15	13.64	12.60	14.13	12.68	12.86	11.95	12.75	15.01	13.06	13.18	14.30	13.55	13.16
H_o	0.94	0.86	0.91	0.82	0.88	0.86	0.91	0.86	0.82	0.86	0.85	0.88	0.90	0.87
H_e	0.90	0.88	0.88	0.90	0.88	0.88	0.89	0.89	0.89	0.88	0.88	0.88	0.90	0.89
n_{eff}	10.00	8.33	8.33	10.00	8.33	8.33	9.09	9.09	9.09	8.33	8.33	8.33	10.00	8.89
F_{is}	−0.047	0.034	−0.035	0.094	0.002	0.024	−0.015	0.031	0.069	0.020	0.020	0.035	0.006	−0.003

Cpa107	AF309792, allele size range 100–172, 2 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	33	126	144	97	85	90	127	99	66	123	97	97	84	1268
n_a	18	25	24	21	22	24	25	25	19	23	20	24	22	31
a	18.00	18.27	16.93	15.96	16.59	17.81	17.65	18.16	14.57	17.29	14.52	18.57	18.07	17.55
H_o	0.94	0.89	0.87	0.84	0.89	0.93	0.91	0.89	0.89	0.84	0.89	0.91	0.94	0.89
H_e	0.90	0.91	0.91	0.90	0.91	0.91	0.91	0.92	0.88	0.91	0.89	0.92	0.93	0.91
n_{eff}	10.00	11.11	10.00	10.00	11.11	11.11	11.11	12.50	8.33	11.11	9.09	12.50	14.29	10.94
F_{is}	−0.044	0.019	0.041	0.073	0.013	0.004	0.001	0.032	−0.012	0.077	0.077	0.005	0.008	−0.013

continued

Appendix (continued)

Cpa108	AF406944, allele size range 227–277, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	47	120	141	94	90	92	126	99	73	123	95	98	90	1288
n_a	9	9	12	10	11	9	12	11	8	13	10	11	9	13
a	8.02	7.76	9.20	8.49	9.46	7.57	9.54	8.77	7.10	8.42	7.41	8.04	6.68	8.38
H_o	0.79	0.73	0.72	0.64	0.68	0.75	0.75	0.78	0.78	0.72	0.75	0.73	0.68	0.73
H_e	0.71	0.73	0.73	0.70	0.72	0.71	0.74	0.75	0.74	0.72	0.71	0.71	0.68	0.72
n_{eff}	3.45	3.70	3.70	3.33	3.57	3.45	3.85	4.00	3.85	3.57	3.45	3.45	3.13	3.58
F_{is}	−0.112	0.000	0.005	0.091	0.061	−0.053	−0.015	−0.038	−0.054	0.012	0.012	−0.049	−0.042	0.004

Cpa111	AF406947, allele size range 162–342, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	50	124	141	97	91	94	124	88	67	124	93	87	92	1272
n_a	19	20	19	19	20	21	19	20	19	19	21	19	23	27
a	17.84	17.08	16.58	16.99	17.89	17.81	17.02	16.75	17.47	16.77	18.45	17.04	18.97	17.29
H_o	0.88	0.92	0.92	0.94	0.97	0.95	0.92	0.82	0.96	0.94	0.96	0.92	0.99	0.93
H_e	0.93	0.93	0.92	0.93	0.93	0.93	0.93	0.92	0.93	0.93	0.93	0.93	0.93	0.93
n_{eff}	14.29	14.29	12.50	14.29	14.29	14.29	14.29	12.50	14.29	14.29	14.29	14.29	14.29	14.01
F_{is}	0.052	0.013	−0.001	−0.006	−0.040	−0.021	0.011	0.112	−0.029	−0.020	−0.020	−0.031	0.007	−0.059

Cpa112	AF406948, allele size range 244–472, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	49	109	137	97	83	94	118	97	74	127	95	99	92	1271
n_a	26	35	32	30	32	34	33	36	35	36	35	38	32	64
a	22.10	24.94	23.02	22.79	23.98	24.61	24.02	24.09	26.02	23.66	23.51	25.12	22.50	23.92
H_o	0.88	0.95	0.99	0.96	0.86	0.93	0.93	0.96	0.91	0.96	0.93	0.95	0.96	0.94
H_e	0.93	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.94	0.94	0.95	0.94	0.95
n_{eff}	14.29	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	16.67	16.67	20.00	16.67	18.79
F_{is}	0.055	−0.005	−0.041	−0.011	0.099	0.025	0.020	−0.009	0.047	−0.020	−0.019	0.011	0.000	−0.013

Cpa113	AF406949, allele size range 109–209, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	49	125	148	96	95	86	126	100	71	113	91	98	90	1288
n_a	18	18	18	20	19	17	17	17	17	18	17	16	16	23
a	16.79	15.13	15.57	17.07	15.90	14.75	14.86	15.59	15.07	15.05	15.15	14.80	14.56	15.36
H_o	0.86	0.90	0.87	0.90	0.92	0.92	0.93	0.92	0.92	0.93	0.96	0.93	0.84	0.91
H_e	0.93	0.91	0.92	0.92	0.92	0.92	0.92	0.92	0.90	0.91	0.92	0.91	0.89	0.92
n_{eff}	14.29	11.11	12.50	12.50	12.50	12.50	12.50	12.50	10.00	11.11	12.50	11.11	9.09	11.86
F_{is}	0.077	0.008	0.051	0.026	0.000	−0.004	−0.013	0.000	−0.016	−0.016	−0.016	−0.037	−0.016	0.056

continued

Appendix (continued)

Cpa114	AF406950, allele size range 196–292, 4 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	47	59	147	94	95	97	128	99	70	126	91	98	90	1241
n_a	16	16	18	18	17	18	17	17	15	17	18	18	16	24
a	14.37	14.74	13.24	12.97	13.76	14.46	13.28	14.35	12.82	13.19	13.93	14.67	13.54	13.70
H_o	0.83	0.97	0.89	0.82	0.91	0.89	0.88	0.90	0.91	0.89	0.88	0.90	0.94	0.89
H_e	0.90	0.90	0.89	0.88	0.90	0.90	0.88	0.88	0.89	0.88	0.90	0.89	0.90	0.89
n_{eff}	10.00	10.00	9.09	8.33	10.00	10.00	8.33	8.33	9.09	8.33	10.00	9.09	10.00	9.28
F_{is}	0.077	−0.069	0.002	0.063	−0.002	0.019	−0.003	−0.016	−0.026	−0.013	−0.013	0.019	−0.004	−0.054

Cpa125	AF309796, allele size range 207–325, 2 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	45	121	148	94	94	96	123	100	69	127	97	99	76	1289
n_a	28	31	37	35	36	33	37	32	29	34	32	33	35	47
a	25.33	23.10	25.30	25.42	25.80	25.74	26.02	23.74	21.75	25.08	22.87	25.48	26.36	25.15
H_o	0.96	0.97	0.95	0.96	0.92	0.95	0.94	0.93	0.97	0.96	0.94	0.94	0.95	0.95
H_e	0.96	0.95	0.95	0.95	0.95	0.96	0.96	0.94	0.94	0.95	0.94	0.96	0.96	0.95
n_{eff}	25.00	20.00	20.00	20.00	20.00	25.00	25.00	16.67	16.67	20.00	16.67	25.00	25.00	21.15
F_{is}	0.001	−0.020	0.002	−0.007	0.040	0.008	0.013	0.015	−0.035	−0.010	−0.010	0.005	0.020	0.009

Cpa134	AF309798, allele size range 119–255, 2 bp repeats													
Collection	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
n	52	125	140	92	88	98	125	100	64	125	96	99	91	1295
n_a	20	30	29	27	22	27	27	29	21	26	27	29	29	57
a	17.06	18.12	18.60	18.07	15.77	17.54	17.46	19.27	16.38	15.90	17.62	18.22	18.88	17.90
H_o	0.92	0.90	0.91	0.92	0.93	0.94	0.92	0.93	0.86	0.88	0.94	0.89	0.97	0.92
H_e	0.91	0.92	0.91	0.92	0.90	0.92	0.92	0.92	0.91	0.91	0.91	0.91	0.93	0.92
n_{eff}	11.11	12.50	11.11	12.50	10.00	12.50	12.50	12.50	11.11	11.11	11.11	11.11	14.29	11.80
F_{is}	−0.015	0.027	0.008	−0.009	−0.032	−0.024	0.000	−0.010	0.057	0.032	0.032	−0.026	0.027	−0.042