Exxon Valdez Oil Spill
Long-Term Herring Research and Monitoring Program Final Report

Genetic Stock Structure of Herring in Prince William Sound

Exxon Valdez Oil Spill Trustee Council Project 16120111-P
Final Report

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May 2018

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Study History:
This multi-year genetics project examined microsatellite loci and mtDNA cytochrome b of spawning Pacific herring (*Clupea pallasii*) from Prince William Sound, collected in 2012 – 2015, and from adjacent stocks collected prior to this study. DNA extraction and genotyping began with the arrival of funds in 2014. Initial analysis showed that fin clips of Prince William Sound herring were contaminated from milt of surrounding spawning fish. A time-consuming refinement of a 'bleaching' procedure of the fin tissue, re-extraction of the DNA, and re-genotyping the collections added 6 months of time to the project. In addition, it was ascertained that one of the microsatellite loci, *Cpa6*, was resolved consistently only in heart tissue. Thus the 2015 collections were obtained from heart tissue, which also eliminated the contamination issue. All genotypes were obtained by early 2016, and analysis and report writing continued during 2016 and culminated in this report. This project builds upon *Exxon Valdez* Oil Spill Trustee Council Project 097165.

Abstract:
Pacific herring (*Clupea pallasii*) stocks have been depressed in Prince William Sound for the majority of the last 20 years and the reasons for their lack of recovery remain unknown. The purpose of our study is to examine the genetic stock structure of herring within Prince William Sound and the connections to herring stocks outside of Prince William Sound. Herring were collected from eastern and western Prince William Sound, and several adjacent locations, including southeast Alaska, Yakutat, Kayak Island, Cook Inlet, Shelikof Strait, and Kodiak Island. Bering Sea herring were included as geographic outliers for comparison. Genetic information was obtained from thirteen microsatellite loci and mtDNA cytochrome b sequences. Analyses of the microsatellite loci show that allele frequencies of herring collections in eastern Prince William Sound are homogenous among bays, between year classes, and over years. Collections from western Prince William Sound indicated a weak signal of genetic divergence from eastern Prince William Sound collections. Prince William Sound herring, as a whole, are genetically similar to herring to the east (Kayak Island and Yakutat), but are significantly different than herring to the west (Kodiak, Cook Inlet, and Shelikof Strait). Bering and Gulf of Alaska herring are highly divergent genetically. Our findings suggest that gene flow is occurring between Prince William Sound herring and adjacent stocks to the east of Prince William Sound, either continuously or episodically. The two genetic marker classes together suggest there is limited herring gene flow from the eastern Gulf of Alaska to the western Gulf of Alaska, and between the Bering Sea and the Gulf of Alaska. Herring in the Bering Sea are genetically divergent enough to potentially be considered a different species; however, our results indicate that Bering Sea individuals may have entered into the Gulf of Alaska at some point in time and successfully reproduced with Gulf of Alaska herring.

Key words: *Clupea pallasii*, Gulf of Alaska, herring, microsatellites, mtDNA, Prince William Sound, population genetics

Project Data: Microsatellite genotypes and mtDNA cytochrome oxidase b sequence data of herring from the Prince William Sound and adjacent Gulf of Alaska collections are available in Excel spreadsheets. Herring DNA and tissue from this study are also retained either frozen or in ethanol at Auke Bay Laboratories, TSMRI in Juneau, Alaska. Contact Sharon Wildes at 907-789-6081/Sharon.wildes@noaa.gov, or the Genetics Program at 907-789-6079.

Data collected for the Herring Research and Monitoring Program projects that contributed to this report are available through the Alaska Ocean Observing System (AOOS) Gulf of Alaska data portal: https://portal.aoos.org/gulf-of-alaska.php#metadata/65c03bfd-2435-4fef-8c8d-c22414cb24ae/project

The data may also be found through the DataONE earth and environmental data archive at https://search.dataone.org/#data and by selecting the Gulf of Alaska Data Portal under the Member Node filter.

The Alaska Ocean Observing System data custodian is Carol Janzen, Alaska Ocean Observing System, 1007 W. 3rd Ave. #100, Anchorage, AK 99501, 907-644-6703, janzen@aoos.org.

Citation:

Wildes S. L., Hv. Nguyen, and J. R. Guyon. 2018. Genetic stock structure of herring in Prince William Sound. *Exxon Valdez* Long-Term Herring Research and Monitoring Program Final Report (*Exxon Valdez* Oil Spill Trustee Council Project 16120111-P), *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

Spatial and temporal patterns of genetic variation were examined from Pacific herring (*Clupea pallasii*) from l6 locations across multiple years within and adjacent to Prince William Sound. Collections were obtained from six eastern Prince William Sound sites in 2012-2014, and two western Prince William Sound sites in 2014 and 2015. An additional pre-spawn collection from western Prince William Sound in 2008 was included in the analyses. Collections of spawning herring from eight adjacent sites were analyzed to examine gene flow between herring inside and outside Prince William Sound. Locations east of Prince William Sound included Yakutat and Southeast Alaska. Sites west of Prince William Sound included Cook Inlet, Shelikof Strait and Kodiak. Samples from Bering Sea herring were included as a geographic outlier.

Because of the high level of polymorphism and the nuclear signal from both parents, microsatellite loci were used to screen genetic variation of all individuals. Of the fifteen microsatellite loci optimized for this study, 13 were used for analyses. A subset of individuals was also screened for mtDNA cytochrome oxidase b to examine historic demography.

Within Prince William Sound and within the eastern Gulf of Alaska, herring are genetically similar. There is weak divergence between collections of herring in eastern and western Prince William Sound, and between Prince William Sound and Southeast Alaska. Herring to the west of Prince William Sound are strongly divergent from each other and from the Prince William Sound herring. The Bering Sea herring appear to be a completely separate stock, with very low, one-way gene flow from the Bering Sea into the Gulf of Alaska. Our results corroborate previous studies.

Principal component analyses of individuals revealed an important pattern of genetic variation. Overall, the collections from within Prince William Sound were largely divergent from collections west of Prince William Sound. However, examination of genotypes from individual herring indicated that about half of the Cook Inlet herring were similar to Prince William Sound herring, and the other half were different. Simulation analyses of stocks assigned Prince William Sound and eastern Gulf of Alaska herring back to the Prince William Sound and eastern Gulf region with 95% accuracy; however, Cook Inlet herring assigned with only 56% accuracy presumably because of the partial overlap with Prince William Sound herring genotypes.

INTRODUCTION

In 1993, the Pacific herring (*Clupea pallasii*) fishery in Prince William Sound (PWS) closed due to low abundance, and has remained closed to the present day. There is a strong need to identify population or reproductively isolated units of herring in PWS in order to uncover reasons for the lack of recovery of this once abundant species. Understanding if there is one PWS herring stock or multiple stocks, or if PWS stocks are receiving recruitment of herring from outside PWS, is important in understanding this lack of recovery. Herring is a quintessential forage species in the PWS ecosystem and has been substantially examined for stock structure with a variety of techniques such as scale patterns (Rowell, 1981), morphology (Schweigert, 1990), tagging (Hourston, 1982), otolith chemistry (Otis and Heintz, 2003), fatty acid analysis (Otis et al., 2010), and genetics (Grant and Utter 1984; O'Connell et al., 1998a; Seeb et al., 1998; Liu et al., 2011; Liu et al., 2012; and Roberts et al., 2012).

There are merits and drawbacks of these techniques as applied to Pacific herring (Otis et al. 2010). The results of most non-genetic methods are environmentally influenced, which means there may be evidence of stock structure in a given year, but ever-changing environmental influences may produce temporal differences. Genetic information from neutral loci is less influenced by the environment, but may be influenced by migration, which can dilute stock structure with a small number of migrants (Waples, 1998).

An early genetic study with allozymes (Grant and Utter, 1984) that surveyed stocks throughout the Gulf of Alaska (GOA) and Bering Sea, but did not include collections from within PWS, was the first to reveal the strong divergence of Bering Sea and GOA herring, and the strong structure between Kodiak Island and the rest of the GOA. Liu et al. (2011) and Liu et al., (2012) examined the same study area and detected three historic lineages of herring with mtDNA, with only one of the lineages (A) found in the Bering Sea. Lineage A is mixed sparingly with lineages B and C throughout the GOA. Because mtDNA reveals only the maternal heritage of an animal, it is important to employ nuclear markers to examine contemporary stock structure. Small scale stock structure has been detected with microsatellite loci in Atlantic herring (*Clupea harengus*; Shaw et al., 1999; Ruzzante et al., 2006), and Pacific herring (O'Connell et al., 1998a; Small et al., 2005; Beacham et al., 2008; Andre et al., 2011; and Wildes et al., 2011). Most structure has been attributed to spawn timing, larval retention, and physical isolation. The growth of genomic resources will likely improve stock discrimination tools (Roberts et al., 2012). However, highly allelic microsatellite loci currently provide the greatest power for genetically detecting stock structure (Speller et al., 2012).

The first study of PWS herring with microsatellite loci detected weak divergence between eastern PWS collections and a western PWS collection at Pt. Chalmers on Montague Island (O'Connell et al., 1998a), and confirmed the strong genetic divergence reported with allozymes among collections from PWS, western GOA, and Bering Sea (Grant and Utter, 1984). Seeb et al., (1998) suggested that further work should focus on different sampling protocols and the striking differentiation between the Bering Sea and the GOA. Thus, in our study we continue to examine the genetic variation of herring within PWS and between PWS and adjacent areas to determine if reduced gene flow may be a factor in the lack of recovery of PWS herring. We increased the number of genetic samples per collection and the number of microsatellite loci

used in Seeb et al. (1998), to increase the statistical power to detect stock structure. We analyzed at least two years of collections to examine temporal stability within sites.

OBJECTIVES

1. Investigate the ability of 15 microsatellite loci to characterize herring populations in PWS.
2. Describe population structure of herring in PWS with microsatellite loci examining:
    a. spatial stability by sampling several locations within PWS,
    b. temporal stability by sampling the same locations for two consecutive years, and
    c. fine-scale structure across multiple age classes at each location.
3. Compare PWS herring populations with adjacent populations outside PWS to gauge overall genetic connectedness.
4. Examine microsatellite genotypes by mtDNA sequences to better understand the connection of herring between the GOA and Bering Sea.

METHODS

Sample collections

Adult spawning herring were collected by cast net and beach seine from PWS, Alaska in 2012 – 2015 (Table 1, Figs. 1 and 2). Three collections were made from eastern PWS in 2012 (n=157-198), and another three collections from a similar area in 2013 (n=401-452). The larger sample sizes in 2013 were collected to examine genetic variation by age. In 2014, three more collections were made from: 1) eastern PWS (n=183), providing another year to examine temporal stability in that area, 2) western PWS near Montague Island (n=82), and 3) Kayak Island, southeast of PWS (n=189). Three more collections were made in 2015 in western PWS, including one collection of 500 to examine genetic variation by age. Additional collections of spawning herring from locations further east and west of PWS were examined for comparison. East of PWS, samples were collected in 2008 by Auke Bay Laboratories, National Oceanic and Atmospheric Administration (NOAA) from the outer coast of Southeast Alaska and from Yakutat Bay. West of PWS, samples were collected by Alaska Department of Fish and Game (ADF&G) from Cook Inlet in 2012 and 2013, Kodiak Island in 2006, the Alaska Peninsula in Shelikof Strait in 2013, and the Bering Sea in 2005-2007. Whole fish were frozen in the field. Lengths and weights were obtained in the laboratory, and fin clips or pieces of heart tissue were archived in 95% ethanol.

Table 1. Collection region, collection site, site number, date of collection, latitude and longitude, sample size of microsatellite (mSat) and mtDNA datasets. * indicates collections that were aged. ^ indicates samples collected prior to this study.

Region	Collection	#	Sample Date	Latitude (*N*)	Longitude (*W*)	mSats	mtDNA
SE Alaska^	Craig	1	4/5/08	55.5241	-133.1288	124	
E. GOA^	Yakutat	2	4/28/09	59.5738	-139.8320	197	
E. GOA	Kayak Island	3	4/10/14	60.0104	-144.2295	189	
E. PWS	Landlock Bay	4	4/14/12	60.8181	-146.5620	157	
E. PWS	Hells Hole	5	4/14/12	60.7017	-146.3799	198	
E. PWS	Sheep Bay	6	4/10/12	60.6427	-146.1235	168	
E. PWS	Knowles Head	7	4/6/13	60.6894	-146.5320	401*	
E. PWS	RedHead	8	4/5/13	60.6733	-146.4747	452*	40
E. PWS	Gravina	9	4/25/13	60.6419	-146.2689	434*	
E. PWS	Gravina	10	4/17/14	60.6261	-146.2357	183	
W. PWS	Stockdale Harbor	11	4/24/14	60.3112	-147.1703	82	
W. PWS	Stockdale Harbor	12	5/2/15	60.2743	-147.1922	500*	40
W. PWS	Rocky Bay North	13	4/30/15	60.3535	-147.1117	220	
W. PWS	Rocky Bay South	14	4/30/15	60.3389	-147.1148	150	
W. PWS	Bishop Rock	15	3/1/08	60.0972	-147.9062	153	
Cook Inlet	Kamishak Bay	16	5/7/12	59.3694	-153.9925	220	40
Cook Inlet	Illiamna Bay	17	5/3/13	59.6364	-153.5790	170	
Shelikof Strait	Kukak	18	2013	58.3145	-154.2471	142	40
Kodiak Is.^	Kiliuda Bay	19	5/5/06	57.3158	-152.9872	52	
Bering Sea^	Hagemeister	20	5/4/06	58.9014	-160.0014	38	
Bering Sea^	Nunavachuk	20	5/4/06	58.9014	-160.0014	37	
Bering Sea^	Bering Sea (H 93)	20	9/26/05	61.9933	-170.1390	39	35
Bering Sea6	Bering Sea (H 77)	20	9/14/07	56.0142	-169.5832	68	
Total						4374	195

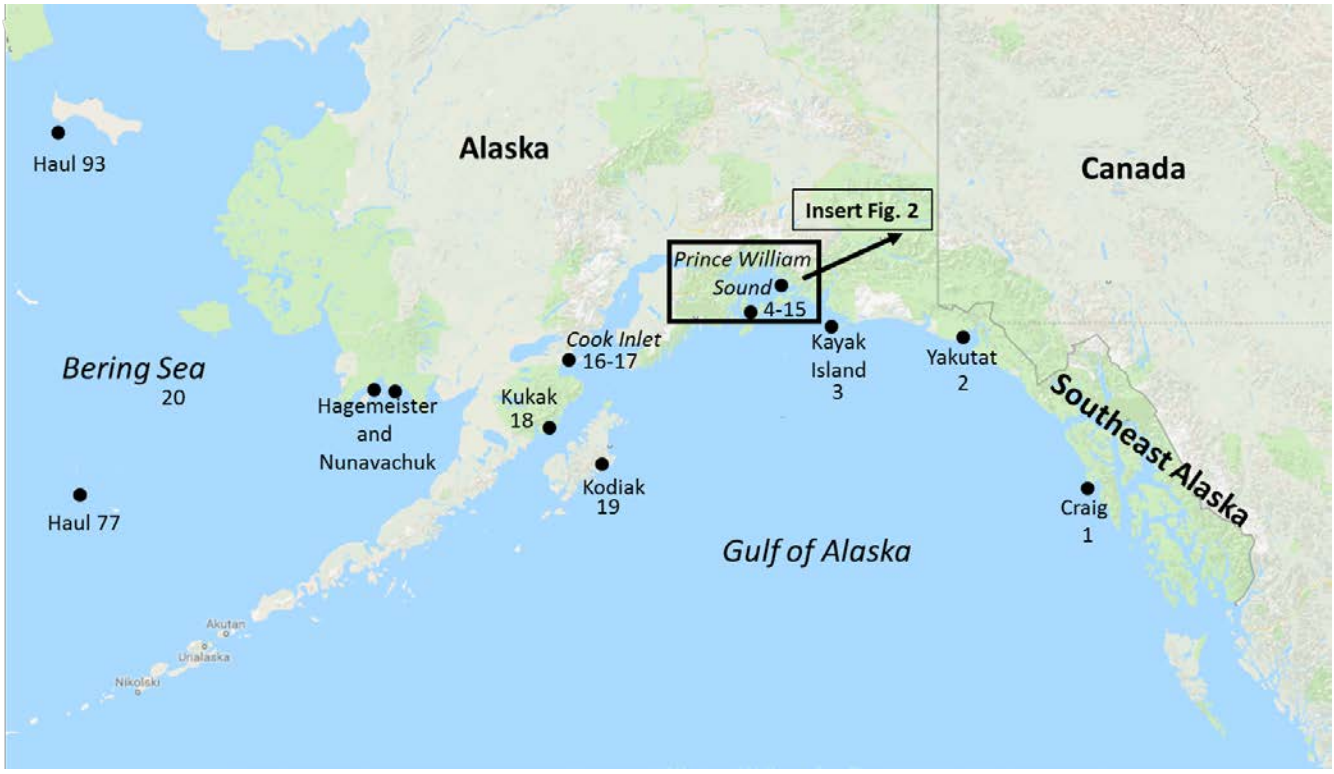

Figure 1. Collection sites of spawning herring in the Gulf of Alaska and Bering Sea listed in Table 1. Haul 77 and Haul 93 are non-spawning herring collections.

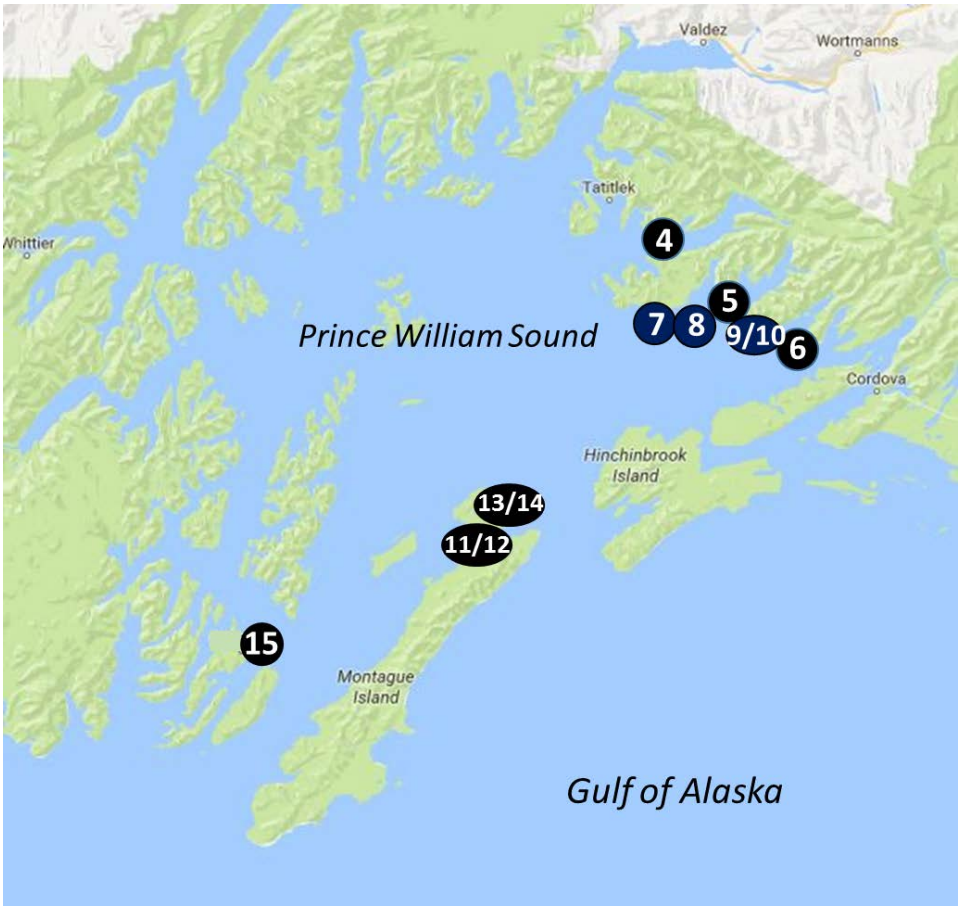

Figure 2. Collection sites 4 – 15 of spawning herring in Prince William Sound. Collection site numbers are defined in Table 1.

Data collection

DNA was extracted from 4,374 tissue samples by using Qiagen® DNeasy[1] Blood and Tissue Kit protocols (Qiagen Inc., Germantown, Maryland, U.S.). Genetic data were obtained from 15 microsatellite loci (Table 2) that were designed in previous herring studies (Miller et al., 2001; McPherson et al., 2001; O'Connell et al., 1998b, Olsen et al., 2002). Microsatellite fragments were amplified by PCR. Ten µl PCR reactions were made with 10 ng DNA, 0.2 µM unlabeled primer, 0.2 µM labeled primer, Qiagen® Multiplex PCR Master Mix 2x, and RNase free water. The PCR reactions were subjected to thermal cycling on a GeneAmp® 9700 (Applied Biosystems, Foster City, CA, USA) with the following protocol: 95°C for 15 min, 28 cycles of amplification (94°C for 30 sec, annealing temperature (dependent on the suite of loci) for 90 sec, and 72°C for 60 sec), final extension cycle of 60°C for 30 min, and a holding temperature of 15°C. Amplified microsatellite fragments were analyzed on a 16-capillary ABI 3130*xl* DNA Analyzer and alleles were determined with GeneMapper 5.0® (Applied Biosystems) software.

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

The initial analysis showed that fin clip samples collected in eastern PWS in 2012 and 2013 were contaminated with milt. Therefore, fin clips, were bleached with 5% Clorox® bleach for 10 minutes using procedures from Mitchell et al. (2008). DNA was re-extracted and re-genotyped. Individuals missing data from more than four loci were removed from analyses.

The mtDNA cytochrome b (*cytb*) gene was sequenced to examine historic demography on 195 samples (Table 1). The numbers of individuals used for microsatellite and mtDNA analyses are given in Table 1.

Table 2. Microsatellite loci examined in this study. Locus name, GenBank accession number, source, and primer sequences. Sources are: 1 = Miller et al., 2001, 2 = McPhearson et al., 2001, 3 = O Connell et al., 1998b, and 4 = Olsen et al., 2002.

Locus	Accession	Source	5'-3' Forward Primer	5'-3' Reverse Primer
Cpa4	AF309800	1	CTTATCTGTCTGACTGCCTATTTG	GTTTCTTCTCTGCTCCACCCAGAA
Cpa6	AF309801	1	GTGTGAGTTTGCTCCAAA	GTTTGTACCAATGAATGATTACAA
Cpa27	AF309799	1	CACATTTATCAATTTCTTTG	GTTTCAGAAAGAGAATCTAACCTCT
Cpa103	AF406939	4	GACTCACAGGTTCTCCTCAACA	TGGAGGGATTGGAACATTT
Cpa107	AF309792	1	GCATTACACAGAGAGGAAT	GTTTAGATACGCCTCTCTCTTT
Cpa108	AF318286	1	CTTGACATACAGTATGTTCAAAT	GTTTCTGTGAGCTGTACACCA
Cpa111	AF406947	4	TGTCCAGTAAAACATGCCTGA	GCTCCGTTCTCTTTCTTGCT
Cpa112	AF406948	4	GAGAGGGAGTTAAAATTGACAGC	GGCACAAGATGAGAGTGCAG
Cpa113	AF309793	1	CAGTCAGAAAGAAGGAGA	GTTTCCTCCTCGTGCTCTTT
Cpa114	AF406950	4	GCGTTTGTCCATACCACATT	CAGCTCTGAAAACCCAGACA
Cpa125	AF309796	1	GCAAGAAAGAGCAGCAGA	GTTTCGACTCAACAGCTGGAA
Cpa134	AF309798	1	CATTCTCTACAAAGGGCATATA	GTTTCATACCATTGAATCCAGCTA
Cha63	AF019987	3	TGCCTGCTGAAGACTTCC	CCCCTAAATGTGTTCTTTTAGC
Cha1017	AF289096	2	GGTCTCATTATCTTCTCACTCTTTTG	TCTCCCTATGTGTATTGTTTTACTGTG
Cha1020	AF289095	2	CCTGGAGAGACAGATAGAAAA	GAGTTTAGCAGACGCTTTA

Microsatellite analysis

Allele and genotype frequencies were estimated at each locus for each collection, and gene diversity was estimated by examining the expected level of heterozygosity in GENEPOP 4.0 (Rousset, 2008) with Markov chain parameters of 10,000 dememorization, 200 batches and 50,000 iterations. Expected Hardy-Weinberg (HW) proportions and F_{IS} (a measure of excess heterozygosity or homozygosity) were calculated for each of the 15 microsatellite loci in GENEPOP 4.0. Sequential Bonferroni corrections were applied for multiple tests. Allelic richness and number of private alleles (alleles unique to a group of samples) were calculated in HP-RARE 1.0 (Kalinowski, 2005) using rarefaction (Kalinowski, 2004), the unbiased estimate of number of alleles per locus used to account for uneven sample sizes (Hurlbert, 1971; Smith and Grassle 1977; and Leberg, 2002). To ensure the loci were independent and randomly associated, they were examined for linkage disequilibrium in both GENEPOP 4.0 and Genetix 4.05 (Belkhir et al., 2004).

Population structure was explored in several ways. Genetic differentiation among pairwise temporal and spatial samples was analyzed by computing F_{ST} (F-STAT 2.9.3, Goudet, 1995; 2001), D_{est} (Jost, 2008, implemented in the software SMOGD vs. 2.6., Crawford, 2010), and G-tests (GENPOP 4.0). P-values were adjusted with a sequential Bonferroni method, and level of significance was assigned at $P<0.05$. Principal component analyses were computed in Minitab (Minitab Inc.) from allele frequencies of herring collections, and from genotypes of herring individuals. Chord distances (Cavalli-Sforza and Edwards, 1967) were calculated among collections by using PHYLIP software, v. 3.69 (Felsenstein, 1989). A neighbor-joining tree was constructed from the chord distances to examine the relationships among collections.

Samples were also partitioned into groups of genetic similarity independent of sampling location by using the model based clustering method in the software program STRUCTURE (Pritchard et al., 2000). This program partitions individual fish to a pre-identified number of genetic groups based on a cluster algorithm to maximize estimations of both Hardy-Weinberg and linkage equilibrium. To investigate whether samples from one location assign to the same or different sample locations, 100% simulation tests were implemented in the software ONCOR (Kalinowski et al., 2008). Simulated mixtures of fish with genotypes from one location were re-assigned back to the entire set of collections (baseline). The more genetically divergent the baseline, the more accurate the simulated mixtures of samples will be correctly re-assigned.

Age composition of herring based on otolith analyses was provided by the ADF&G for the three 2013 eastern PWS collections, and the 2015 Stockdale Harbor collection in western PWS. Individuals were partitioned by brood year at each of these four collections. Pairwise comparisons of microsatellite allele frequencies at locations by brood year were evaluated with G-tests in GENEPOP 4.0.

mtDNA analyses

A subset of individuals from five locations (Table 1) were sequenced at the mtDNA *cytb* gene with the primers: Forward: 5'-AACCACCGTTGTCATTCAACTA-3'and Reverse: 5'-TTGTAAGAGTACGGGTGGAA-3'. The *cytb* region was amplified by PCR in a 50-µL reaction

volume containing 1X GoTaq® Buffer (Promega Corp., Madison, WI, USA), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM each of forward and reverse primer, 1 unit of GoTaq®Flexi DNA Polymerase (Promega), 10 ng sample DNA, and deionized water. Thermal cycling conditions of the PCR reactions were as follows: 95°C for 2 min, 30 cycles of amplification (95°C for 40 sec, 55°C for 40 sec, and 72°C for 1 min), 72°C for 7 min, and 4°C indefinitely. Forward sequences were obtained from the PCR product by the Sanger sequencing method on an ABI 3130xl DNA Analyzer. Sequences were aligned and edited with the software program CodonCode Aligner, vers. 3.7.1 (CodonCode Corp., Dedham, MA). Ambiguous end regions of mtDNA *cytb* sequences, particularly on the 3′ end, were trimmed to 628 bp in length to maximize the number of high-quality sequences.

From the mtDNA sequences, the following genetic indices were calculated in the software program Arlequin, v. 3.5 (Excoffier and Lischer, 2010): the number of polymorphic sites (*S*), average nucleotide diversity (π), and haplotype diversity (H). The number of nucleotide differences between sequences was visually represented by a haplotype network (minimum spanning tree) created with the program Sneato, v. 2 (Wooding, 2004; http://user.xmission.com/~wooding/Sneato/). Phylogenetic relationships among unique mtDNA haplotypes were evaluated with the program MEGA 6 (Tamura et al., 2013), wherein a tree was constructed with the neighbor-joining method (Saitou and Nei, 1987) using the distance algorithm of Tamura and Nei, (1993). Support for nodes of the tree was evaluated with 1,000 bootstrap replicates (Felsenstein, 1985). The number of substitutions per site between all unique sequences was examined with the Tamura and Nei (1993) model. A PCA was computed to compare mtDNA haplotypes with microsatellite data to ascertain if nuclear genotypes aligned with mitochondrial haplotypes.

RESULTS

Initial analysis of microsatellite genotypes from PWS samples collected in 2012 and 2013 revealed contamination of DNA from multiple individuals, likely via milt sticking to fins from collecting spawning fish. The bleaching process removed the milt and provided clean DNA in 90% of the samples re-processed (Fig. 3).

Two loci were dropped from analyses: 1) *Cpa107*, because six collections departed from Hardy-Weinberg proportions due to excess homozygosity, possibly indicating null alleles (lack of amplification of one allele copy due to a mutation in the primer binding region), and 2) *Cpa6* due to poor resolution in fin tissue, although resolution was high in heart tissue. Nearly all of the excess homozygosity at *Cpa107* occurred in the PWS collections. The remaining 13 loci were in HW equilibrium, with the exception of *Cha1017* in the Illiamna collection and *Cpa113* in the Bering 2007 collection (Table 3). F_{IS} values (Appendix 1) ranged from -0.155 (excess heterozygosity) to 0.299 (excessive homozygosity). The largest departures from zero were in *Cha1017*, and half of these were detected in the Bering Sea collections.

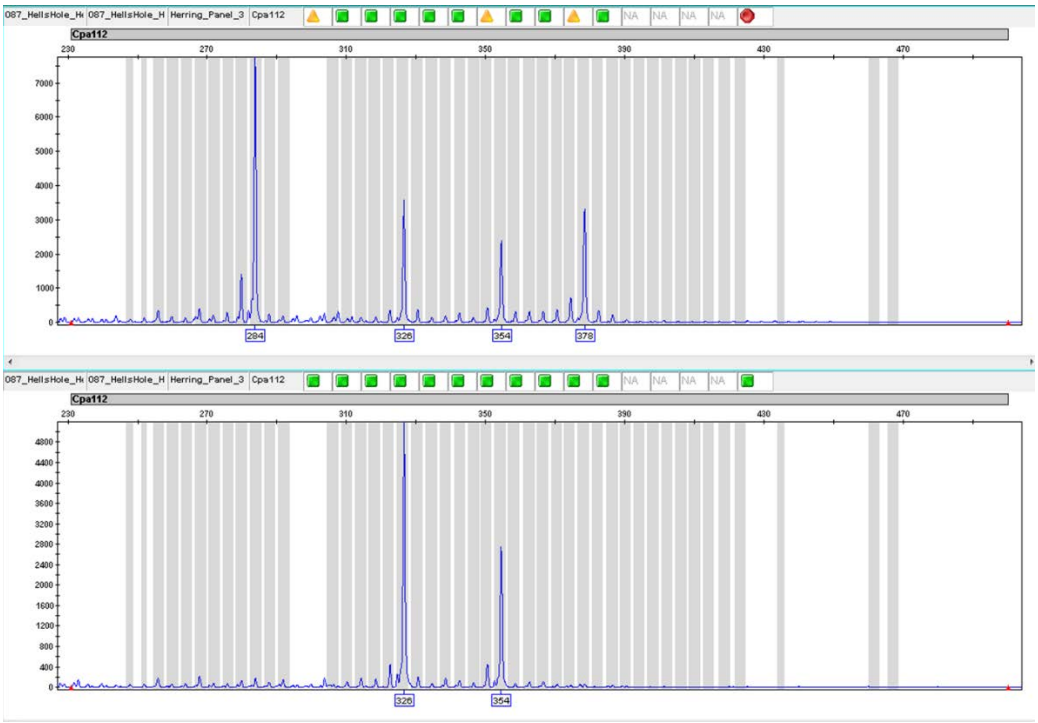

Figure 3. Genotypes present before (top panel) and after (bottom panel) herring samples were bleached. The top panel shows 4 ‘peaks’, likely representing at least two individuals. The lower panel shows the same individual after bleaching with the expected two ‘peaks’ for a single individual (one peak inherited from each parent).

Table 3. P-values less than 0.05 for tests of Hardy-Weinberg equilibrium at 14 microsatellite loci for 23 herring collections (Table 1), across all loci and collections with and without locus *CPA107*. Significant P-values after Bonferroni correction for multiple tests are highlighted in gray.

Collections	*Cpa 27*	*Cpa 103*	*Cha 1020*	*Cpa 113*	*Cpa 108*	*Cpa 114*	*Cpa 4*	*Cpa 134*	*Cha 1017*	*Cpa 107*	*Cpa 112*	*Cpa 125*	*Cpa 111*	*Cpa 63*	Total	Total no 107
1 Craig 08															0.543	0.427
2 Yakutat 08							0.033			0.014					0.160	0.334
3 Kayak Is. 14					0.046						0.017				0.007	0.004
4 Landlock 12			0.042												0.024	0.036
5 Hells Hole 12			0.009		0.039				0.025						0.046	0.031
6 Sheep Bay 12										0.001					0.013	0.156
7 Knowles 13										0.001					0.011	0.229
8 Red Head 13									0.045	0.000					0.001	0.123
9 Gravina 13					0.017			0.007							0.005	0.007
10 Gravina 14	0.041									0.046			0.044	0.029	0.015	0.025
11 Stockdale 14					0.045										0.189	0.235
12 Stockdale 15									0.041	0.000		0.037			0.015	0.453
13 Rocky N. 15										0.000					HS	0.883
14 Rocky S. 15										0.020					0.139	0.370
15 Bishop R. 08								0.004							0.229	0.226
16 Kamishak 12															0.216	0.219
17 Illiamna 13									0.001	0.000					0.007	0.140
18 Kukak 13	0.048											0.017			0.026	0.108
19 Kiliuda 14		0.005												0.021	0.004	0.028
20 Hagemeiste 14															0.572	0.624
20 Nunavachuk14									0.034						0.483	0.498
20 Bering 05															0.596	0.475
20 Bering 07				0.000											0.059	0.055
Total	0.199	0.207	0.072	0.153	0.018	0.383	0.203	0.121	0.000	HS	0.005	0.185	0.233	0.271		

Of the 13 loci analyzed, only 7 of the 156 pairwise comparisons suggested possible linkage. None of the 7 locus pairs were significant for more than a single collection and no collection had more than one locus pair out of equilibrium, indicating that the 13 loci independently assort.

The number of alleles per microsatellite locus ranged from 5 in *Cha1017* to 46 in *Cpa112*. The overall level of observed heterozygosity was 0.85 and ranged from 0.59 in *Cha1017* to 0.94 in *Cpa125* (Appendix 1).

Bering Sea samples were homogenous among the four collections, as determined by G-tests. Because statistical power decreases if sample sizes are unbalanced (Goudet et al., 1996; Waples and Gaggiotti, 2006), the Bering Sea samples were consolidated and subsequently analyzed as a single collection. The Bering Sea collection had significantly different allele frequencies, lower allelic richness, and fewer private alleles, averaged over all loci than the GOA collections. Allelic richness averaged over all loci ranged from 11.6 in Bering Sea samples to 15.2 in Kamishak, Cook Inlet samples. Private allele (alleles detected only in one collection) frequencies ranged from a low of 0.02 in Bering Sea samples to a high of 0.28 in Yakutat samples. Eastern PWS samples had fewer private alleles, averaging 0.18, than samples from both western PWS (average 0.20) and outside PWS (average 0.20) (Appendix 1 and 2).

Herring among eastern PWS collections were genetically homogenous as examined by D_{est}, F_{ST} and G-test values (Tables 4 and 5). Weak divergence was detected among pairwise comparisons of some eastern and western PWS collections and among some western PWS collections. Results of the pairwise comparisons were not consistent across all tests, except for the Rocky South collection which diverged from both Bishop Rock and nearby Stockdale 2015 in all tests. Collections to the west of PWS were significantly different from those within PWS and from each other. Kamishak and Illiamna herring, collected in Cook Inlet in consecutive years, were divergent from each other. Kukak samples collected in the Shelikof Strait, and Kiliuda samples collected from eastern Kodiak Island were significantly different from each other and all other collections.

Table 4. Pairwise D_{est} values are above the diagonal, and F_{ST} values are below the diagonal. Herring collections (1-20) correspond with locations outlined in Table 1 and Figure 1. 1= Craig, 2=Yakutat, 3=Kayak, 4=Landlock, 5=Hells Hole, 6=Sheep, 7=Knowles, 8=Red, 9=Gravina13, 10=Gravina14, 11=Stock14, 12=Stock15, 13=RockyN, 14=RockyS, 15=Bishop, 16=Kamishak, 17=Illiamna, 18=Kukak, 19=Kiliuda, and 20=Bering Sea. Light gray highlights eastern PWS, and darker gray highlights, western PWS.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1		0	0.002	0.002	0.001	0	0.001	0.002	0.003	0.001	0.001	0.003	0.001	0.002	0	0.022	0.041	0.086	0.016	0.328
2	0.001		0	0	0	0	0	0	0	0	0	0	0	0	0	0.008	0.027	0.063	0.008	0.284
3	0.001	0		0	0	0	0	0	0	0	0	0	0	0	0.001	0.006	0.021	0.054	0.005	0.262
4	0.002	0	0		0	0	0	0	0	0	0	0	0	0	0	0.008	0.020	0.054	0.008	0.288
5	0.002	0	0	0		0	0	0	0	0	0.001	0.002	0	0.001	0	0.009	0.022	0.058	0.005	0.279
6	0.002	0	0	0	0		0	0	0	0	0	0	0	0	0	0.008	0.028	0.062	0.004	0.295
7	0.002	0	0	0	0	0		0	0	0	0	0	0	0	0	0.007	0.025	0.058	0.006	0.288
8	0.002	0	0	0	0	0	0		0	0	0	0	0	0	0	0.008	0.028	0.060	0.007	0.295
9	0.002	0	0	0	0	0	0	0		0	0	0	0	0	0	0.007	0.021	0.053	0.006	0.280
10	0.001	0	0	0	0	0	0	0	0		0	0	0	0	0	0.005	0.022	0.052	0.005	0.274
11	0.001	0	0	0	0	0	0	0	0	0		0	0.001	0	0	0.005	0.023	0.041	0.007	0.276
12	0.002	0.001	0.001	0.001	0.001	0	0.001	0.001	0.001	0.000	0.001		0	0.001	0	0.010	0.025	0.059	0.007	0.287
13	0.001	0	0	0	0.001	0	0	0	0	0	0	0.001		0.001	0	0.012	0.024	0.063	0.010	0.281
14	0.001	0	0	0	0.001	0	0	0	0.001	0	0	0.002	0		0.001	0.006	0.020	0.053	0.005	0.277
15	0.002	0	0.001	0	0	0	0	0	0	0	0.001	0.001	0.001	0.001		0.010	0.022	0.062	0.005	0.285
16	0.009	0.004	0.004	0.004	0.003	0.004	0.004	0.004	0.003	0.003	0.004	0.005	0.004	0.005	0.004		0.001	0.016	0.002	0.195
17	0.018	0.011	0.010	0.009	0.009	0.010	0.011	0.010	0.009	0.010	0.010	0.013	0.010	0.010	0.010	0.001		0.008	0.003	0.138
18	0.033	0.024	0.022	0.021	0.020	0.022	0.022	0.022	0.020	0.021	0.022	0.025	0.022	0.023	0.022	0.008	0.003		0.029	0.105
19	0.011	0.006	0.005	0.006	0.004	0.004	0.005	0.005	0.005	0.004	0.005	0.006	0.006	0.006	0.005	0.002	0.005	0.012		0.230
20	0.076	0.066	0.060	0.063	0.061	0.066	0.064	0.064	0.061	0.062	0.065	0.073	0.063	0.063	0.063	0.043	0.030	0.026	0.052	

Table 5. P-values from pairwise G-tests of herring collections (1-20). Collection numbers correspond with locations outlined in Table 1 and Figure 1. Genic (allele frequency) comparisons are above the diagonal; genotypic comparisons are below. Significant P-values after Bonferonni correction for multiple tests are in bold. Light gray highlights eastern PWS and darker gray, Western PWS. 1=Craig, 2=Yakutat, 3=Kayak, 4= Landlock, 5=Hells, 6=Sheep, 7=Knowles, 8=Red, 9=Gravina13, 10=Gravina14, 11=Stock14, 12=Stock15, 13=RockyN, 14=RockyS, 15=Bishop, 16=Kamishak, 17=Illiamna, 18=Kukak, 19=Kiliuda, 20=Bering Sea. HS = highly significant (P<0.0001).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1		0.17	**0.03**	**0.00**	0.38	0.20	**0.04**	0.05	**0.00**	0.18	0.26	0.14	**0.02**	**0.03**	**0.02**	**HS**	**HS**	**HS**	**HS**	**HS**
2	0.22		0.32	0.26	0.28	0.64	0.49	0.31	0.37	0.54	0.77	0.76	0.14	0.63	**0.04**	**HS**	**HS**	**HS**	**0.00**	**HS**
3	**0.03**	0.38		0.21	0.29	0.96	0.45	0.29	0.73	0.54	0.60	0.68	0.48	0.19	0.24	**HS**	**HS**	**HS**	**0.00**	**HS**
4	**0.00**	0.32	0.22		0.40	0.51	0.21	0.21	0.05	0.32	0.43	0.32	0.27	0.17	**0.01**	**HS**	**HS**	**HS**	**0.00**	**HS**
5	0.46	0.35	0.32	0.50		0.92	0.44	0.52	0.34	0.51	0.47	0.29	0.47	0.20	0.09	**HS**	**HS**	**HS**	**0.00**	**HS**
6	0.21	0.67	0.96	0.55	0.94		0.84	0.78	0.60	0.75	0.96	0.88	0.39	0.17	0.35	**HS**	**HS**	**HS**	**0.00**	**HS**
7	0.08	0.58	0.55	0.31	0.55	0.87		0.18	0.10	0.97	0.78	0.08	0.20	0.25	**0.03**	**HS**	**HS**	**HS**	**0.00**	**HS**
8	0.07	0.37	0.33	0.26	0.58	0.81	0.26		0.04	0.18	0.90	0.34	0.12	0.30	0.14	**HS**	**HS**	**HS**	**0.00**	**HS**
9	**0.00**	0.49	0.77	0.09	0.41	0.65	0.17	0.06		0.65	0.94	0.06	0.28	0.32	**0.00**	**HS**	**HS**	**HS**	**0.00**	**HS**
10	0.24	0.61	0.61	0.41	0.58	0.80	0.98	0.21	0.74		0.65	0.56	0.53	0.26	0.26	**HS**	**HS**	**HS**	**0.00**	**HS**
11	0.32	0.81	0.62	0.52	0.58	0.97	0.82	0.93	0.96	0.72		0.19	0.56	**0.02**	0.39	**0.00**	**0.00**	**HS**	**0.00**	**HS**
12	0.17	0.81	0.71	0.41	0.35	0.90	0.11	0.42	0.09	0.61	0.73		0.13	0.63	0.66	**0.00**	**HS**	**HS**	**0.00**	**HS**
13	**0.03**	0.19	0.52	0.33	0.56	0.43	0.28	0.14	0.36	0.63	0.16	0.45		**0.01**	0.39	**HS**	**HS**	**HS**	**0.00**	**HS**
14	**0.04**	0.69	0.20	0.21	0.21	0.19	0.31	0.33	0.39	0.31	0.71	**0.01**	0.06		**0.04**	**HS**	**HS**	**HS**	**0.00**	**HS**
15	**0.02**	0.05	0.24	**0.01**	0.13	0.35	0.05	0.18	**0.01**	0.32	0.45	0.24	0.59	**0.03**		**HS**	**HS**	**HS**	**0.00**	**HS**
16	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**0.00**	**HS**	**HS**	**HS**	**0.00**		**0.03**	**HS**	0.08	**HS**
17	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	0.04		**HS**	**0.00**	**HS**
18	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**0.00**		**0.00**	**HS**
19	**HS**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**		**HS**
20	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	

Principal component analyses by collection mirror the statistical tests and indicate: 1) weak divergence of the western PWS collections, 2) similarity of eastern PWS collections to each other and to collections east of PWS, and 3) strong divergence of each collection west of PWS. Bering Sea collections were highly divergent from the GOA collections and were omitted from Fig. 4 to show the GOA collection structure. Small sample size may be driving divergence of Stockdale14 (western PWS) and Kiliuda (Kodiak)

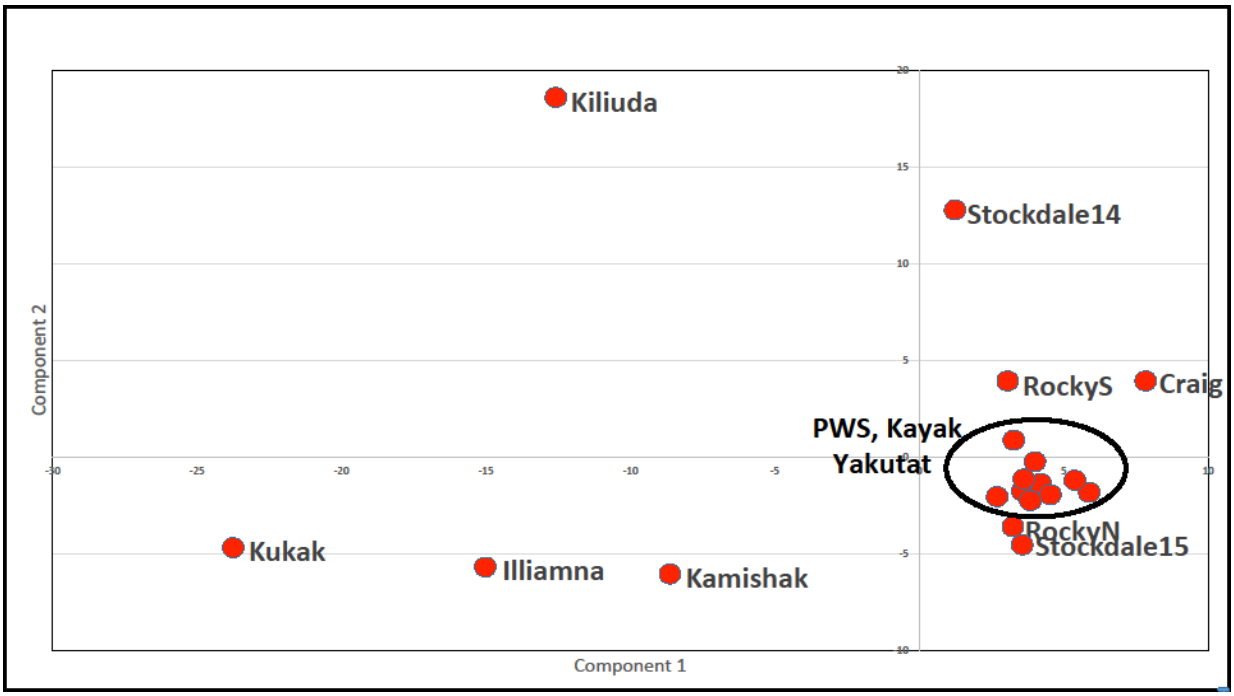

Figure 4. Principal component analysis of Gulf of Alaska herring collections based on allele frequencies of 13 microsatellite loci.

A principal component analysis of genotypes of individual herring provides a more in-depth look at the gene flow between PWS, western GOA, and the Bering Sea. It is difficult to discern results from all 4,000 individuals in a single plot, so principal components 1 and 2 from 13 microsatellite loci are plotted for individuals from representative collections in eastern PWS (Landlock Bay), Cook Inlet (Kamishak Bay), Shelikof Strait (Kukak Inlet), and the Bering Sea in Figure 5. A similar pattern was observed when individuals from other collections were plotted (not shown). A strong east-west geographic pattern in the genotypes was evident. The Bering Sea individuals were nearly discrete from the Gulf of Alaska individuals. The Shelikof Strait individuals overlap about half of the Cook Inlet individuals and PWS individuals overlap the other half of the Cook Inlet individuals.

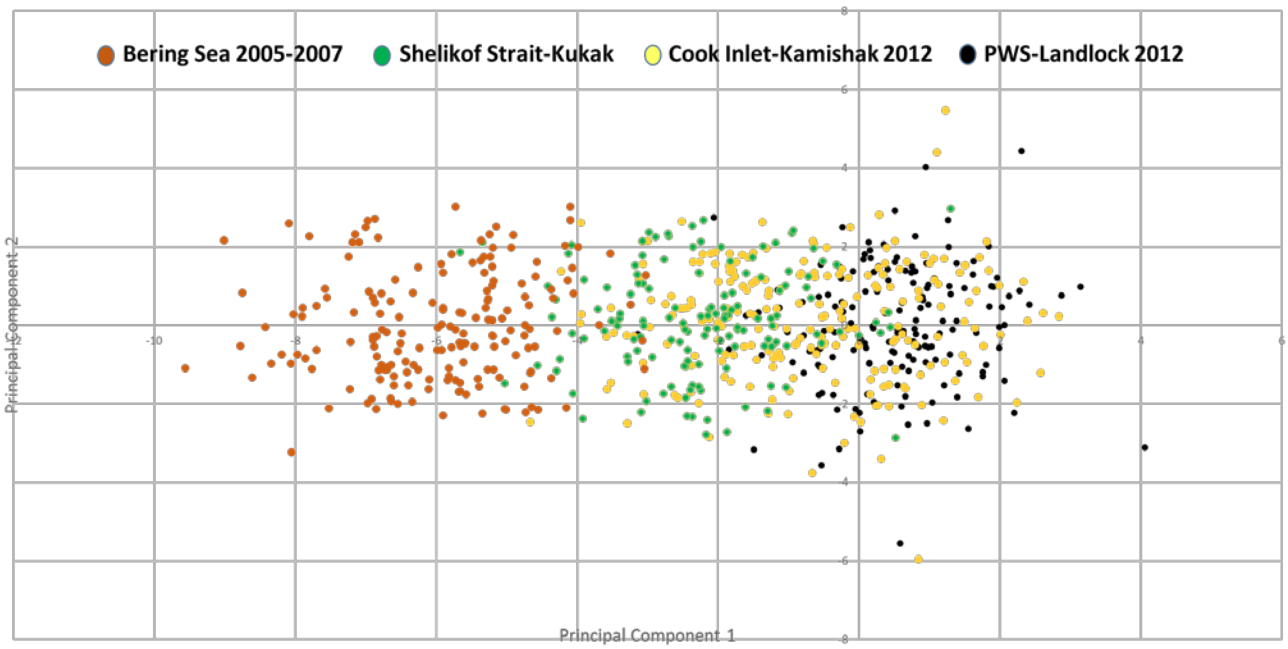

Figure 5. Principal component analysis of individual herring from selected collections, based on genotypes at 13 microsatellite loci.

A neighbor joining tree illustrates the genetic similarity of herring within and to the east of PWS, and the large divergence among collections west of PWS (Fig. 6). The tree corroborates the structure in the PCA of collections (Fig. 4).

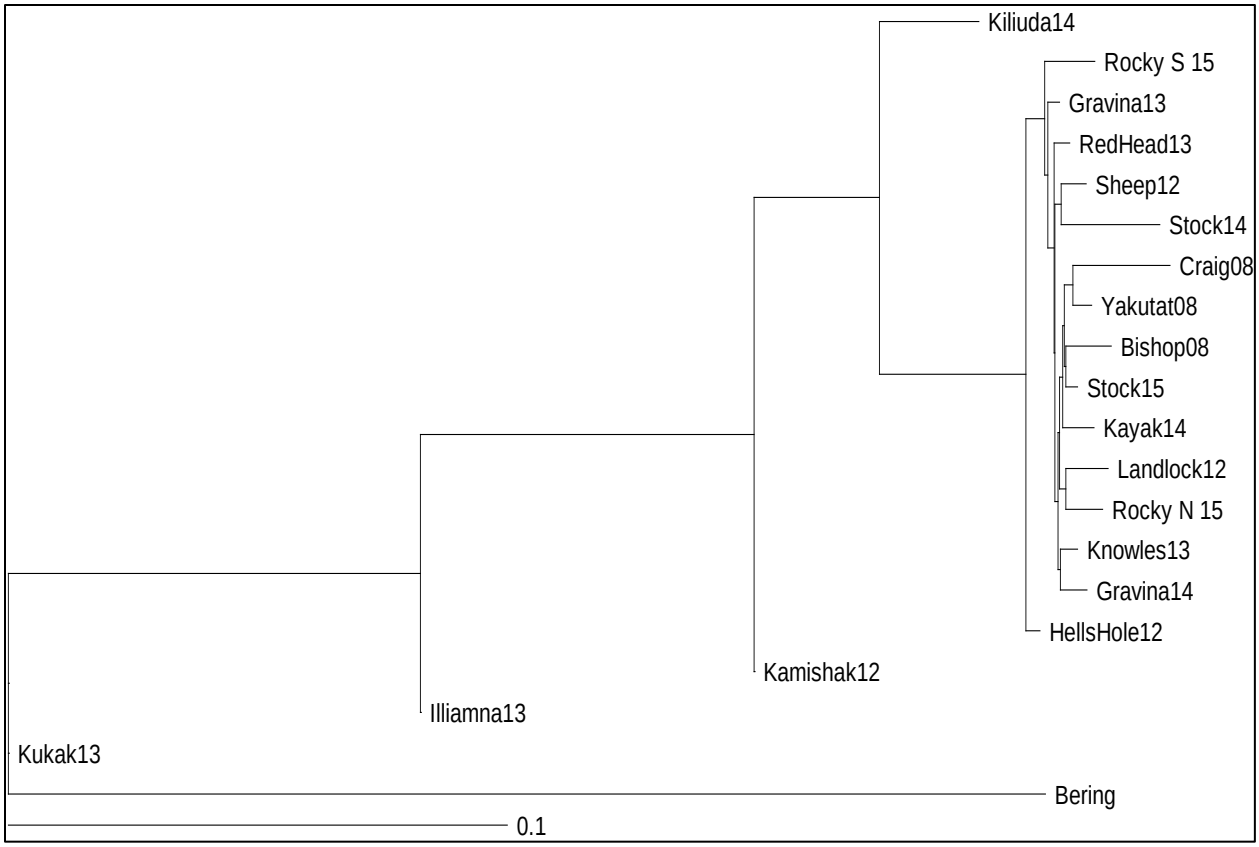

Figure 6. Neighbor-joining tree of herring collections from chord distances of microsatellite allele frequencies.

The program STRUCTURE indicated a similar pattern across all eastern GOA and PWS collections (Fig. 7). These collections were portioned primarily into inferred clusters 1 and 2 (red and blue), with a low percent of assignment to the 3rd inferred cluster (green). Craig (1) in Southeast Alaska had the lowest percent of assignment to the 3rd cluster. The Bering Sea (20) had a 95% or greater assignment to the 3rd cluster, and the four collections from the western GOA (16-19) had an increased percent assignment to the 3rd cluster, with the largest proportion in Kukak (18) on the Shelikof Strait, and the smallest proportion in Kiliuda (19), from eastern Kodiak Island.

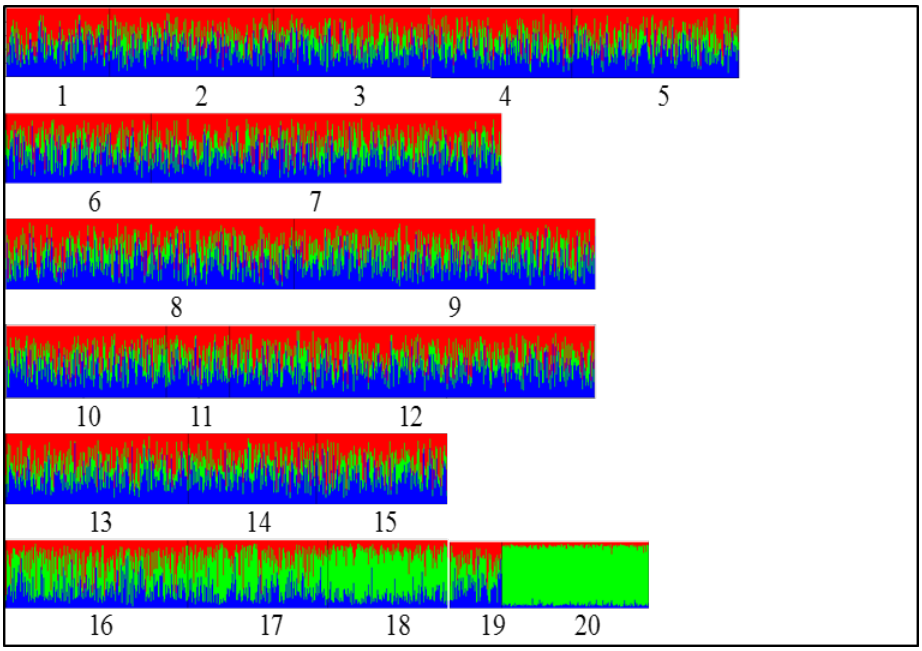

Figure 7. Population assignment of individual herring at 20 collections with the program STRUCTURE. 1= Craig, 2=Yakutat, 3=Kayak, 4=Landlock, 5=Hells Hole, 6=Sheep, 7=Knowles, 8=Red, 9=Gravina13, 10=Gravina14, 11=Stock14, 12=Stock15, 13=RockyN, 14=RockyS, 15=Bishop, 16=Kamishak, 17=Illiamna, 18=Kukak, 19=Kiliuda, and 20=Bering Sea.

Results from 100% mixture simulations examined with the program ONCOR revealed an inability to correctly assign an individual back to its collection of origin in the GOA (Table 6). Individuals were, however, assigned back to the eastern GOA and PWS as a reporting group with over 95% accuracy. Assignment of individuals to western GOA reporting groups (2-4) was less accurate due to the overlap of genotypes with PWS fish (see Fig. 5). Bering Sea herring were detected with near perfect accuracy and precision to the Bering Sea reporting group (5).

Table 6. ONCOR results of 100% stock estimate simulations. The mean estimates, 1 standard deviation, and 95% confidence intervals are provided by collection (left) and for five reporting groups (right) separated by bold lines. Group 1 = eastern GOA and PWS, Group 2 = Cook Inlet, Group 3 = Shelikof Strait, Group 4 = Kodiak Island, and Group 5 = Bering Sea.

Reporting		Collection Estimates			Reporting Group Estimates		
Group	Collection	Average	SD	95% CI	Average	SD	95% CI
1	Craig 08	0.10	0.05	(0.03, 0.19)	1.00	0.00	(0.99, 1.00)
1	Yakutat 09	0.05	0.04	(0.00, 0.12)	0.99	0.01	(0.96, 1.00)
1	Kayak 14	0.02	0.03	(0.00, 0.10)	0.96	0.03	(0.90, 1.00)
1	Landlock 12	0.11	0.04	(0.02, 0.20)	0.99	0.01	(0.97, 1.00)
1	Hells Hole 12	0.08	0.04	(0.00, 0.17)	0.98	0.02	(0.93, 1.00)
1	Sheep Bay 12	0.00	0.01	(0.00, 0.03)	0.99	0.01	(0.95, 1.00)
1	Knowles 13	0.12	0.07	(0.00, 0.25)	0.98	0.02	(0.94, 1.00)
1	Red Head 13	0.16	0.07	(0.03, 0.30)	0.99	0.02	(0.95, 1.00)
1	Gravina 13	0.20	0.07	(0.06, 0.31)	0.98	0.02	(0.94, 1.00)
1	Gravina 14	0.04	0.04	(0.00, 0.15)	0.98	0.02	(0.93, 1.00)
1	Stockdale 14	0.00	0.01	(0.00, 0.02)	0.97	0.03	(0.90, 1.00)
1	Stockdale 15	0.18	0.07	(0.06, 0.33)	0.99	0.02	(0.95, 1.00)
1	Rocky North 15	0.11	0.05	(0.00, 0.22)	0.99	0.01	(0.95, 1.00)
1	Rocky South 15	0.05	0.04	(0.00, 0.13)	0.98	0.02	(0.93, 1.00)
1	Bishop 08	0.05	0.04	(0.00, 0.11)	0.98	0.02	(0.94, 1.00)
2	Kamishak 12	0.42	0.06	(0.31, 0.53)	0.62	0.05	(0.53, 0.73)
2	Illiamna 13	0.32	0.05	(0.23, 0.41)	0.67	0.05	(0.58, 0.76)
3	Kukak 13	0.59	0.05	(0.51, 0.68)	0.59	0.05	(0.51, 0.68)
4	Kiliuda 06	0.10	0.04	(0.04, 0.17)	0.10	0.04	(0.04, 0.17)
5	Bering Sea 05-07	1.00	0.01	(0.98, 1.00)	1.00	0.01	(0.98, 1.00)

The herring in the four largest collections were aged: three collections from eastern PWS and one collection from western PWS (Table 7, Fig. 8). A wide span of ages were present, especially in eastern PWS, with a gap of absent intermediate ages. For example, the 2013 Knowles Head collection had fish that ranged in age from 3 to 20 years old, but no fish in this collection were ages 12-16. A similar pattern was found for the Red Head and Gravina 2013 collections in eastern PWS. Twelve individuals from Knowles Head and Red Head were estimated to be 18-20 years old at time of capture in 2013. The Gravina 2013 collection had the youngest herring on average at 6.15 years. The herring from the one western PWS collection, Stockdale 2015, had no fish older than 13 years and were about one year younger on average (6.54 years) than the Knowles Head and Red Head collections in eastern PWS at 7.35 and 7.44 years respectively. The Stockdale 2015 herring were primarily from brood years 2009 and 2010, whereas the three eastern PWS collections had more evenly distributed and older age classes, from the 2004-2010 brood years, with lower numbers from brood year 2008, and low numbers from brood year 2010 at Knowles Head and Red Head. Herring of different ages were genetically similar within

collections (Table 8). All significant G-Tests were between fish from different locations, both from the same brood years, and different brood years. Although only 10 of the 171 pairwise tests were significant, a result expected by chance alone, 8 of the significant tests were between the Gravina collection and other collections.

Table 7. Age composition of four collections of herring from PWS. Numbers of individuals by brood year at each location. Samples not used in genetic analysis of microsatellite data due fewer than 40 individuals per collection and brood year are highlighted in gray.

Brood year	Knowles 2013	RedHead 2013	Gravina 2013	Stockdale 2015
1993	0	1	0	0
1994	1	3	0	0
1995	2	5	0	0
1996	9	22	18	0
1997	0	0	0	0
1998	0	0	0	0
1999	0	0	0	0
2000	0	1	0	0
2001	0	5	2	0
2002	3	0	3	1
2003	11	6	3	3
2004	65	60	33	3
2005	101	98	52	18
2006	74	60	48	32
2007	77	84	83	57
2008	21	18	26	52
2009	47	86	121	215
2010	2	11	57	108
2011	0	0	0	9
2012	0	0	0	1
Total	413	460	446	499

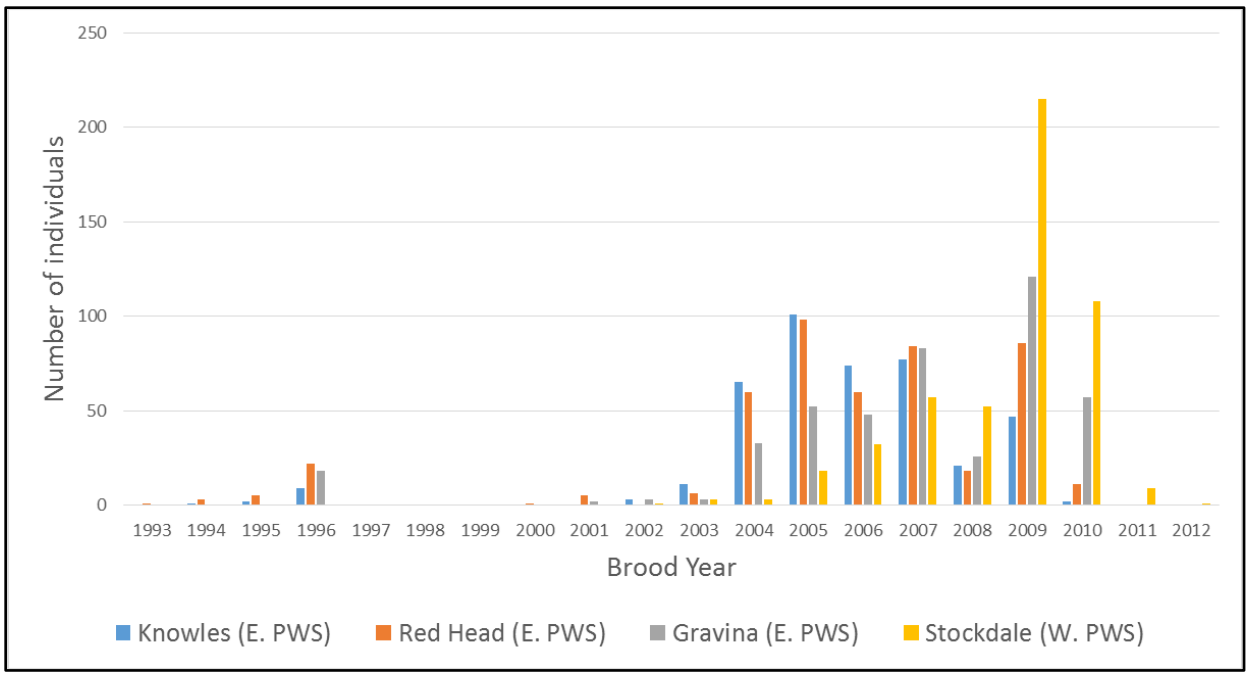

Figure 8. Frequency distribution by brood year of four herring collections from PWS. Knowles, Red Head, and Gravina samples were collected from eastern PWS in 2013, and Stockdale samples were collected from western PWS in 2015.

Table 8. P-values from pairwise G-tests by brood year. Genic (alleles) on top, genotypic (genotypes) below. Collections are partitioned by brood year and location with accordance to Table 6. P values<0.05 are in bold.

		Knowles					Red Head					Gravina					Stockdale			
		2004	2005	2006	2007	2009	2004	2005	2006	2007	2009	2005	2006	2007	2009	2010	2007	2008	2009	2010
Knowles	2004		0.25	0.09	0.37	0.20	0.05	0.74	0.20	0.07	**0.00**	0.14	**0.01**	0.20	0.22	**0.03**	**0.03**	0.32	0.15	0.44
	2005	0.32		0.70	0.74	0.25	0.22	0.23	0.58	0.12	0.10	0.76	0.37	0.54	0.34	**0.02**	0.20	0.40	0.18	0.84
	2006	0.13	0.73		0.86	0.62	0.38	0.90	0.96	0.41	0.63	0.43	**0.04**	0.52	0.11	0.37	0.50	0.38	0.08	0.48
	2007	0.49	0.82	0.90		0.36	0.87	0.88	0.85	0.19	0.20	0.42	0.17	0.54	**0.04**	0.20	0.42	0.25	0.30	0.72
	2009	0.20	0.26	0.69	0.48		0.39	0.95	0.38	0.25	0.28	0.53	0.24	0.64	**0.05**	0.07	0.46	0.35	0.29	0.61
Red Head	2004	0.08	0.24	0.45	0.93	0.41		0.77	0.59	0.45	0.18	0.46	0.33	0.47	0.11	0.39	0.61	0.07	0.73	0.92
	2005	0.80	0.26	0.94	0.93	0.96	0.82		0.96	0.84	0.64	0.59	0.07	0.80	0.14	0.75	0.73	0.48	0.86	0.99
	2006	0.28	0.66	0.97	0.93	0.42	0.72	0.98		0.45	0.69	0.73	0.54	0.43	0.15	0.44	0.63	0.20	0.23	0.96
	2007	0.09	0.14	0.46	0.25	0.28	0.44	0.87	0.57		0.43	0.38	0.07	0.30	0.19	0.39	0.97	0.11	0.51	0.75
	2009	**0.01**	0.13	0.72	0.29	0.33	0.20	0.67	0.77	0.49		0.81	0.09	0.36	0.06	0.16	0.08	0.14	0.05	0.76
Gravina	2005	0.18	0.81	0.51	0.63	0.60	0.56	0.67	0.83	0.44	0.87		0.58	0.31	0.12	0.16	0.74	0.38	0.61	0.73
	2006	**0.01**	0.43	0.06	0.19	0.25	0.36	0.08	0.69	0.07	0.10	0.67		0.55	0.15	0.07	0.45	0.14	0.07	0.36
	2007	0.21	0.56	0.53	0.61	0.63	0.53	0.85	0.53	0.34	0.40	0.42	0.62		0.51	0.45	0.30	0.35	0.24	0.70
	2009	0.28	0.41	0.15	0.07	0.07	0.14	0.18	0.23	0.26	0.10	0.20	0.19	0.57		0.08	0.36	**0.02**	0.20	0.20
	2010	**0.04**	**0.02**	0.43	0.32	0.08	0.44	0.82	0.50	0.43	0.17	0.21	0.09	0.50	0.11		0.16	0.20	**0.02**	0.30
Stockdale	2007	**0.03**	0.21	0.50	0.53	0.45	0.59	0.74	0.69	0.98	0.10	0.76	0.49	0.32	0.41	0.15		0.19	0.66	0.89
	2008	0.35	0.50	0.52	0.34	0.39	0.12	0.61	0.29	0.15	0.22	0.57	0.22	0.41	**0.04**	0.27	0.24		0.57	0.62
	2009	0.20	0.22	0.11	0.40	0.34	0.78	0.89	0.28	0.56	0.07	0.68	0.08	0.29	0.27	**0.03**	0.71	0.63		0.92
	2010	0.51	0.88	0.52	0.82	0.64	0.94	1.00	0.98	0.80	0.80	0.82	0.43	0.73	0.26	0.34	0.90	0.71	0.94	

As with the microsatellites, the diversity of mtDNA *cytb* sequences was significantly lower in Bering Sea samples (Table 9). Bering Sea haplotypic diversity was 0.038 and nucleotide diversity was 0.0033, whereas the average GOA haplotypic and nucleotide diversity was 0.065 and 0.0086, respectively. Only lineage A was found in the Bering Sea. Lineage B and C were predominate in the GOA samples, and lineage A, although present in the GOA collections, was observed in smaller proportions (Table 9, Fig. 9), especially in the eastern PWS collections.

Table 9. Summary statistics of mtDNA *cytb* sequence data. N = sample size (628 nucleotides each sample), S= # polymorphic sites (unique haplotypes), H = average haplotype diversity (S/N), π = average nucleotide diversity, and proportion of lineage A, B or C, in each collection.

	N	S	H	π	A	B	C
Red Head E. PWS	40	38	0.061	0.0084	0.075	0.450	0.475
Stockdale W. PWS	40	46	0.073	0.0084	0.100	0.350	0.550
Kamishak, Cook Inle	40	37	0.059	0.0079	0.125	0.400	0.475
Kukak, Shelikof St.	40	42	0.067	0.0096	0.175	0.400	0.425
Bering Sea	35	24	0.038	0.0033	1.000	0	0
Total	195	89					

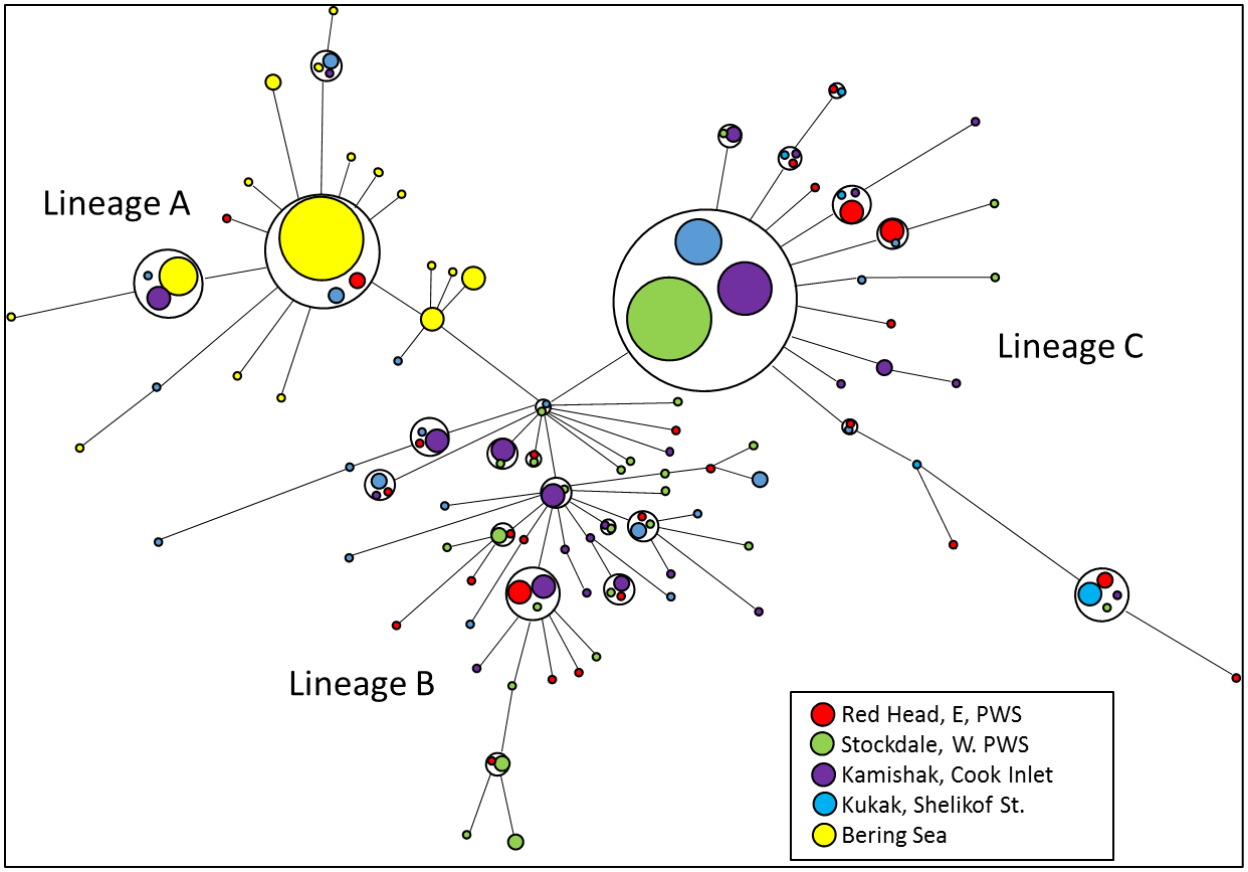

Figure 9. Minimum spanning tree of mtDNA *cytb* sequence data of herring from the Bering Sea, Gulf of Alaska, and PWS. The size of the circle is proportional to the frequency of the haplotype. The length of the lines is proportional to the number of single nucleotide differences (1 to 5 base pair differences).

The principal component analysis of microsatellite data by individual herring with known mtDNA lineage resulted in a nearly complete separation of Bering Sea individuals (lineage A), indicated in red (Fig. 10). Individuals collected from the GOA with known mitochondrial lineage A are indicated in black in order to distinguish them from Bering lineage A individuals. GOA microsatellite genotypes with mtDNA lineage A are genetically divergent from Bering Sea microsatellite genotypes, which are entirely of mtDNA lineage A. There appears to be no relationship between the three mtDNA lineages and microsatellite data from the GOA individuals (green, yellow and black dots), which share the same space in the PCA. The individuals from PWS with lineage A are separated in space from the other GOA individuals with lineage A: the eight lineage A individuals detected in PWS are the eight black dots furthest to the right of the graph.

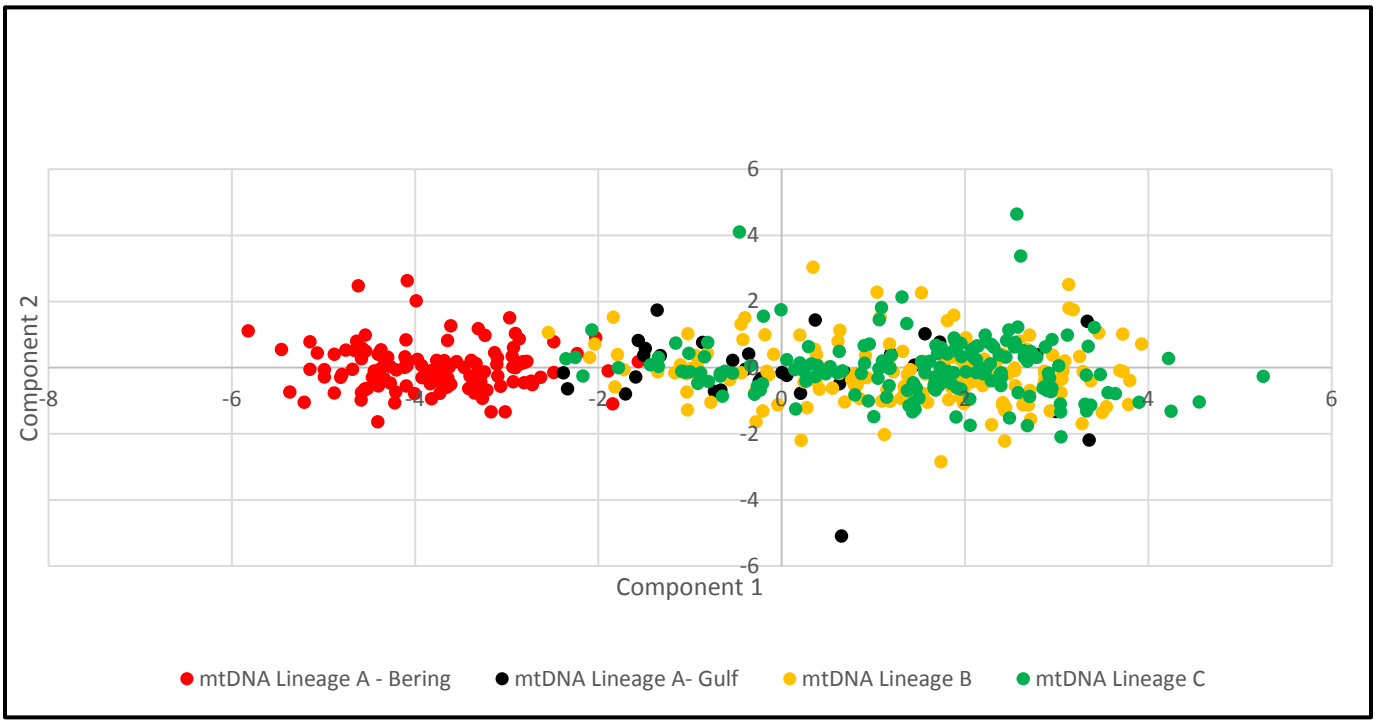

Figure 10. PCA of individual herring analyzed for both mtDNA and microsatellite loci. Each point represents a distillation of 13 microsatellite loci for a single herring. The individuals are color coded to indicate mtDNA haplotype lineage. Red = Bering Sea lineage A; black, yellow, and green = Gulf lineages A, B, and C respectively.

DISCUSSION

The once productive Pacific herring (*Clupea pallasii*) fishery in Prince William Sound (PWS) has been closed since 1993, due to low abundance. Conclusions of our study are based on results from mtDNA and microsatellite analyses to identify population or reproductively isolated units of herring in PWS in order to uncover reasons for the lack of recovery. Knowing whether there is one PWS herring stock or multiple stocks, or if PWS stocks are receiving recruitment of herring from outside PWS, is important in understanding this lack of recovery. Genetic data from spawning herring collected outside PWS (in the larger GOA and Bering Sea), provided a framework to better understand the stock structure inside PWS. Our study adds to the body of previous herring studies in the Pacific and includes a substantial increase in collection size, new locations in the Gulf of Alaska, and additional genetic markers. Both marker types (mtDNA *cytb* and microsatellite loci) and all analyses indicate the same pattern of genetic population structure of herring within and outside PWS and corroborate the results of previous population structure studies.

PWS

Our results show that there is sufficient gene flow to make it difficult to genetically distinguish herring among spawning sites within PWS. Statistical testing for pairwise comparisons of multiple years of spawning sites within PWS, produced fairly homogenous results. Results from D_{est}, F_{st}, and G-test's of collections 4-15, showed eastern PWS collections 4-10 (light gray) were

highly similar, however, western PWS collections 11-15 (dark gray), while not significant after correction, did reveal a trend toward differentiation from eastern PWS. Our results confirm this trend of western PWS differentiation as a dense cluster of eastern PWS for multiple years of collection with some western PWS outliers. This was also noted by O'Connell et al., (1998a) where Pt. Chalmers on Montague Island produced a signal of differentiation from eastern PWS collections. Stockdale 14 divergence may be exaggerated by the small sample size (n=84), which presents the possibility of less diversity than the other collections (n > 150).

In forage species, the effective population size is typically large enough that even reproductively isolated populations can be difficult to detect because the effect of genetic drift is insignificant, except on very long time scales (Kliman et al., 2008), and migration, even episodically or by just a few individuals per generation can be enough to dilute structure (Waples, 1998). Therefore, even weak structure can be indicative of some level of spawning site fidelity. This differentiation is not significant or consistent enough to utilize for stock identification within PWS with this set of markers; nonetheless, a reduced level of connectivity between eastern and western PWS is indicated, as well as among western PWS collections, as identified in statistical tests (dark gray areas of Tables 4 and 5), and identified as slight outliers to the dense grouping of eastern PWS in the PC analysis (Fig. 4). One explanation for the weak differentiation between western and eastern PWS stocks may be the proximity to Montague Strait and outside waters with recruitment of spawners from outside and to the west of PWS.

No temporal (age) genetic differentiation was detected in pairwise comparisons of collections from different years. Year classes from the four larger collections from PWS, (eastern-Gravina, Knowles and Red Head, collected in 2013, and western, Stockdale 2015) allowed for pairwise tests of 3 to 9 year old fish and although 10 of 171 tests were significant, those could be produced by chance alone.

In age-structured populations with overlapping generations, allele frequencies may differ among age classes because of "sweepstakes" recruitment, in which a small number of spawners are disproportionately successful in reproducing offspring, relative to the massive number of spawners who fail to leave offspring (Jorde and Ryman, 1996). In our study, all significant divergence was between collections from different locations. We note that the age of herring from the western PWS collection, Stockdale, was younger (brood years 2007-2010) than herring from the eastern PWS collections (brood years 2004-2007) and may reflect time of collection during spawning, wherein older herring spawn first, and younger fish spawn later within a season (McPhearson, 2003; Jorgenson, 2005), or may undergird the genetic data illustrating divergence of western and eastern PWS stocks.

PWS and GOA

All statistical tests indicate that eastern PWS, Kayak Island, and Yakutat are genetically homogenous, including temporal collections. Although not significant, indications of weak divergence from this meta-population were detected for the Craig collection from Southeast Alaska. The collections west of PWS include Kodiak, Cook Inlet and Shelikof Strait on the Gulf side of the Alaska Peninsula, and those were highly different from PWS and from collections east of PWS (Kayak Island, Yakutat, and Southeast Alaska). This signal is temporally stable since the first report over 30 years ago (Grant and Utter, 1984), and concordant across genetic

marker types, including allozymes (Grant and Utter, 1984), microsatellites (O'Connell et al., 1998a and this study), and single nucleotide polymorphism's (Roberts et al., 2012).

The large allele frequency differences between collections from PWS and western GOA indicate a barrier to gene flow between these two areas (Tables 4 and 5; Figs. 4 and 6), however PCA of a western GOA collection, when examined by individuals (Fig. 5), was shown to have both overlapping genotypes with PWS, and genotype combinations not detected in PWS. The drawback to this hypothesis is that eastern GOA and most PWS collections have rare alleles not detected in Cook Inlet, Shelikof Strait, Kodiak, particularly at loci *Cpa4*, *Cpa134*, and *Cpa112* (Appendix 2).

GOA and Bering Sea

Herring collections from the Bering Sea show species level divergence from the GOA. Statistical comparisons of Bering Sea and GOA microsatellite allele frequencies resulted in highly significant values from G-tests and large F^{ST} values. This basin level divergence corroborates previous genetic studies (Grant and Utter, 1984; O'Connell et al., 1998a; Liu et al., 2011; Liu et al., 2012; Roberts et al., 2012).

Mitochondrial data, in addition to providing historic demography, may also provide a more contemporary view of gene flow of herring. Bering Sea herring have mtDNA lineage A exclusively, corroborating a previous studies (Liu et al., 2011; Liu et al., 2012). Our results show that a small percentage of lineage A is found throughout the GOA, most frequently in Shelikof Strait, and least frequently in PWS. The presence of lineage A in the GOA indicates one-way migration of herring out of the Bering Sea and into the GOA, either contemporaneously or in the past. Microsatellite data of Bering Sea and GOA individuals, supports the migration from the Bering Sea into the GOA hypothesis. While nuclear microsatellite data of individuals in the GOA indicate no contemporary migration between GOA and Bering Sea herring, the mtDNA lineage A, passed through maternal generations, is now integrated among GOA fish, with GOA microsatellite genotypes. A small overlap of contemporary microsatellite data between the Bering Sea and western GOA such as Shelikof Strait, are also illustrative of this one way migration. GOA individuals are not likely moving into the Bering Sea however, as illustrated by the complete absence in the Bering Sea of GOA mtDNA lineages B and C, and many microsatellite alleles (clearest illustration is *Cpa112*).

This study represents the most comprehensive analysis of herring in PWS to date which included over 20 collections and two marker classes. The results suggest that glacial age herring isolation resulted in two species, and we are likely witnessing secondary contact in the western GOA (Liu et al., 2012). This data suggest gene flow is from east to west in the GOA, and from the Bering Sea into the GOA, where the western GOA is a mixing zone. Herring in the Bering Sea appear to be genetically divergent enough to be considered a different species, however, our results indicate that Bering Sea individuals have entered into the GOA at some point in time and have successfully reproduced.

Conclusions

The first objective was to evaluate the ability of 15 microsatellite markers to characterize PWS herring. Allele frequencies were out of Hardy-Weinberg equilibrium with marker *Cpa107*, likely

due to null alleles and this locus was removed from analyses. Marker *Cpa6* was only resolved in heart tissue, which was only available for a limited number of samples, and it was also removed from analyses. The remaining 13 markers provided data that were used for subsequent population genetic analyses in our study.

The second objective was to describe population structure in PWS. Eastern PWS herring are homogenous both spatially and temporally. Western PWS collections near Montague Island are weakly differentiated from eastern PWS collections, and show inconsistent temporal differences. No genetic differentiation was detected among year classes within the four largest PWS collections. A signal of weak divergence was evident between year classes of Gravina (E. PWS) and Knowles (E. PWS) and Stockdale (W. PWS) collections. Eastern PWS collections contained older fish.

The third objective was to examine collections adjacent to PWS. PWS herring are genetically similar to neighboring stocks to the east (Kayak Island and Yakutat), but highly divergent from stocks to the west (Cook Inlet, Kodiak).

The final objective was to examine both mtDNA and microsatellite markers to better understand basin level divergence of herring (Bering Sea and GOA). We found species level differences in both the nuclear microsatellite loci and the mtDNA cytb sequences. Herring from the two basins are now experiencing secondary contact in the western GOA region, as discussed in Liu et al. (2012). As genetic resources and techniques continue to develop, exploring the depth of herring divergence in this area would provide an interesting study in secondary contact of species, as well as the tracking of herring movement to recolonize places of once abundant herring numbers, like Prince William Sound.

ACKNOWLEDGEMENTS

We thank the following people for collections and aging information: Jim O'Rourke, Angelina Kelly, Angela Kuntz, Chantel Caldwell, Martin Schuster, Rich Brenner, Steve Moffitt, Cassidy Somerville, Cheng Xiong, Joe Loboy, Ted Otis, and Stew Grant (ADF&G); Jim Murphy and Johanna Vollenweider (NOAA); Scott Pegau (Prince William Sound Science Center).Thank you to Chris Kondzela for her review and suggestions. The findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the *Exxon Valdez* Oil Spill Trustee Council.

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Appendix 1. Microsatellite locus information of herring by collection. Name of locus, GenBank accession number, type of repeat, and allele size range of GOA and Bering Sea (in parentheses) collections. Collections: 1=Craig08, 2=Yakutat08, 3=Kayak Island14, 4=Landlocked12, 5=HellsHole12, 6=SheepsBay12, 7=Knowles13, 8=Red Head13, 9=Gravina13, 10=Gravina14, 11=Bishop08, 12=Stockdale14, 13=Stockdale15, 14=RockyNorth15, 15=RockySouth15, 16=Kamishak12, 17=IlliamnaBay13, 18=Kukak13, 19=Kiliuda14, 20-23=Hagemeister, Nunavachak, Bering05, and Bering 07. Collection sizes (N), number of alleles (N_A), allele richness (N_S), observed heterozygosity (H_O), expected heterozygosity (H_E), and estimated inbreeding coefficient (F^{IS}).

Cpa4 AF309800, 4 bp repeats (GACA), allele size range 83-204 (105-174)

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	123	195	188	165	195	166	406	459	430	193	154	65	499	213	149	220	166	138	65	38	36	40	68
N_A	16	23	19	19	20	17	21	21	20	19	18	16	24	18	17	18	19	18	16	12	10	11	13
N_S	13.4	15.0	14.2	15.4	14.7	13.3	14.7	14.4	15.1	13.8	14.1	14.0	14.5	14.3	14.1	14.4	15.3	14.3	14.2	11.8	9.8	10.5	11.3
H_o	0.93	0.88	0.91	0.93	0.90	0.92	0.90	0.90	0.89	0.86	0.88	0.91	0.91	0.91	0.90	0.93	0.90	0.90	0.86	0.87	0.92	0.90	0.82
H_E	0.91	0.91	0.91	0.92	0.92	0.91	0.91	0.91	0.91	0.91	0.91	0.90	0.91	0.91	0.91	0.91	0.92	0.91	0.91	0.91	0.88	0.88	0.88
F_{is}	-0.02	0.04	0.00	-0.01	0.02	-0.01	0.02	0.01	0.02	0.06	0.03	0.00	0.00	0.00	0.02	-0.02	0.02	0.01	0.05	0.04	-0.04	-0.02	0.07

Cpa27 AF309799, 4 bp repeats (GACA), allele size range 86-205 (108-176)

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	124	195	189	162	197	169	392	450	393	187	152	71	493	210	150	220	170	145	46	38	31	40	69
N_A	13	13	13	12	12	13	14	17	15	12	11	12	18	13	13	14	16	12	10	7	9	8	9
N_S	9.80	10.27	9.42	9.56	9.85	10.14	10.27	10.08	9.95	9.90	9.94	9.97	10.63	10.00	9.81	9.10	10.50	9.30	8.59	6.78	9.00	7.50	7.71
H_o	0.77	0.78	0.76	0.83	0.76	0.77	0.75	0.76	0.79	0.73	0.82	0.77	0.79	0.77	0.77	0.77	0.81	0.72	0.67	0.71	0.84	0.78	0.78
H_E	0.73	0.79	0.78	0.81	0.79	0.78	0.77	0.78	0.81	0.78	0.79	0.72	0.80	0.78	0.80	0.79	0.83	0.83	0.73	0.72	0.80	0.77	0.77
F_{is}	-0.06	0.02	0.03	-0.03	0.03	0.02	0.03	0.03	0.02	0.06	-0.04	-0.07	0.01	0.02	0.04	0.02	0.03	0.14	0.07	0.01	-0.05	-0.01	-0.02

Cpa103 AF406939, 4 bp repeat (TAGA), allele size range 146-274 (188- 232)

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	123	194	188	162	197	166	399	447	436	194	143	69	491	210	150	218	168	145	62	38	37	39	69
N_A	17	23	19	22	17	18	21	24	24	23	19	12	25	21	21	19	17	13	14	6	9	10	10
N_S	13.31	13.51	12.21	13.79	12.28	12.85	12.72	13.45	12.92	12.99	13.19	10.70	12.79	13.54	13.55	12.69	11.18	10.14	12.30	5.93	8.76	9.30	7.86

H_o	0.88	0.88	0.87	0.85	0.86	0.86	0.88	0.87	0.84	0.82	0.90	0.86	0.86	0.88	0.91	0.86	0.80	0.78	0.76	0.61	0.78	0.74	0.65
H_E	0.89	0.88	0.88	0.89	0.89	0.88	0.88	0.89	0.88	0.88	0.89	0.84	0.88	0.89	0.89	0.86	0.85	0.81	0.87	0.69	0.78	0.78	0.72
F_{is}	0.01	0.00	0.01	0.05	0.03	0.02	0.00	0.02	0.04	0.07	-0.02	-0.01	0.03	0.01	-0.02	0.00	0.06	0.04	0.13	0.13	0.00	0.05	0.09

Cpa108 AF406944, 2 bp repeat (CA), allele size range 225-285 (232-277)

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	120	197	184	159	196	166	388	437	413	194	153	56	498	211	149	219	170	143	63	38	37	40	68
N_A	11	12	11	11	13	11	12	13	13	12	10	8	13	11	11	11	10	9	10	7	7	9	8
N_S	7.87	8.81	8.87	7.87	8.01	7.86	8.41	8.37	7.84	8.66	7.54	7.06	8.78	7.63	8.77	8.30	7.77	7.24	8.30	6.78	6.64	8.40	7.06
H_o	0.73	0.80	0.71	0.70	0.74	0.75	0.70	0.69	0.73	0.73	0.71	0.70	0.70	0.68	0.69	0.62	0.61	0.56	0.75	0.58	0.41	0.58	0.59
H_E	0.73	0.74	0.75	0.71	0.71	0.71	0.72	0.73	0.72	0.74	0.71	0.69	0.74	0.70	0.72	0.66	0.63	0.54	0.71	0.56	0.47	0.62	0.60
F_{is}	0.00	-0.08	0.05	0.01	-0.04	-0.05	0.03	0.05	-0.01	0.01	0.00	0.00	0.05	0.03	0.05	0.06	0.02	-0.03	-0.06	-0.03	0.13	0.08	0.03

Cpa111 AF406947, 4 bp repeat, (TAGA), allele size range 219-371 (235-307)

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	123	197	186	163	197	169	402	453	435	172	152	69	490	206	144	214	169	135	58	38	37	40	69
N_A	21	24	22	21	19	22	23	23	21	19	20	19	23	20	19	20	19	19	20	14	13	11	16
N_S	16.80	17.81	16.79	17.52	16.70	17.29	16.95	17.14	16.74	16.64	16.82	17.47	17.21	16.36	16.76	15.91	16.09	15.29	18.08	13.01	12.62	10.05	12.41
H_o	0.89	0.92	0.94	0.91	0.94	0.93	0.93	0.92	0.93	0.92	0.95	0.97	0.93	0.9	0.93	0.94	0.92	0.88	0.97	0.79	0.84	0.85	0.74
H_E	0.92	0.93	0.92	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.94	0.93	0.92	0.93	0.91	0.92	0.90	0.93	0.81	0.81	0.81	0.76
F_{is}	0.04	0.01	-0.02	0.02	-0.02	0.00	0.00	0.01	0.00	0.01	-0.02	-0.04	0.00	0.02	0.00	-0.04	-0.01	0.02	-0.04	0.02	-0.04	-0.05	0.03

Cpa112 AF406948, 4 bp repeat (TAGA), allele size range 240-476 (250-292)

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	122	195	183	160	193	165	394	444	434	194	153	58	488	205	149	220	170	146	56	37	36	40	69
N_A	37	38	35	32	37	37	41	46	45	35	36	26	42	36	35	38	32	27	23	11	10	10	12
N_S	24.06	21.97	22.86	21.60	22.95	22.30	22.88	23.08	23.46	23.10	23.67	22.26	23.20	23.05	22.40	23.47	20.51	16.22	18.98	10.94	9.99	9.72	10.67
H_o	0.93	0.95	0.95	0.91	0.95	0.93	0.91	0.94	0.93	0.95	0.92	0.95	0.95	0.94	0.95	0.91	0.89	0.84	0.86	1.00	0.89	0.85	0.90

H_E	0.95	0.94	0.95	0.95	0.95	0.94	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.94	0.94	0.93	0.88	0.92	0.87	0.87	0.86	0.87
F_{is}	0.02	-0.01	0.00	0.04	0.00	0.02	0.04	0.01	0.02	0.00	0.03	-0.003	0.00	0.01	-0.004	-0.012	0.04	0.05	0.07	-0.155	-0.025	0.01	-0.035

Cpa113	AF406949, 2 bp repeat (CT), allele size range 106-216 (122-190)																						
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	123	197	189	168	196	168	407	456	436	194	152	62	499	211	149	220	169	145	65	37	37	40	68
N_A	17	20	19	21	20	20	24	21	21	19	20	16	25	21	19	20	23	21	18	15	13	15	14
N_S	14.62	15.23	15.49	15.92	14.93	15.95	15.50	15.59	15.60	15.18	15.07	14.97	15.71	15.62	15.22	15.65	17.17	16.62	15.80	13.81	12.30	14.03	12.39
H_o	0.93	0.90	0.94	0.90	0.90	0.95	0.91	0.91	0.92	0.92	0.88	0.87	0.90	0.92	0.91	0.91	0.91	0.95	0.83	0.76	0.78	0.93	0.91
H_E	0.91	0.91	0.92	0.91	0.91	0.92	0.91	0.92	0.92	0.92	0.90	0.92	0.91	0.92	0.92	0.92	0.93	0.93	0.90	0.87	0.87	0.90	0.90
F_{is}	-0.014	0.010	-0.03	0.010	0.020	-0.04	0.00	0.00	0.00	0.00	0.03	0.05	0.01	0.00	0.01	0.01	0.02	-0.03	0.07	0.13	0.10	-0.03	-0.02

Cpa114	AF406950, 4 bp repeat (ATCT), allele size range 180-291 (193-266)																						
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	122	197	187	164	197	165	404	454	438	194	154	60	499	212	149	220	169	142	60	38	37	39	65
N_A	19	19	21	14	20	20	23	22	20	21	19	13	24	20	18	20	21	18	14	14	16	15	17
N_S	13.22	13.46	12.97	11.89	13.36	13.46	13.50	13.16	13.55	14.70	13.63	11.65	13.12	14.02	13.21	14.42	13.44	14.24	12.06	13.43	15.46	14.30	14.79
H_o	0.91	0.91	0.91	0.90	0.87	0.90	0.88	0.91	0.87	0.89	0.88	0.87	0.86	0.92	0.89	0.92	0.94	0.87	0.82	0.58	0.84	0.90	0.92
H_E	0.89	0.90	0.89	0.88	0.89	0.88	0.89	0.89	0.89	0.90	0.89	0.88	0.89	0.89	0.89	0.90	0.88	0.89	0.89	0.89	0.90	0.90	0.90
F_{is}	0.00	-0.01	-0.03	-0.02	0.01	-0.02	0.02	-0.03	0.02	0.01	0.00	0.02	0.03	-0.03	-0.01	-0.02	-0.02	0.03	0.08	-0.01	0.07	0.00	-0.03

Cpa125	AF309796, 2 bp repeat (GA)(GT), allele size range 228-325 (245-309)																						
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	121	191	185	123	194	167	381	435	416	171	151	68	465	188	137	215	167	129	40	34	36	37	68
N_A	32	35	40	33	37	34	41	42	42	38	34	34	39	36	38	40	33	33	26	18	15	19	23
N_S	22.16	23.39	24.88	22.88	22.50	23.52	23.33	24.02	23.19	23.82	23.79	25.59	23.35	23.26	23.31	24.58	21.85	23.16	24.01	17.46	14.55	17.64	16.81
H_o	0.90	0.95	0.95	0.94	0.93	0.95	0.95	0.97	0.95	0.94	0.91	0.96	0.94	0.94	0.93	0.94	0.93	0.84	1.00	0.91	0.97	0.81	0.94
H_E	0.94	0.95	0.95	0.95	0.94	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.94	0.95	0.94	0.94	0.95	0.92	0.90	0.91	0.90

F_{is}	0.04	0.00	0.01	0.01	0.02	0.00	0.00	-0.02	0.00	0.01	0.04	0.00	0.01	0.01	0.01	0.01	0.02	0.04	-0.05	0.01	-0.08	0.11	-0.04

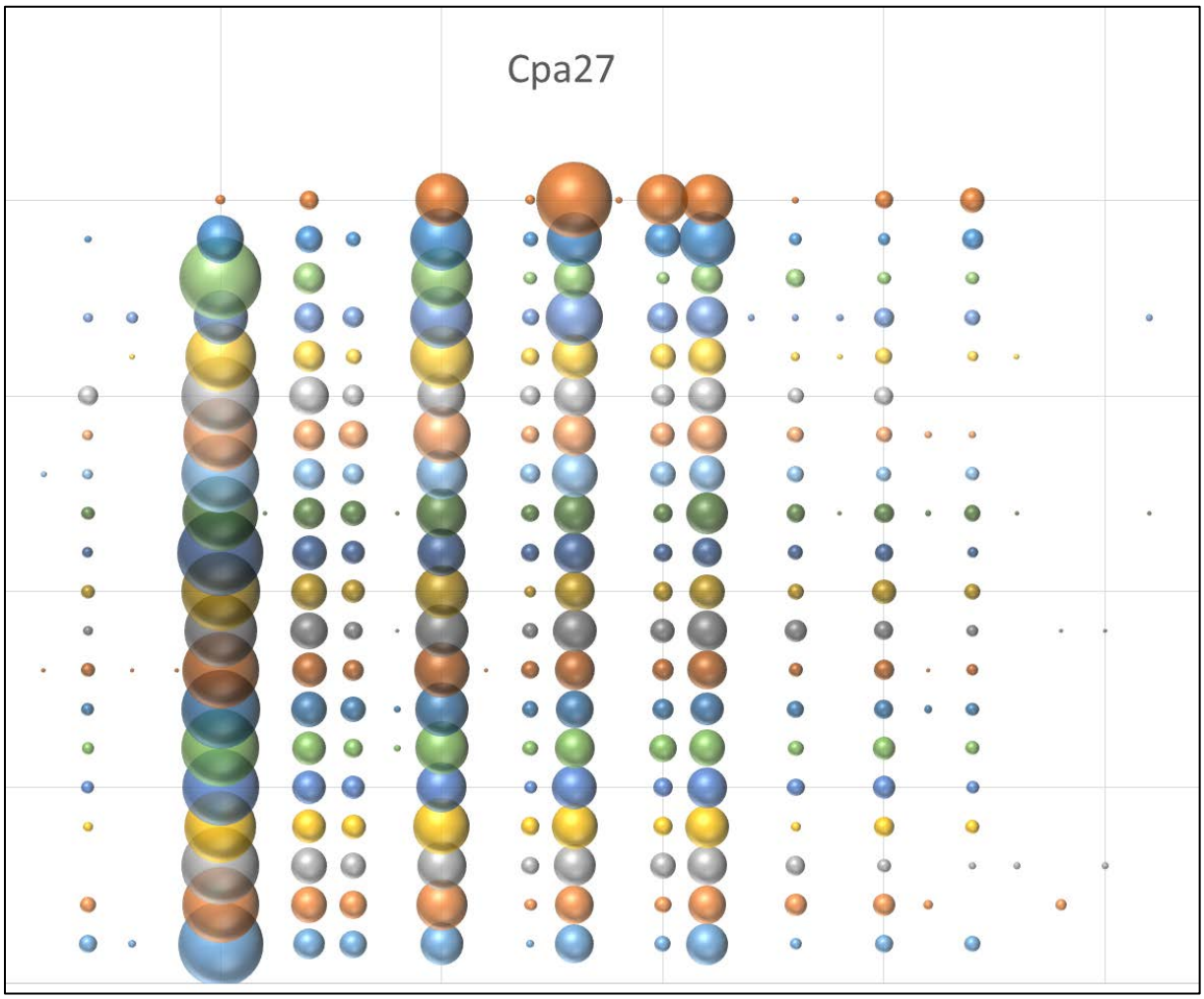
Cpa134	AF309798, 2 bp repeat (CA), allele size range 111-227 (115-192)																						
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	123	194	189	167	197	167	409	459	439	192	149	72	495	207	150	219	169	142	64	37	37	40	69
N_A	27	28	32	28	34	32	37	37	33	29	32	21	40	34	28	33	28	27	20	20	19	20	26
N_S	16.67	16.25	17.23	16.51	17.96	17.54	16.37	16.64	16.99	17.17	17.18	16.53	16.07	17.43	16.85	17.86	16.00	16.38	17.08	19.17	18.08	18.77	19.45
H_o	0.93	0.92	0.92	0.92	0.91	0.91	0.91	0.88	0.90	0.88	0.81	0.86	0.91	0.93	0.91	0.88	0.92	0.87	0.81	0.92	0.89	0.98	0.93
H_E	0.92	0.91	0.92	0.91	0.92	0.92	0.91	0.91	0.91	0.91	0.91	0.92	0.91	0.92	0.92	0.92	0.92	0.91	0.92	0.92	0.89	0.90	0.90
F_{is}	-0.01	-0.01	0.01	-0.01	0.01	0.01	0.01	0.04	0.01	0.04	0.12	0.06	0.00	-0.02	0.01	0.04	-0.01	0.05	0.12	0.01	0.00	-0.08	-0.03

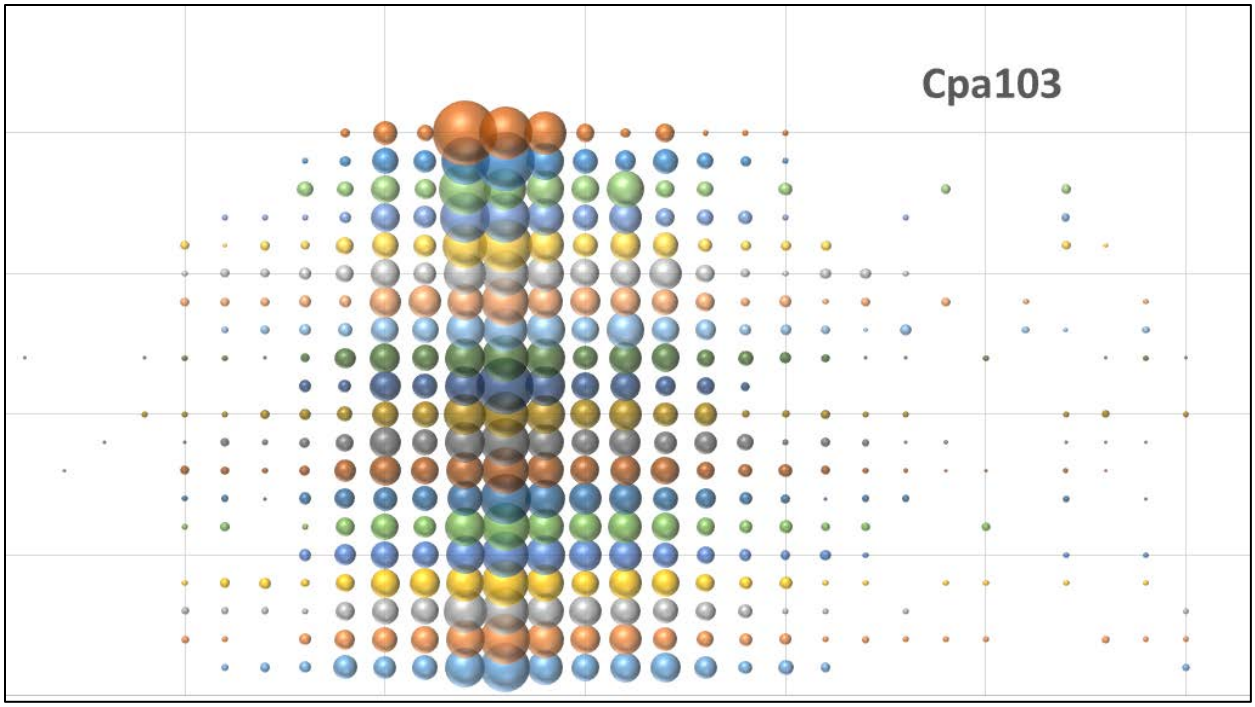
Cha63	AF019987, 2 bp repeat (GT), allele size range 121-193 (130-179)																						
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	124	194	189	161	198	167	401	455	432	173	154	71	485	210	141	220	169	139	62	37	36	40	69
N_A	22	27	25	24	27	23	28	26	26	25	25	19	30	25	23	22	27	24	21	11	13	11	19
N_S	17.54	18.55	17.58	17.98	18.32	17.06	17.63	18.05	18.07	17.52	17.22	16.95	18.17	18.22	17.51	17.23	17.70	17.73	18.15	10.64	12.65	10.71	14.94
H_o	0.90	0.95	0.92	0.90	0.91	0.86	0.92	0.90	0.92	0.90	0.86	0.92	0.90	0.92	0.89	0.88	0.92	0.90	0.90	0.84	0.78	0.83	0.86
H_E	0.90	0.92	0.92	0.91	0.91	0.90	0.91	0.91	0.91	0.91	0.90	0.90	0.91	0.91	0.91	0.92	0.92	0.92	0.93	0.80	0.84	0.87	0.86
F_{is}	0.00	-0.04	0.00	0.01	0.00	0.05	-0.01	0.01	-0.01	0.01	0.04	-0.02	0.01	-0.01	0.03	0.04	-0.01	0.02	0.03	-0.05	0.07	0.05	0.01

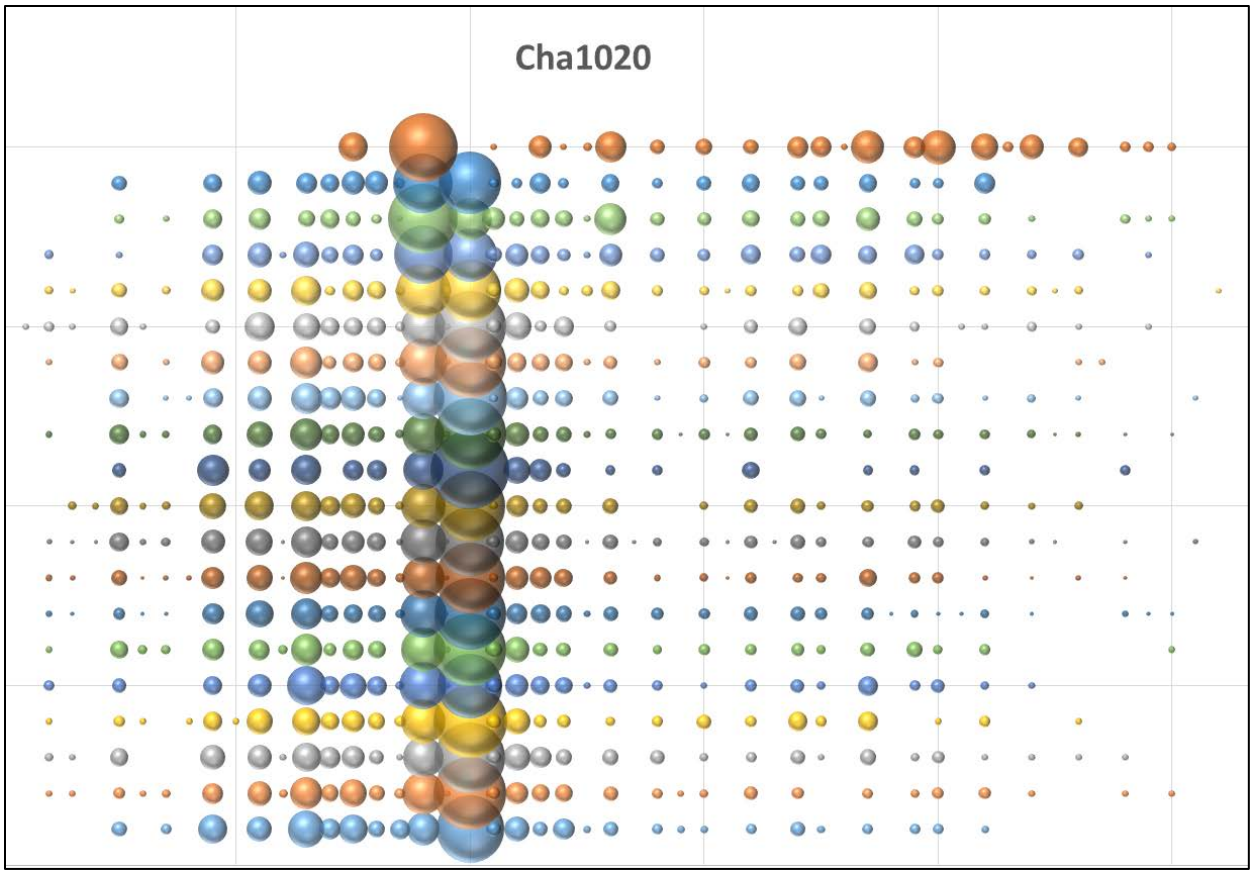
Cha1017	AF289096, 4 bp repeat, (GATA), allele size range 147-179 (155-175)																						
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	124	190	189	164	191	170	404	451	426	194	153	70	492	212	150	220	168	145	65	37	37	40	69
N_A	7	6	6	7	5	6	8	7	6	6	7	6	6	6	5	7	7	6	6	6	6	6	5
N_S	5.24	5.02	5.27	5.45	4.79	5.06	5.27	5.31	5.07	5.12	5.94	5.35	5.19	5.31	4.69	5.42	5.96	5.80	5.40	5.97	6.00	5.77	4.81
H_o	0.52	0.53	0.60	0.56	0.50	0.55	0.55	0.53	0.51	0.53	0.59	0.44	0.52	0.57	0.45	0.46	0.53	0.60	0.49	0.68	0.54	0.58	0.49
H_E	0.57	0.58	0.58	0.59	0.53	0.53	0.57	0.56	0.54	0.58	0.62	0.47	0.59	0.61	0.49	0.59	0.64	0.65	0.53	0.70	0.77	0.70	0.64
F_{is}	0.08	0.08	-0.02	0.04	0.06	-0.03	0.02	0.07	0.06	0.09	0.04	0.06	0.11	0.07	0.09	0.07	0.17	0.07	0.08	0.04	0.30	0.18	0.24

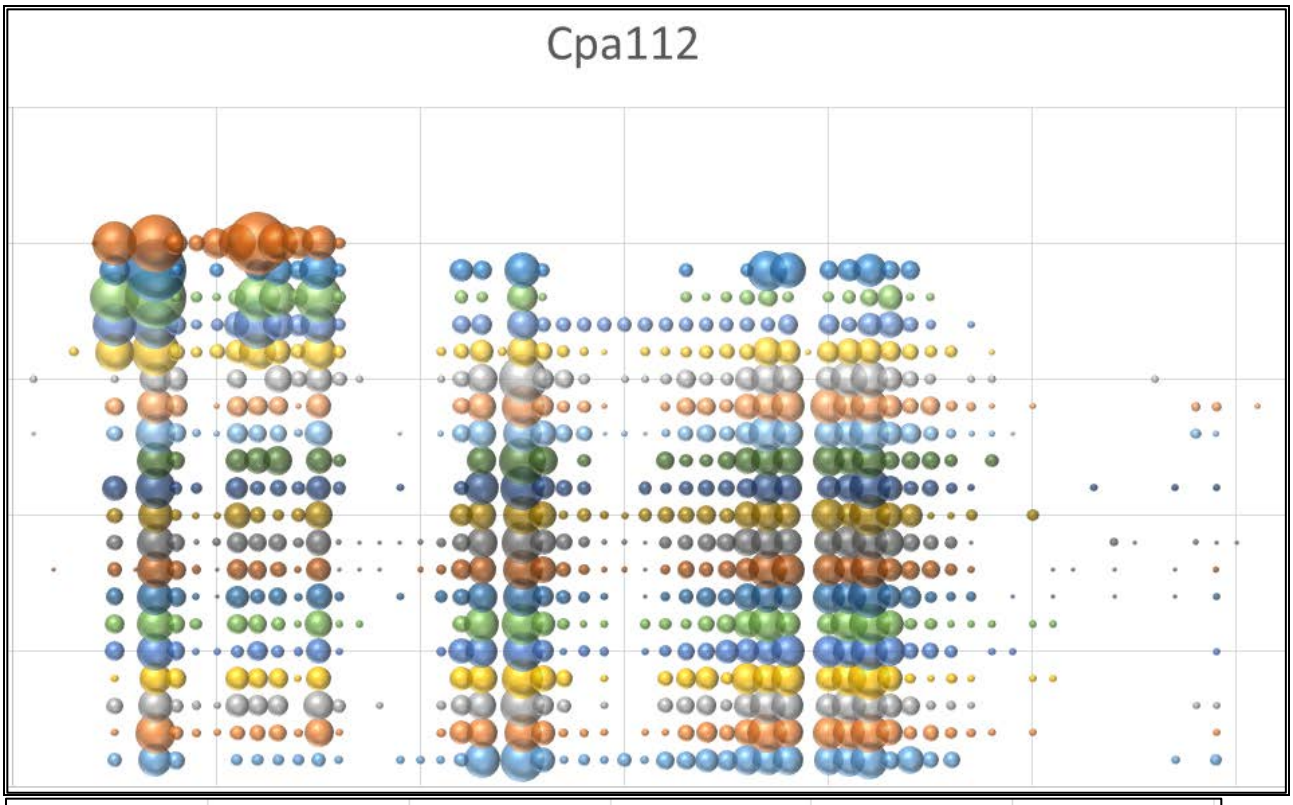
Cha1020	AF289095, 4 bp repeat, (GACA), allele size range 122-248 (162-240)																						
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
N	122	195	184	164	194	162	395	443	432	190	148	68	492	211	150	219	170	146	63	38	37	40	69
N_A	27	33	30	28	28	29	36	35	39	29	30	18	36	29	28	34	29	30	24	18	16	18	21
N_S	17.77	17.20	17.32	16.73	17.14	17.58	16.64	16.54	17.44	16.93	17.09	13.71	17.44	16.22	16.74	18.58	19.06	18.84	18.50	16.91	14.77	16.51	16.71
H_o	0.82	0.83	0.86	0.79	0.83	0.77	0.78	0.82	0.82	0.82	0.78	0.74	0.82	0.81	0.77	0.84	0.85	0.8	0.79	0.89	0.78	0.85	0.87
H_E	0.85	0.81	0.83	0.81	0.86	0.81	0.81	0.83	0.83	0.83	0.82	0.76	0.82	0.81	0.82	0.86	0.88	0.84	0.84	0.85	0.81	0.85	0.86
F_{is}	0.04	-0.03	-0.03	0.03	0.03	0.05	0.04	0.02	0.02	0.01	0.05	0.03	0.00	0.00	0.06	0.03	0.03	0.05	0.06	-0.06	0.04	0.00	-0.01

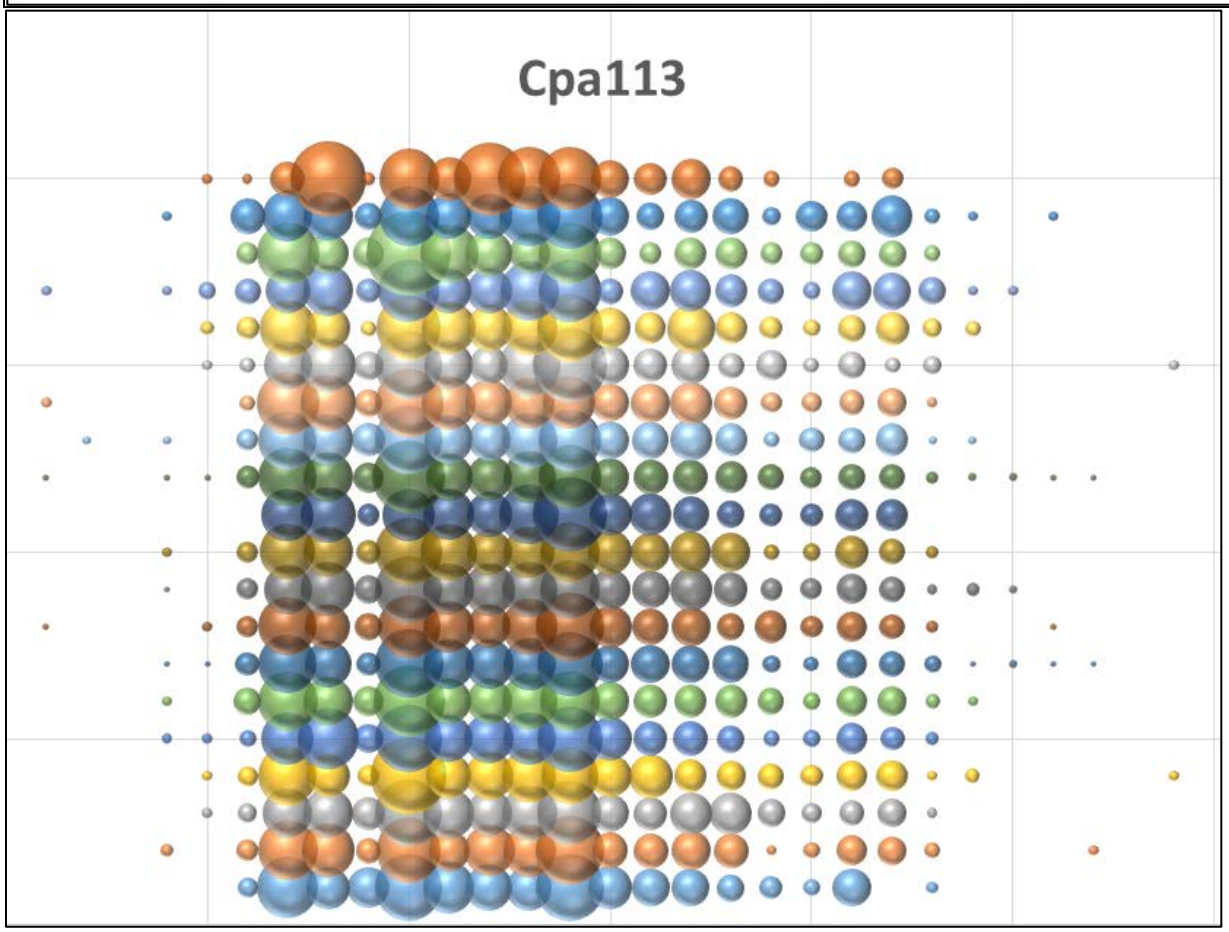
Appendix 2. Plots of microsatellite loci allele frequencies for 20 collections of herring. Each row is a collection of fish. Collections order following Table 1.
The bottom row 1=Craig, SE AK, row 2=Yakutat, row 3=Kayak Island, Rows 4-10=east PWS, rows 5-15=west PWS, row 16 and 17=Cook Inlet, row 18=Kodiak Island, row 19= Shelikof Strait, and the top row (20) is the Bering Sea (4 collections pooled).

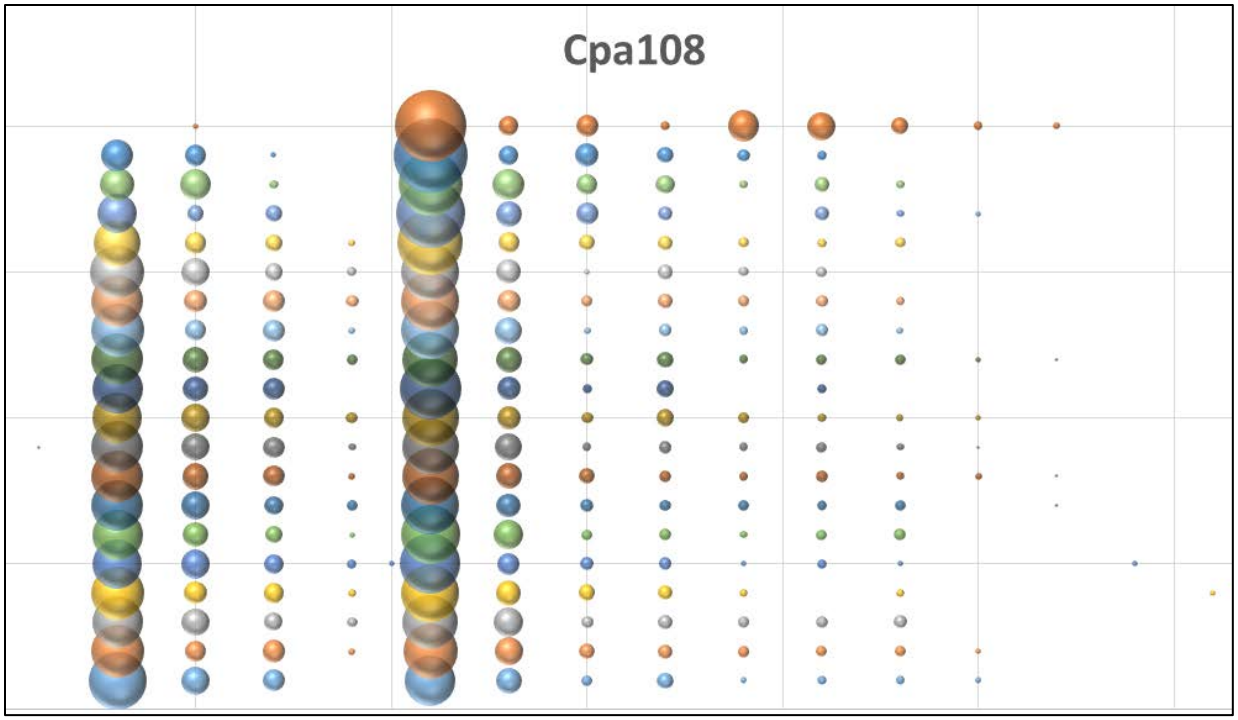


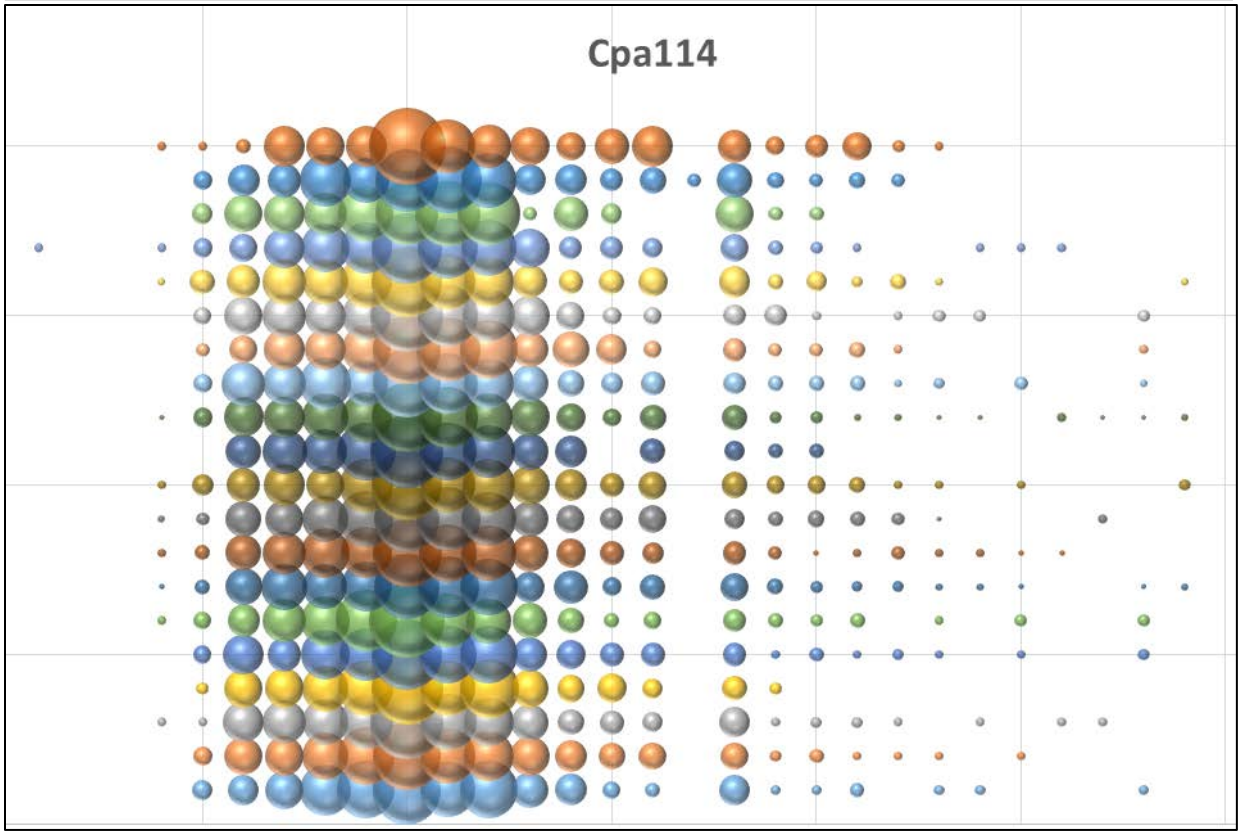

Cpa103


Cha1020


Cpa112


Cpa113


Cpa108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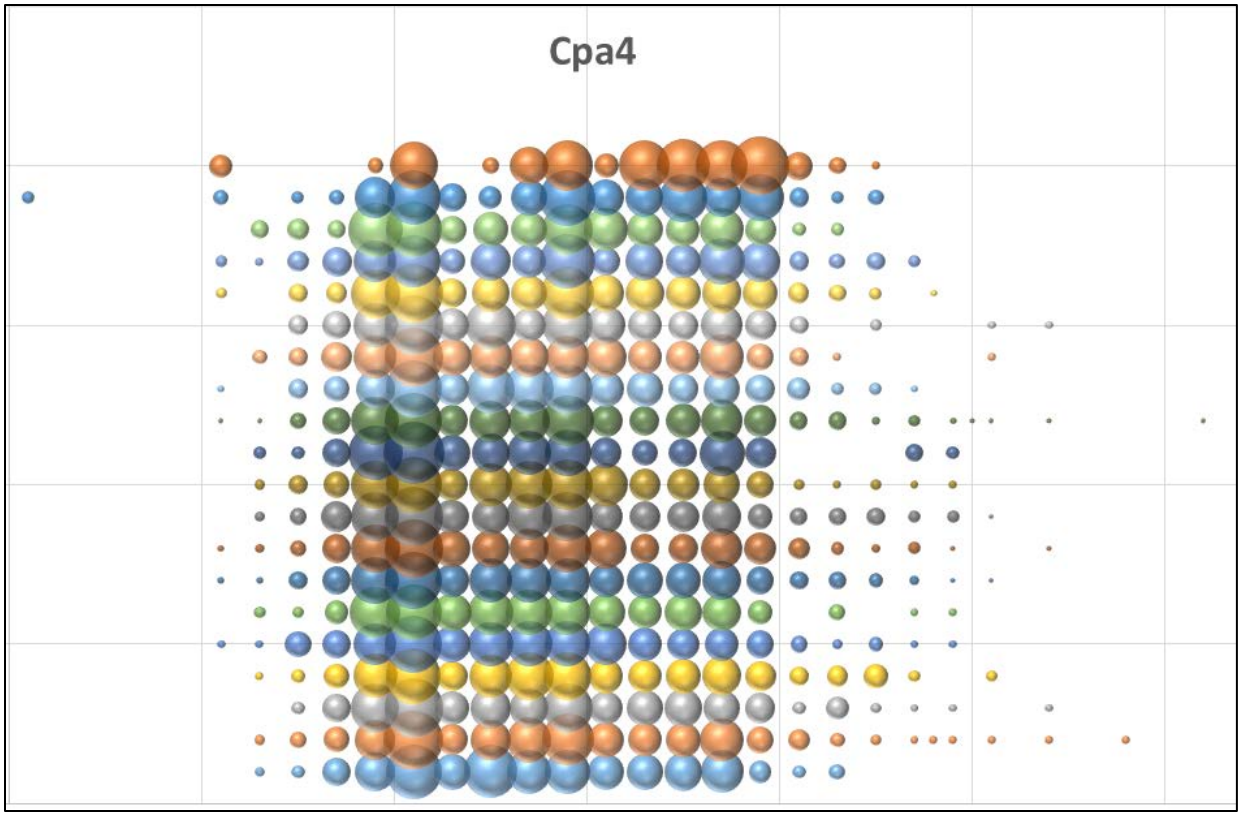
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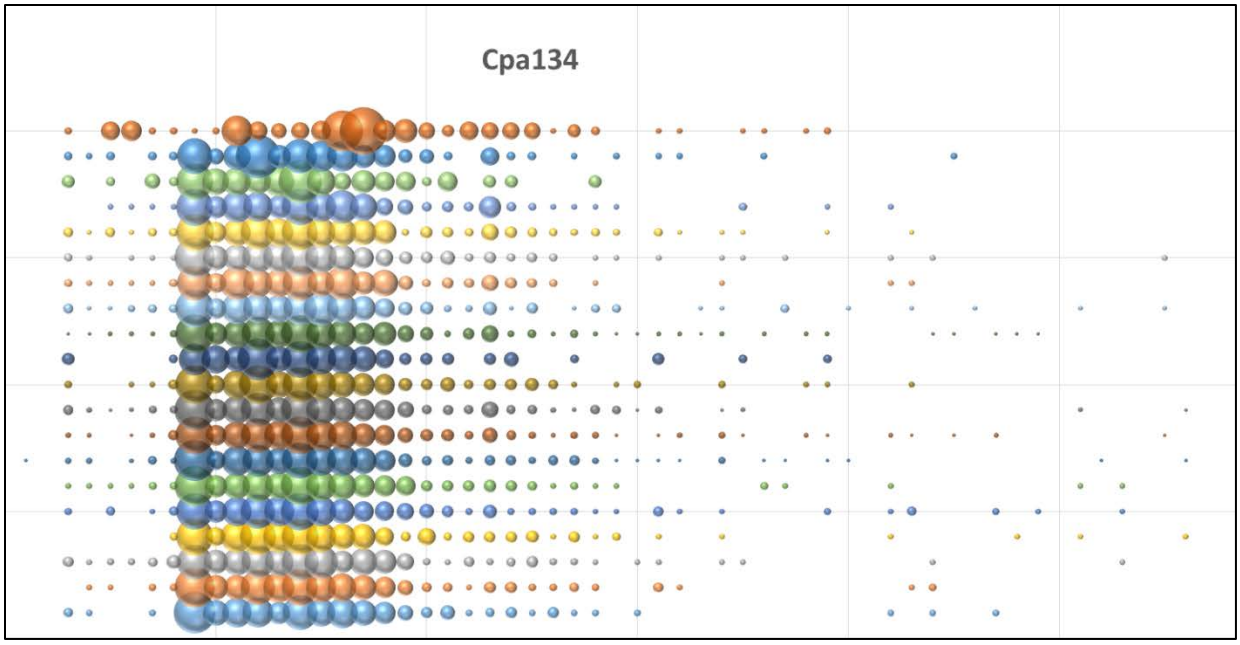
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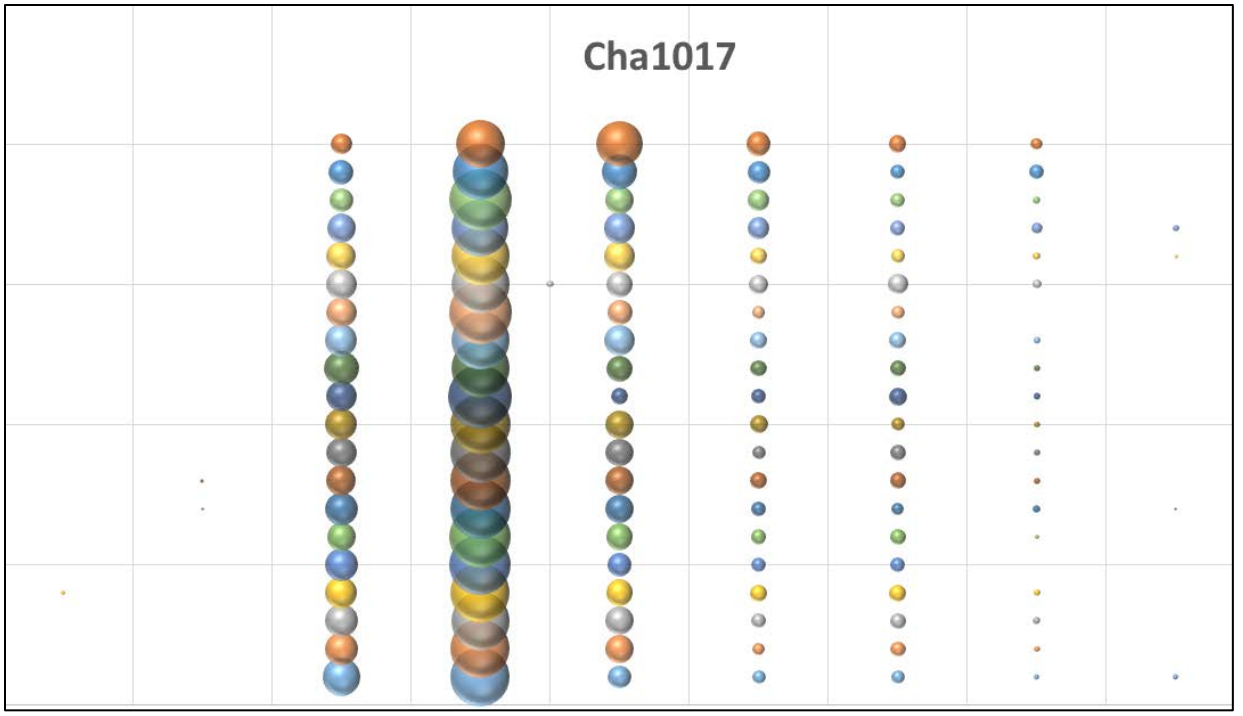
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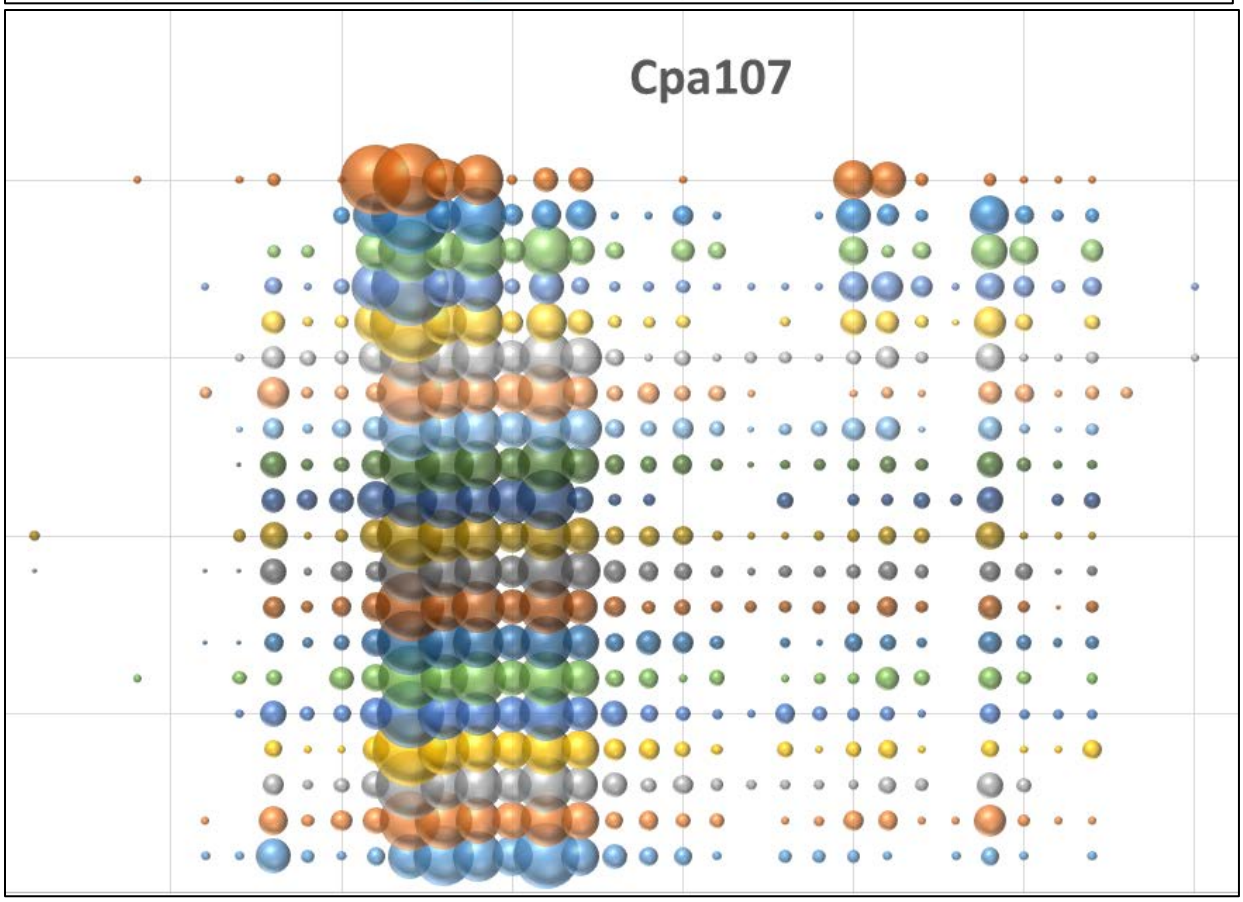
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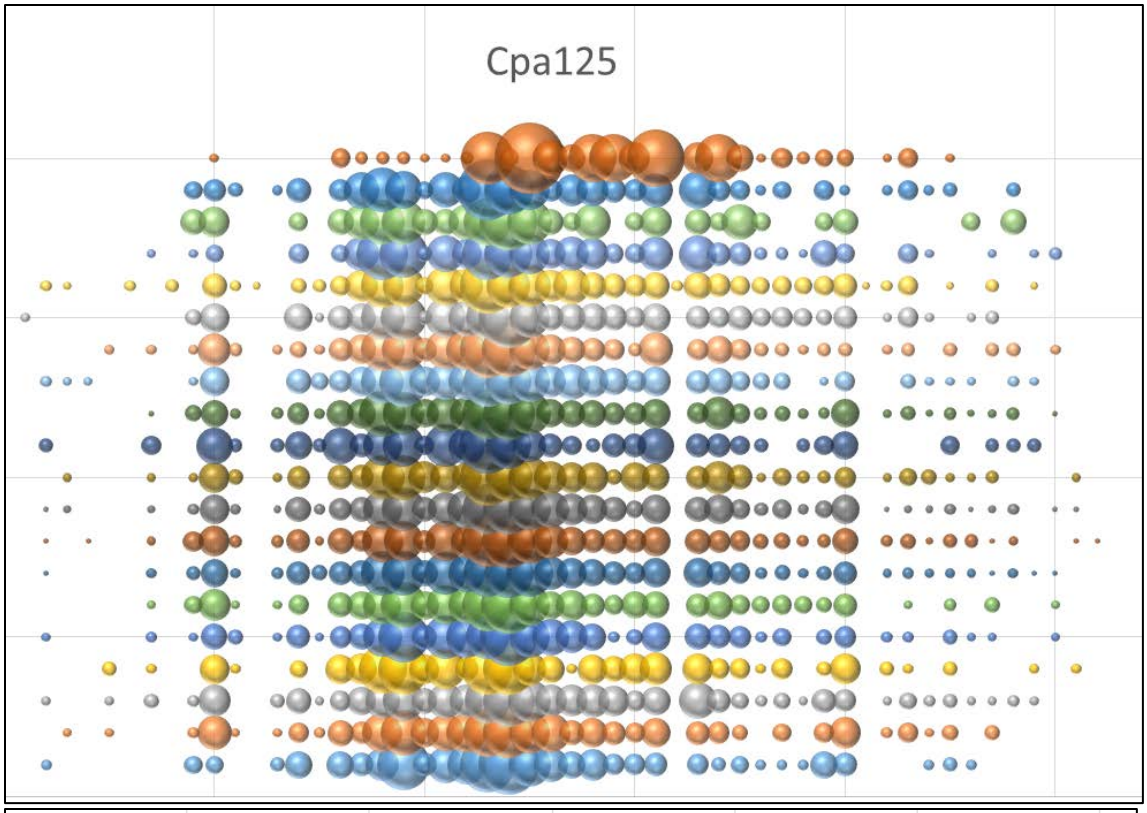
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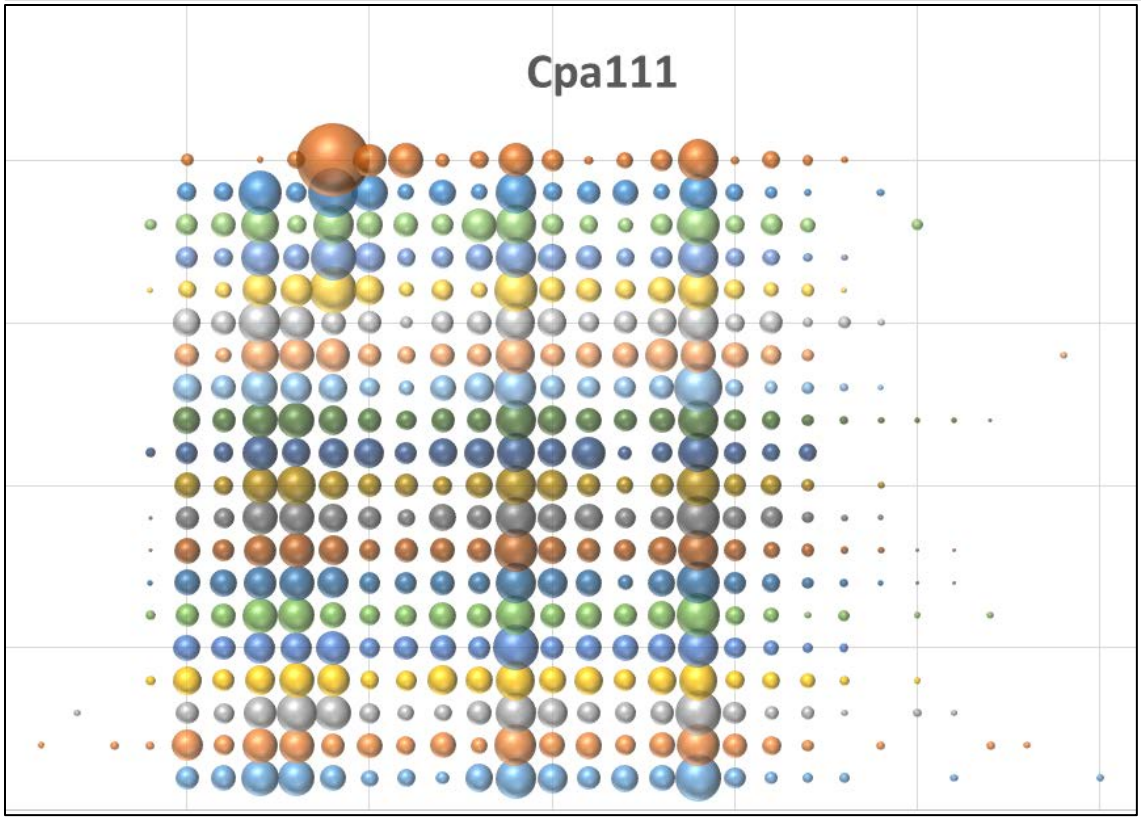
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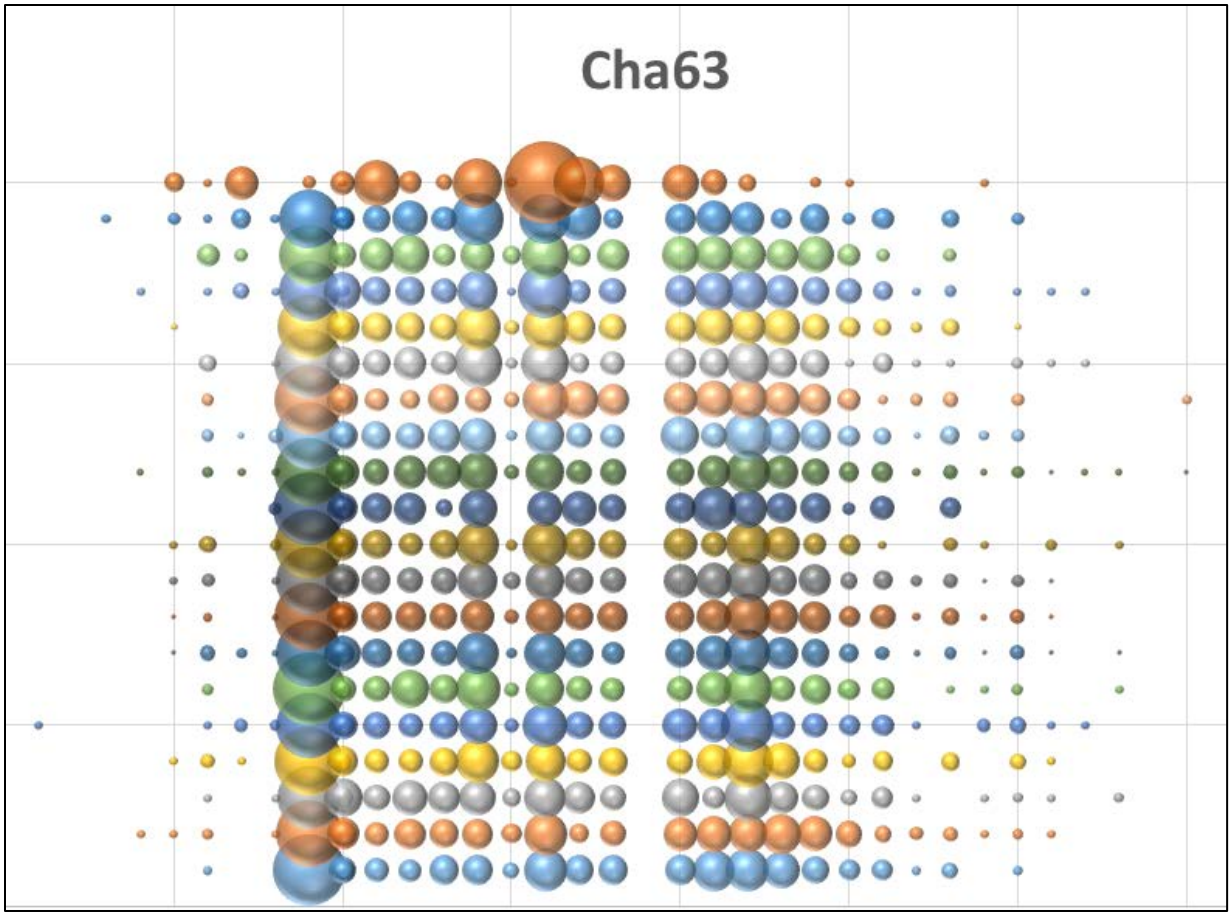
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