Running Head:
Divergence of chum salmon in Japan

Title:

Enhanced genetic differentiation of Japanese chum salmon identified from a meta-analysis of allele frequencies

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Abstract
The number of individuals returning to Japan, the location of the world's largest chum salmon hatchery program, has declined substantially over two decades. To find the genetic cause of this severe decline never previously experienced, we analyzed published genetic data sets for adult chum salmon, namely, 10 microsatellites, 53 single nucleotide polymorphisms (SNPs) and a combined mitochondrial DNA locus (mtDNA3), and three isozymes, from 576 locations in the distribution range (n = 76,363). The SNPs were selected for stock identification to achieve high accuracy, were highly differentiated in the distribution range and included important genes related to reproduction, growth and immune responses. By contrasting the genetic differentiation of these genes with the population structure estimated from the neutral microsatellite markers, we identified genes that distort the neutral population structure. We matched the sampling locations of SNPs and isozymes with those of microsatellites based on geographical information, and performed regression analyses of SNP and isozyme allele frequencies of matched locations on the population structure. TreeMix analysis indicated two admixture events, from Japan/Korea to Russia and the Alaskan Peninsula. Meta-analysis of allele frequencies identified three outliers, mtDNA3 (control region and NADH-3), GnRH373 (gonadotropin-releasing hormone) and U502241 (unknown), which showed enhanced differentiation in Japanese/Korean populations compared with the others. GnRH improves stream odor discrimination and has increased expression in adult chum salmon brains during homing migration, suggesting that the current admixture was caused by GnRH373 differentiation. mtDNA plays a key role in endurance exercise training, energy metabolism and oxygen consumption, suggesting that the significant reduction in mtDNA3 allele frequencies reduced aerobic athletic ability, as observed in YouTube videos. Our analyses relied on limited data sets, though they were the best available. Clearly, genome-wide data will be needed to fully address this issue.

Keywords:
demographic history, hatchery, microsatellite, mtDNA, population structure, SNPs

1 | INTRODUCTION

Pacific salmon have the longest history of artificial propagation, which started in the middle of the eighteenth century, and management of these species constitutes the world's largest set of hatchery enhancement programs (reviewed by Naish et al., 2007). Chum salmon (*Oncorhynchus keta*) comprises the world's largest marine stock enhancement and sea-ranching program (Amoroso, Tillotson, & Hilborn, 2017; Kitada, 2018). Canada, South Korea (hereafter, Korea), Russia, the United States and Japan currently operate Pacific salmon hatcheries in the North Pacific. According to North Pacific Anadromous Fish Commission statistics (NPAFC, 2020), the total number of chum salmon released into the North Pacific during six decades (1952–2019) was 137 billion, which included 90 billion juveniles (66%) released by Japan. Currently, ~60% of captured chum salmon is of hatchery origin (Ruggerone & Irvine, 2018). The biomass of chum salmon is at a historical maximum in the North Pacific. However, the proportion of chum salmon in the Japanese catch was 72% in 2005, but steeply decreased to 23% in 2019 (Figure S1).

Japan runs the world's largest chum salmon hatchery release program (Supplemental Note). At present, 262 salmon hatcheries operate in Japan. The number of chum salmon juveniles released from Japan was 0.5 billion in 1970 and has increased remarkably since the 1970s—to 1.8 billion in 2019 (Figure S2). Supported by natural shifts in marine productivity, the number of chum salmon returning to Japan sharply increased after the 1970s (Beamish, Mahnken, & Neville, 1997). Despite continuous hatchery releases, the number of returning chum salmon steeply decreased after 1996, the year when the recorded catch reached a historical maximum of 81 million fish (266 thousand tons) (Figure S2). In 2019, the catch was 17 million fish (56 thousand tons), which was ~20% of the maximum. The 1992 closure of high-sea salmon fisheries (Morita et al., 2006), which harvested a mixed stock of North American and Asian chum salmon populations (e.g., Beacham et al., 2009b; Myers, Klovach, Gritsenko, Urawa, & Royer, 2007; Seeb et al., 2011; Urawa et al., 2009; Wilmot et al., 1994), might have been expected to increase the homing of mature fish to their natal rivers. A marked increase after the closure of the high-sea salmon fisheries was only observed in Russia (Figure S2b), however, which suggests that the Sea of Okhotsk, an indispensable common feeding ground for juvenile chum salmon originating in Russia and Japan (Beamish, 2017; Mayama & Ishida, 2003), has been a favorable environment. This outcome further suggests that Japanese chum salmon might lose out to Russian chum salmon in the competition among juveniles for food in the Sea of Okhotsk. A regression analysis explained 62% of variation in the decreasing Japanese catch after 1996 by Russian catch and the coastal sea surface temperature in Hokkaido and northern Honshu (Kitada, 2018).

The genetic effects of hatchery-reared animals on wild populations have long been a major concern (e.g., Allendorf, 1991; Gharrett & Smoker, 1993; Hilborn, 1992; Laikre, Schwartz, Waples, & Ryman, 2010; Waples, 1991, 1999; Waples & Drake, 2004). Salmonids are genetically the most well-studied non-model organisms (Glover et al., 2017; Waples, Naish, & Primmer, 2020). Evidence exists for reduced survival rates (Reisenbichler & McIntyre, 1977; Reisenbichler & Rubin, 1999) and reproductive success of hatchery-reared salmonids in the wild (Araki, Cooper, & Blouin, 2007, 2009; Fleming et al., 2000; McGinnity et al., 2003, reviewed by Christie, Ford, & Blouin, 2014). Genetic adaptation to hatchery environments can occur even in early generation hatchery-born steelhead (*O. mykiss*) (Christie, Marine, French, & Blouin, 2012; Christie, Marine, Fox, French, & Blouin, 2016). Artificial selection in hatcheries may also drive maladaptation to climate change and act in a direction opposite to

that of natural selection in sockeye salmon (*O. nerka*) (Tillotson, Barnett, Bhuthimethee, Koehler, & Quinn, 2019). Moreover, hatchery environments can favor genotypes associated with domestication when genes from farmed Atlantic salmon (*Salmo salar*) are substantially introgressed into hatchery broodstock (Hagen et al., 2019). In a marine fish, red sea bream (*Pagrus major*), the reduction in survival rates of hatchery-reared fish born from captively reared parents was constant over time (Kitada et al., 2019). Almost all chum salmon returning to Japan are hatchery-released fish (Kaeriyama, 1999) or possibly wild-born hatchery descendants at present (i.e., no real wild fish may exist) (Ruggerone & Irvine, 2018; Kitada, 2020), and huge numbers of returning fish are used for broodstock in hatcheries every year (Supplemental Note). It was hypothesized that the significant decline in Japanese chum salmon populations was induced by genetic effects of long-term hatchery releases over 130 years (Kitada, 2020). However, no empirical study has examined the causal mechanisms for the rapid decline in Japanese chum salmon populations.

To find the genetic cause of the population decline, we analyzed published genetic data sets of adult chum salmon collected from the species' distribution range. First, we inferred the population structure and demographic history of chum salmon based on microsatellites, which can be used as neutral markers. Second, we performed a meta-analysis of allele frequencies of SNPs, mtDNA and isozymes. The SNPs had been developed for stock identification (Elfstrom, Smith, & Seeb, 2007; Smith et al., 2005a; Smith, Elfstrom, Seeb, & Seeb, 2005b). To achieve high accuracy, rapidly evolving genes associated with fatty acid synthesis, testis-specific expression, olfactory receptors, immune responses, and cell growth and differentiation (Nielsen et al., 2005), were purposely selected. By contrasting the genetic differentiation of these important genes with the population structure estimated by the neutral microsatellite markers, we identified a unique environmental and anthropologic selection force.

2 | MATERIAL AND METHODS

2.1 | Changes in chum salmon catch in the North Pacific and Japan

We reviewed changes in regional catch statistics in the North Pacific. Chum salmon catch and release data were retrieved from NPAFC statistics (NPAFC, 2020) (Table S1). We organized the numbers of released and returning chum salmon by prefectures/areas in Japan (Table S2).

2.2 | Screening of genetic data for chum salmon

We screened studies in the published literature after 1990 using the Google Scholar online database search system. We also added studies known to us. Population genetics have been extensively studied for chum salmon over the last four decades in the North Pacific. International sampling of chum salmon has been conducted cooperatively across its distribution range to establish baseline genotype data for effective genetic stock identification in high-sea fisheries (Beacham, Candy, Le, & Wetklo, 2009a; Beachamet al., 2009b; Seeb, Crane, & Gates, 1995; Seeb & Crane, 1999; Urawa, Azumaya, Crane, & Seeb, 2005; Urawa et al., 2009; Wilmot et al., 1994). Population structure has also been extensively studied using various markers, such as isozymes (Kijima & Fujio, 1979; Okazaki, 1982; Wilmot et al., 1994; Seeb, Crane, & Gates, 1995; Sato & Urawa, 2015; Seeb & Crane, 1999; Winans, Aebersold, Urawa, & Varnavskaya, 1994) and mtDNA (Garvin, Saitoh, Churikov, Brykov, & Gharrett, 2010; Park, Brainard, Dightman, & Winans, 1993; Sato et al., 2004; Yoon et al., 2008). Minisatellite (Taylor, Beacham, & Kaeriyama, 1994) and microsatellite markers (Beacham, Sato, Urawa, Le, & Wetklo, 2008a; Beacham, Varnavskaya, Le, & Wetklo, 2008b;

Beacham, Candy, Le, & Wetklo, 2009a; Beacham et al., 2009b; Olsen et al., 2008) have also been used. In the 2000s, SNPs were developed for accurate stock identification and also used for population genetics (Garvin et al., 2013; Sato, Templin, Seeb, Seeb, & Urawa, 2014; Seeb et al., 2011; Petrou et al., 2014; Small et al., 2015).

Through the data screening, we found several publicly available genetic data sets covering the distribution range of the species, including Japan. These data, which comprised microsatellite allele frequencies (Beacham, Candy, Le, & Wetklo, 2009a), nuclear SNP genotypes with a combined mtDNA locus (Seeb et al., 2011), and isozyme allele frequencies (Winans, Aebersold, Urawa, & Varnavskaya, 1994; Seeb, Crane, & Gates, 1995), were subsequently analyzed in this study. Note that all Japanese samples were caught in weirs in hatchery-enhanced rivers and hatcheries and were therefore hatchery-reared fish and/or hatchery descendants.

2.3 | Microsatellites

Microsatellite allele frequencies of chum salmon were retrieved from the Molecular Genetics Lab, Fisheries and Oceans Canada website (https://www.pac.dfo-mpo.gc.ca/science/facilities-installations/pbs-sbp/mgl-lgm/data-donnees/index-eng.html, accessed on August 1st, 2020), whose data consisted of allele frequencies at 14 loci from 381 localities throughout the distribution range (n = 51,355) (Table S3; Figure S3) (Beacham, Candy, Le, & Wetklo, 2009a). The data set comprised baseline allele frequencies throughout the Pacific Rim obtained from Canada, Korea, Russia, the USA and Japan. The data were used to infer the population structure and demographic history of chum salmon.

2.4 | SNPs

SNP genotypes retrieved from the Dryad data repository included 58 SNPs from 114 samples throughout the whole distribution range (Table S4; Figure S4) (Seeb et al, 2011). We excluded four loci following the original study, namely, *Oke_U401-220*, *Oke_GHII-2943*, *Oke_IL8r-272* and *Oke_U507-87*, thus leaving 53 nuclear SNPs and a combined mtDNA locus (mtDNA3) in our analysis (n = 10,458). The mtDNA locus included three gene loci, namely, *Oke-Cr30* and *Oke-Cr386* (mtDNA control region) and *Oke-ND3-69* (mtDNA NADH-3). The mtDNA locus (mtDNA3) had five alleles and the major allele was used in our meta-analysis.

The SNPs (Seeb et al, 2011) were genotyped using previously developed SNPs (Elfstrom, Smith, & Seeb, 2007; Smith et al. 2005a; Smith, Elfstrom, Seeb, & Seeb, 2005b). To improve the resolution of genetic stock identification of chum salmon by increasing the chances of observing large allele frequency differences among populations (Elfstrom, Smith, & Seeb, 2007), these markers were chosen from SNPs that have been previously characterized as rapidly evolving, including genes associated with fatty acid synthesis, testis-specific expression, olfactory receptors, immune responses, cell growth and differentiation (Nielsen et al. 2005). Thus, the chum salmon SNP markers included important genes such as GnRH (gonadotropin-releasing hormone), growth hormone (GH), heat shock protein (HSP), interleukin, transferrin, mtDNA control region and mtDNA NADH-3 genes (Elfstrom et al. 2007; Smith et al. 2005a).

GnRH is a key regulator of reproduction in vertebrates, including salmonids (Khakoo, Bhatia, Gedamu, & Habibi, 1994). GH has an important role in controlling mammalian longevity (Bartke & Quainoo, 2018) and somatic growth in salmonids (Björnsson, 1997). The GH-

insulin-like growth factor (IGF) system regulates muscle growth in vertebrates including fish (Fuentes, Valdés, Molina, & Björnsson, 2013). HSP is expressed in response to a wide range of stressors, and HSP protein is elevated when heat stress is experienced in salmonids (von Biela et al., 2020). Interleukins are a subgroup of cytokines that play a major role in the intercellular regulation of the fish immune system (Secombes, Wang, & Bird, 2011). Transferrin is related to iron storage and resistance to bacterial disease in Pacific salmon (Ford, 2001). As for mtDNA, the control region is related to the endurance capacity and/or trainability of individuals (Murakami et al., 2002), and is associated with athletes' endurance and contributes to individual differences in aerobic exercise performance (reviewed by Stefàno et al., 2019). By observing the geographical distributions of allele frequencies of the diverged SNPs, we could obtain signals of natural adaptation and hatchery-derived selection on related genes.

2.5 | Isozymes

We focused on LDH isozymes, which can be useful for understanding physiological thermal adaptation and the evolutionary consequences of artificial selection in salmonids and marine fish populations (Chen, Farrell, Matala, & Narum, 2018; Nielsen, Hemmer-Hansen, Larsen, & Bekkevold, 2009). *LDH-A* and *LDH-B* genes are predominantly expressed in various fish species in skeletal muscle and the heart (Powers, Lauerman, Crawford, & DiMichele, 1991; Somero, 2004), respectively. The loci have two codominant alleles, and their allele frequencies exhibit clear latitudinal clinal variation (Karabanov & Kodukhova, 2018; Merritt, 1972; Powers, Lauerman, Crawford, & DiMichele, 1991; Somero, 2004). For comparative purposes, we used tripeptide aminopeptidase (PEPB), a digestive enzyme of fish (Govoni, Boehlert, & Watanabe, 1986).

We organized publicly available allele frequencies of *LDH-A*, *LDH-B* and *PEPB-1* loci in the Pacific Rim, namely those collected in Japan (Kijima & Fujio, 1979; Okazaki, 1982), Japan and Russia (Winans, Aebersold, Urawa, & Varnavskaya, 1994), and northwest Alaska, Yukon, the Alaskan Peninsula, southeast Alaska (SE Alaska), British Columbia (BC) and Washington (Seeb, Crane, & Gates, 1995). To avoid problems associated with standardization of electrophoretic bands obtained in different laboratories, we used the data from the two latter studies because they used the same protocol (Aebersold, Winans, Teel, Milner, & Utter, 1987) and followed the genetic nomenclature of the American Fisheries Society (Shaklee, Allendorf, Morizot, & Whitt, 1990). Samples collected from three hatcheries were excluded from the North American samples. We obtained allele frequencies of the three isozyme loci in 81 chum salmon populations in the distribution range (n = 14,550) (Table S5 and Figure S5). Four alleles were found at the *LDH-A1* locus, two of which were very minor and found in only seven populations. The *LDH-B2* locus also had four alleles, but two were again very minor and found in only three populations. The *PEPB-1* locus had five alleles. We used the most common alleles—LDH-A1*100, LDH-B2*100 and PEPB-1*-100—in our meta-analysis.

2.6 | Accounting for ascertainment bias in microsatellite and SNP data sets

Significant deviations from Hardy–Weinberg equilibrium at four loci (*Oke*3, *Ots*103, *One*111 and *OtsG68*) found from a 14-microsatellite locus panel in Japan, Russia and representative North American populations (Beacham, Sato, Urawa, Le, & Wetklo, 2008a; Beacham, Varnavskaya, Le, & Wetklo, 2008b) were likely because of ascertainment bias (Seeb et al., 2011). Ascertainment bias can occur when markers are selected from unrepresentative

individual samples and then used to infer population structure for a much larger set of samples (Bradbury et al., 2011; Templin, Seeb, Jasper, Barclay, & Seeb, 2011). To account for ascertainment bias, we excluded four loci, and 10 loci were used in our analysis for estimating population structure and demographic history.

For the SNP data, the highest levels of allelic richness observed in part of the Alaskan region might be affected by ascertainment bias, though the effects of the bias were expected to be minimal within Alaska (Seeb et al., 2011). Allele/site frequency spectrums are useful to examine potential ascertainment bias in SNPs (Nielsen, Hubisz, & Clark, 2004). We calculated observed allele frequency spectrums in six geographical areas according to the major lineage used in the original study (Seeb et al., 2011), which were Western Alaska, Yukon/Kuskokwim upper, Alaskan Peninsula, SE Alaska/BC/Washington, Russia, and Japan/Korea. The allele frequency spectrums showed a similar pattern in all areas except Western Alaska (Figure S6), where a much smaller frequency was observed for the smallest allele frequency, suggesting a possible ascertainment bias caused by cutting off rare alleles in this region. A straightforward way to account for ascertainment bias is to exclude populations that are susceptible to potential bias (Templin, Seeb, Jasper, Barclay, & Seeb, 2011). We prepared a subset of data that excluded populations sampled from Western Alaska. Using the full data (114 populations) and subset data (77 populations), we performed a meta-analysis of allele frequencies to detect genes affected by hatchery-derived selection and compared the results.

2.7 | Population structure and demographic history based on microsatellites

To infer the population structure of chum salmon, we used the bias-corrected G_{ST} moment estimator to compute pairwise F_{ST} (Nei & Chesser, 1983) based on the microsatellite allele frequencies collected from 381 locations (Beacham, Candy, Le, & Wetklo, 2009a). Our previous coalescent simulations found that Nei and Chesser's bias-corrected G_{ST} moment estimator performed the best among F_{ST} estimators when estimating pairwise F_{ST} values (Kitada, Nakamichi, & Kishino, 2017). As only allele frequency data (no genotypes) were provided, we used the 'read.frequency' function and computed pairwise F_{ST} values averaged over loci using the 'GstNC' function in the R package FinePop ver.1.5.1 on CRAN. Multi-dimensional scaling (MDS) was applied to the pairwise F_{ST} distance matrix using the 'cmdscale' function in R. As an explained variation measure, we used the cumulative contribution ratio up to the kth axis $(j = 1, \dots, k, \dots, K)$, which was computed in the function as $C_k = \sum_{j=1}^{k} \lambda_j / \sum_{j=1}^{K} \lambda_j$, where λ_j is the eigenvalue and $\lambda_j = 0$ if $\lambda_j < 0$. Following the original study (Beacham, Candy, Le, & Wetklo, 2009a), the populations were classified into eight geographical areas: Japan, Korea, Russia, Alaskan Peninsula, Western Alaska, Yukon/Tanana/Upper Alaska, SE Alaska/BC, and Washington (Table S3). Colors specific to populations are used consistently throughout this paper.

We applied TreeMixv1.13 (Pickrell &Pritchard, 2012) to identify population splits and admixture. The analyses were performed using six regional genetic groups, where Japan/Korea and SE Alaska/BC/Washington were combined to reduce the number of parameters to be estimated under the limited number of loci. Based on allele frequencies and numbers of repeats (Beacham, Candy, Le, & Wetklo, 2009a), we computed the mean and variance in length at each microsatellite, which were used to run TreeMix. We tested up to five migration events and found the best tree was based on the Akaike Information Criterion (AIC, Akaike, 1973); $AIC = -2 \log \hat{L} + 2 \times m$, where $\hat{L}$ is the maximum composite

likelihood and m is the number of parameters to be estimated, which was three per migration event, namely, migration edge, branch length and migration weight. For example, the number of parameters was six for the model with two migration events. TreeMix maximizes composite likelihood and the 2log-likelihood ratio is expected to approximately follow a χ^2 distribution of df = 3. Because TreeMix calculates composite likelihoods, the penalty in AIC needs to account for the correlation in the likelihoods. To avoid unconscious bias toward selection of parameter-rich models, we kept the candidate models unless the AIC values were decisively different.

2.8 | Detecting genetic differentiations that distort the population structure of neutral microsatellite markers

We performed a meta-analysis of the combined allele frequencies of 53 nuclear SNPs, a combined mtDNA locus and three isozymes. The functions of all 57 analyzed gene loci were confirmed by referring to the GeneCards database system (https://www.genecards.org/) and published literature. For the data sets used in our analyses, we recorded longitudes and latitudes of sampling locations for genotyping using Google Maps, relying on the names of rivers and/or areas and maps of the sampling locations in the original studies.

To contrast the SNPs genetic differentiation against the population structure based on the neutral microsatellite markers, we first matched the 114 sampling locations of the SNPs and 81 locations of the isozymes to the nearest locations among the 381 sampling points of the microsatellites. One Korean sample was included in the microsatellite and SNP data, but not in the isozyme data. In addition, the same locations were not necessarily used for genotyping. When we did not find the same location names, we assigned the closest locations of microsatellites to those of SNPs and isozymes using the longitudes and latitudes of the sampling locations.

We then identified the genetic differentiations that distort the population structure of the microsatellite markers. Specifically, we performed regression analyses of SNP and isozyme allele frequencies on the two axes of MDS:

$$p_j^{(i)} = \beta_0^{(i)} + \beta_1^{(i)} mds1_j + \beta_2^{(i)} mds2_j + \varepsilon_j^{(i)}, \varepsilon_j^{(i)} \sim N(0, \sigma^2).$$

Here, $p_j^{(i)}$ is the allele frequency of locus $i\ (= 1, \ldots, L)$ in population $j\ (= 1, \ldots, J)$. The explanatory variables $mds1_j$ and $mds2_j$ are the first and second MDS coordinates of pairwise F_{ST} values estimated from the microsatellite allele frequencies of 381 locations, which were assigned to the matched populations of SNPs and isozymes. The regression analysis was performed for each marker, from which we obtained L sets of coefficients $(\beta_0^{(i)}, \beta_1^{(i)}, \beta_2^{(i)})$.

To identify genes that best characterize geographical areas, we conducted a principal component analysis (PCA) of the allele frequencies of the 53 nuclear SNPs and the combined mtDNA3 locus using the 'prcomp' function in R. Finally, we visualized the geographical distributions of the genes detected beyond neutrality using boxplots with a color gradient of allele frequencies for population j rendered as rgb $(p_{j0}, 0, 1 - p_{j0})$, where $p_{j0} = (p_j - \min p)/(\max p - \min p)$. This conversion represents the standardized allele frequencies value at the sampling point, with colors between blue (for the smallest allele frequencies) and

red (the largest ones). In the box plot, allele frequencies for geographically close areas were combined: Japan/Korea, Yukon/Kuskokwim upper and SE Alaska/BC.

2.9 | Athletic ability of Japanese chum salmon

We searched the social network YouTube for videos that recorded the athletic ability of returning chum salmon in Japan and other countries, collecting as many as possible without any intentional sampling. We counted the number of times salmon successfully crossed weirs and/or falls when possible.

3 | RESULTS

3.1 | A historical overview of chum salmon catches in the North Pacific and Japan

Hatchery-originated Japanese chum salmon occupied the bulk of the highest-ever recorded catch, 117 million fish in 1996, but this number had drastically shrunk by 2019 (Figure 1). In contrast, the total catch was relatively stable in Alaska, with some regional variation, while slight decreases were observed in SE Alaska, BC and Washington.

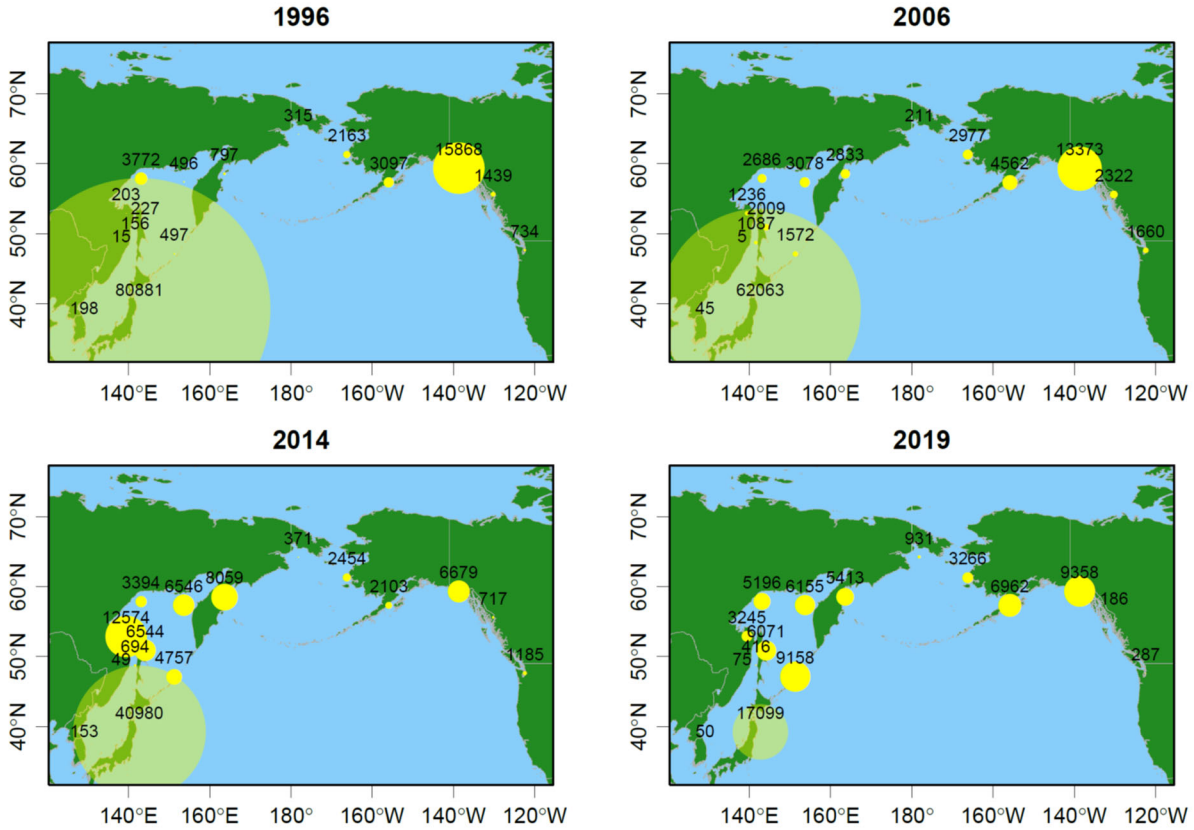

FIGURE 1 Changes in the chum salmon catch in the North Pacific over recent decades. Numbers (in thousands) are shown for 1996—the year of historical maxima in the North Pacific (117 million fish) and Japan (81 million fish)—and 2006, 2014 and 2019. Circle sizes are proportional to the number of salmon.

In Japan, the largest numbers of returning chum salmon were caught in the Sea of Okhotsk and on the Pacific coast, the latter affected by the cold-water Oyashio current. The catch in Iwate Prefecture (Figure B2 in Supplemental Note) was particularly notable in 1996, when the total catch in Japan was at a historical maximum (Figure 2). In contrast, very small catches

were obtained on the coast of the Sea of Japan, which is affected by the warm-water Tsushima current, and on the southern Pacific coast. In 2006, populations on the Pacific coast of Honshu rapidly decreased, whereas decreases on the Pacific coast of Hokkaido were insubstantial. Substantial declines occurred in all areas in 2014, particularly on the Pacific coast, with further shrinkage in 2019 even though the number of released chum salmon had been stable for two decades throughout Japan (Figure S7). These data show that the catch decline might be associated with warming sea temperature. In contrast, the Russian catch on Iturup Island markedly increased and reached a historical maximum of 8.3 million in 2019, which was equivalent to 55% of the total catch in Hokkaido.

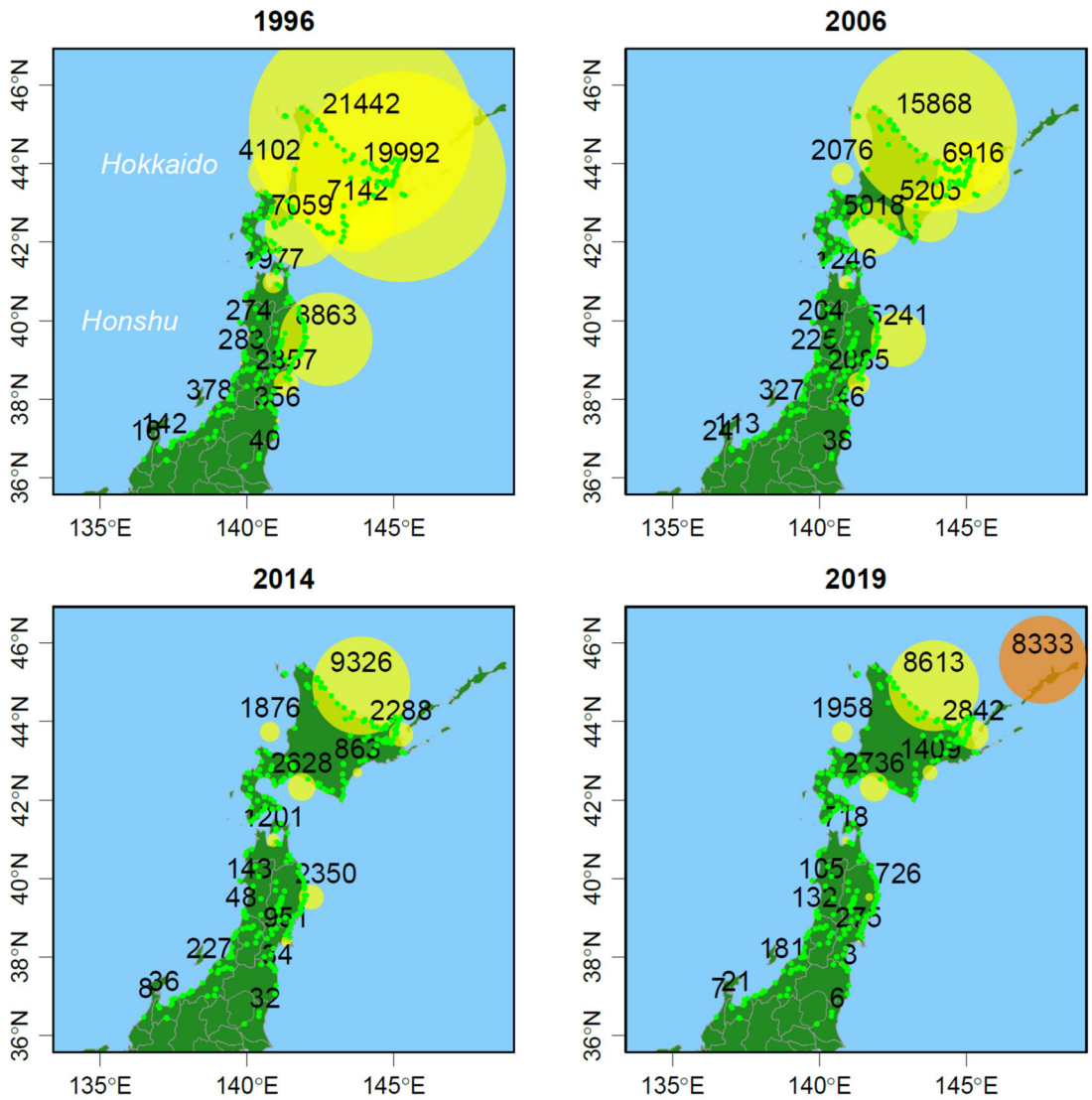

FIGURE 2 Changes in the number of chum salmon returning to Japan. Numbers (in thousands) are shown for 1996 (the historical maximum catch year), 2006, 2014 and 2019 (including the catch on Iturup Island, Russia). Circle sizes are proportional to the number of salmon. Green dots show salmon hatcheries.

3.2 | Population structure and demographic history in the distribution range

Pairwise F_{ST} values based on the microsatellite allele frequencies were 0.0185 ± 0.0103 (SD).

The MDS of pairwise F_{ST} values (Figure 3a) revealed a latitudinal cline and a separation of Alaskan populations from the others along the first axis (mds1) explaining 29% of the variance. The second axis (mds2, 16% of variance) showed a separation of Japanese/Korean populations from the others, but they were closely related to southern Russian populations from Amur and Primorye. Plots of mds1 and mds3, and mds1 and mds4 produced similar results, showing most differentiation was driven by divergence from a latitudinal cline (Figure S8). TreeMix analysis indicated two periods of admixture (migration events), from Japan/Korea to Russia and the Alaskan Peninsula (Figure S9; Figure 3b). Japanese/Korean populations had the highest level of drift among all groups, while the drift was the lowest in the Alaskan Peninsula and SE Alaska/BC/Washington.

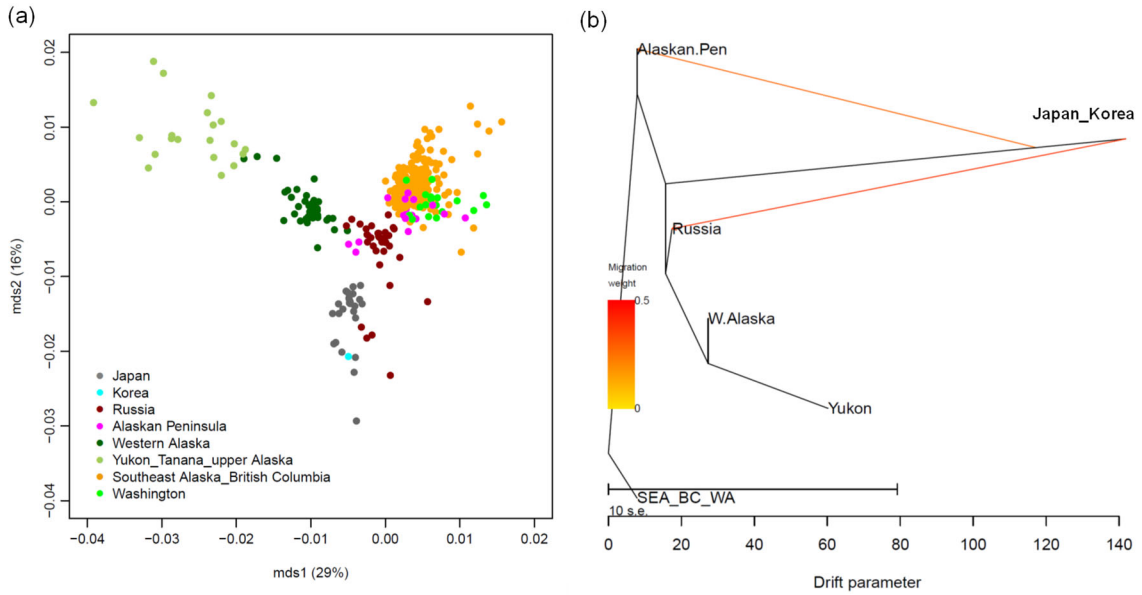

FIGURE 3 Population structure and admixture events of chum salmon based on microsatellite markers. (a) MDS plots (mds1 vs mds2) of the population structure of chum salmon in the distribution range based on pairwise F_{ST} values estimated from 10 microsatellite loci in 381 populations. (b) Admixture graph inferred with TreeMix with two migration events. The scale bar (drift parameter) is 10 times the average standard error of the sample covariance matrix of allele frequencies between populations (Pickrell1 & Pritchard, 2012).

3.3 | Genetic differentiations that distort the population structure of neutral markers

We summarized 57 genes with functions (if known), locus names and raw data locus names, which we used in our analysis (Table S6). In this list, locus names followed by an asterisk indicate five outlier SNPs identified as potential candidates for selection in the original study (Seeb et al., 2011), while *U* and *u* designate unknown SNPs.

Figure 4a shows three outliers, mtDNA3 (control region and NADH-3), GnRH373 (gonadotropin-releasing hormone) and U502241 with large contributions to mds2, which represents the differentiation of Japanese/Korean populations from the others. The PCA (Figure 4b) identified differences between Japanese/Korean and other populations as a primary component (PC1, 43% variance), while the second component (PC2, 20% variance) corresponded to the latitudinal cline among Russian/American samples. The PCA consistently indicated that Japanese/Korean populations were characterized by the three genes detected by

the regression analysis. Biplots of PCA1 and PCA3, and PCA1 and PCA4 described outliers (Susitna, and Sturgeon River on Kodiak Island), but most differentiation was caused by divergence of Japanese/Korean populations (Figure S10). Figure 5 confirms that their allele frequencies were distinct in Japanese/Korean populations in the distribution range. To reduce the effect of ascertainment bias, we also conducted the analysis excluding Western Alaskan populations, and confirmed the results were qualitatively unchanged (Figure S11).

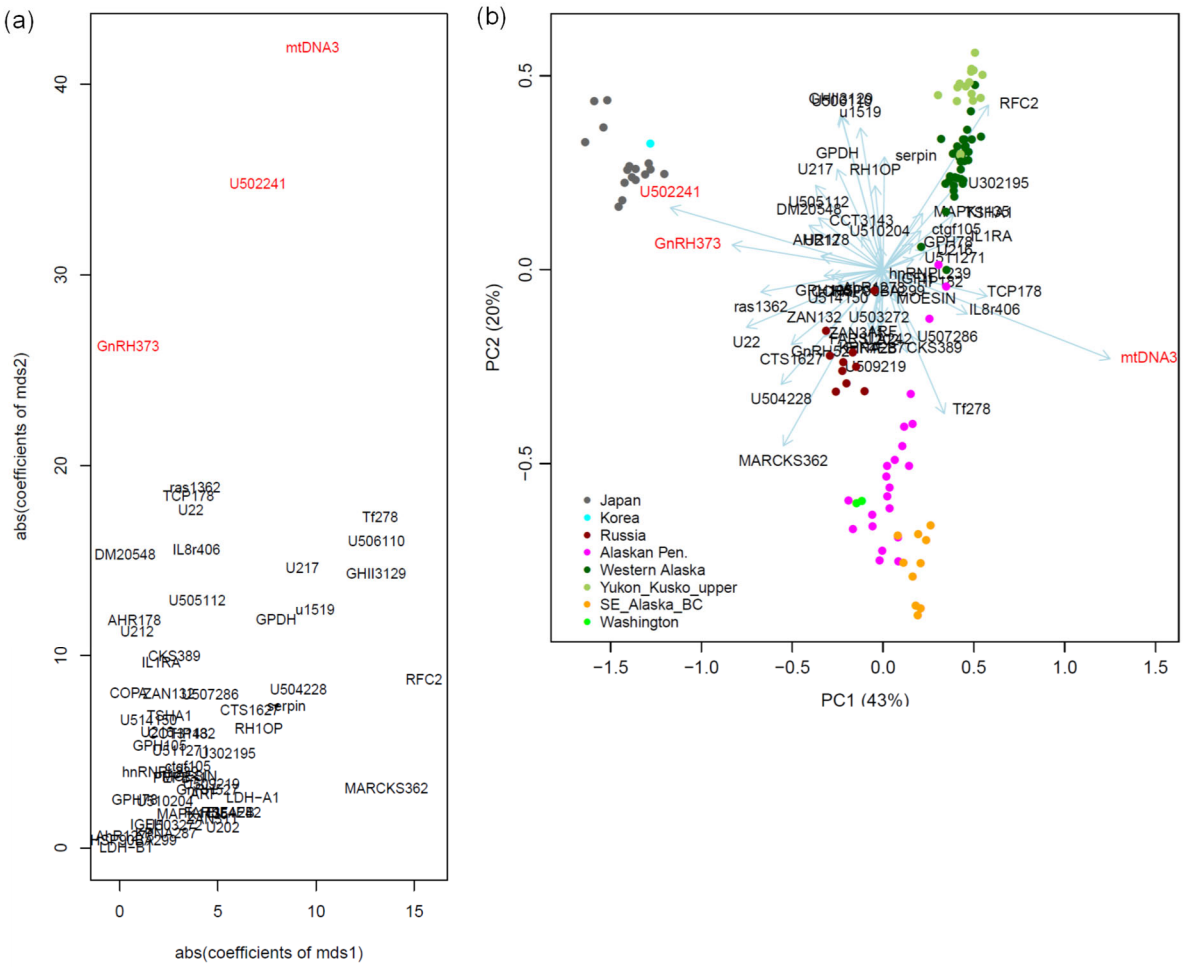

FIGURE 4 Particular genes characterizing the distinctiveness of Japanese/Korean chum salmon populations inferred from the full data set. (a) Absolute values of the regression coefficients of mds1 vs those of mds2 based on 53 nuclear SNPs, a combined mtDNA locus and three isozyme loci. The same scale was used for the first and second axes. (b) PCA biplot for 53 nuclear SNPs and a combined mtDNA locus.

All isozyme loci were plotted in the range of neutrality (Figure 4a). LDH-A1*100 allele frequencies were distributed along a south-to-north gradient in Russian and American samples, while the frequency in Japan was lower than those of Southeast Alaska and Washington (Figure S12). LDH-B2*100 allele frequencies were fixed at or near 1.0 in all populations. A north-to-south gradient was also found in PEPB-1*-100 allele frequencies.

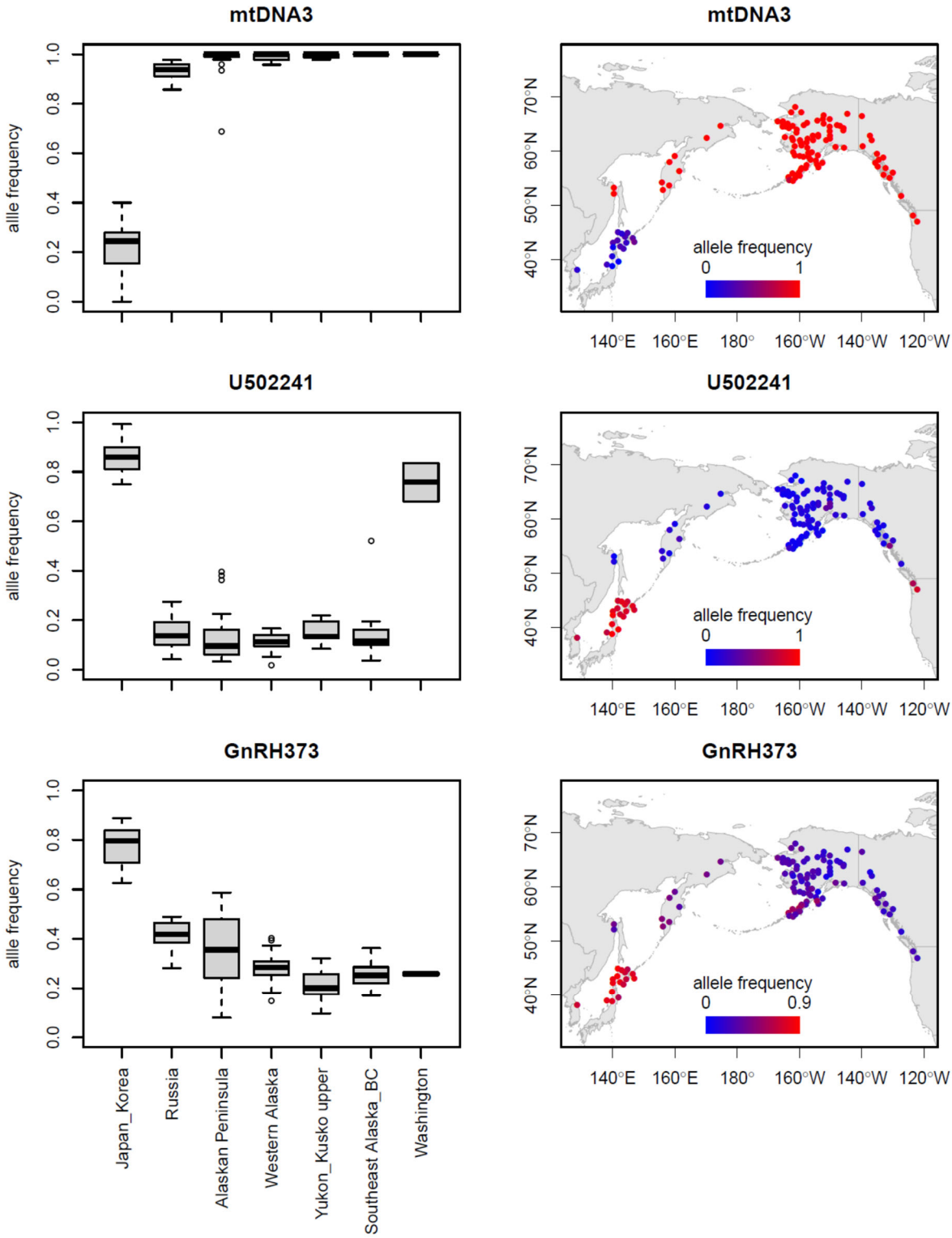

FIGURE 5 Geographical distribution of the three particular genes characterizing the distinctiveness of Japanese/Korean chum salmon populations. The points on the maps shows allele frequencies at sampling locations with a color gradient.

3.4 | Athletic ability of Japanese chum salmon

We found YouTube videos—seven from Japan and one from Washington, USA—that recorded the athletic ability of returning chum salmon. These eight videos were all we could

find and were chosen without any intentional selection.

In the Okushibetsu River, Shiretoko Peninsula, Hokkaido, a representative enhanced river to which thousands of chum salmon return every year (Hokkaido Shimbun Press, 2017), fish movement looked slow. Jumping trials of returning Japanese chum salmon at a small fall in Hokkaido at Esashi Town facing the Sea of Okhotsk (Sapporo TV House Stock Footage, 2015) resulted in no success after 64 jumps, excluding two jumps that were difficult to judge. When we counted these two jumps as successful, the rate of success was 3.0% (= 2/66). At another small fall in northern Hokkaido (Kawamura, 2008), the success rate was 1 out of 47 jumps (2.1%). At a small weir in the Hamamasu River, Hokkaido (Onishi, 2009), only one chum salmon succeeded out of 219 jumps (0.5%). Even at a much lower small fall in the Haboro River, Hokkaido (haruma1108, 2017), only three jumps were successful out of eight (37.5%). In the hatchery-enhanced Kido River, Fukushima, Honshu, none of the eight observed jumps were successful (The Asahi Shimbun, 2013). In this river, which is located in the southern distribution area in Honshu, body damage was severe (KyodoNews, 2014). In contrast, on the coast and along Puget Sound, Washington, USA, chum salmon were powerful swimmers, even swimming across roads during floods (Allison, 2016).

4 | DISCUSSION

The goal of this study was to identify the genetic cause of the decline in Japanese chum salmon populations. We first inferred population structure and admixture in the distribution ranges based on microsatellite allele frequencies. We then analyzed the pattern of genetic differentiation of rapidly evolving SNPs and isozymes, and identified mtDNA3, GnRH373 and U502241 as outliers that had enhanced differentiation in Japanese/Korean populations compared with the others.

The inferred population structure (Figure 3a) was consistent with TreeMix analysis (Figure 3b). The large standard error of the drift parameter might be related to the relatively small number of markers (10 microsatellite loci) as compared to the number of groups, which was six. However, a higher level of drift among Japanese/Korean populations was clear relative to Russian and American ones. This result suggested that Japanese/Korean populations are the most diverged from the ancestral population and that they diverged between Russia and the Alaskan Peninsula. Two suggested admixture events may reflect the stray of Japan/Korean populations into the Russian/Alaskan rivers. The result also indicated that modern chum salmon populations might be recolonized from source populations between the Alaskan Peninsula and SE Alaska/BC/Washington.

mtDNA encodes some proteins of the oxidative phosphorylation enzymatic complex, and plays a key role in aerobic ATP production, contributing to the ability to respond to endurance exercise training (reviewed by Stefàno et al., 2019). The mtDNA control region functions in dNTP metabolism (Nicholls & Minczuk, 2014) and oxygen consumption (Kong et al., 2020). Subunits of the NADH dehydrogenase complex in mtDNA are involved in the pathway from NADH to oxygen (Rivera et al., 1998). Evidence from previous studies suggested that a significant reduction in mtDNA3 (a combined locus of the control region and NADH-3) allele frequencies (Figure 5) reduces the efficiency of aerobic exercise ability and endurance, energy metabolism and oxygen consumption. The slow movement and lower athletic ability of Japanese chum salmon observed in YouTube videos agreed with this inference, suggesting physical exhaustion after long distance migration.

U502241 allele frequencies were much higher in Japan/Korea and Washington than in Russian and American populations (Figure 5). This phenomenon was found in the original study (Seeb et al. 2011). No function is associated with the chum U502241 locus (Elfstrom et al., 2007), but the Atlantic salmon immunoglobulin IgH locus B genomic sequence, which includes multiple HSPs, is the most likely match for U502241 (Seeb et al. 2011). HSP is elevated when heat stress is caused by warmer temperatures beyond suitable ranges and can cause mortality of salmonids (von Biela et al., 2020). However, the causes of the elevated levels of U502241 allele frequencies in Japan/Korea and Washington are unknown.

GnRH improves stream odor discrimination in adult chum salmon (Ueda, 2019), and expression of GnRH increases in adult chum salmon brains during homing migration (Ueda et al., 2016). The differentiation of GnRH373 (Figure 5) might influence homing migration timing, potentially leading to the current introgression from Japan into Russian populations, as suggested in our TreeMix analysis. GnRH is also involved in gonadal maturation during the early and final phases of upstream migration (Kudo et al., 1996). Efforts to enhance early-run (September to October) populations since the 1980s changed the run timing in Hokkaido, with late-run populations (December to January) having almost disappeared by the late 1990s (Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013). The artificial selection of run timing might affect GnRH373 allele frequencies.

Our analyses relied on limited data sets, though they were the best available covering the distribution range including Japan. Clearly, genome-wide data in the distribution range are needed to fully address the decline of chum salmon in Japan. Further study will provide more precise views of demographic history, and environmental and anthropogenic selection in this important species.

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AUTHOR CONTRIBUTION
S.K. and H.K conceived the study, analyzed the data and wrote the manuscript.

CONFLICT OF INTEREST
None declared.

DATA AVAILABILITY STATEMENT
The authors affirm that all data necessary for confirming the conclusions of this article are present within the article, figures and supporting information.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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