Genomic insights into environmental and anthropological selection on the physiology of Pacific salmon

Running head: Genomic insights into salmon physiology

Shuichi Kitada[1*] and Hirohisa Kishino[2]

[1] Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan.
[2] Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan.

Correspondence
Shuichi Kitada, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan.
Email: kitada@kaiyodai.ac.jp

Although chum salmon (*Oncorhynchus keta, Salmonidae*) biomass in the North Pacific is at a historical maximum, 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 search for potential causes of this decline in the context of evolutionary history, we synthesized catch/release and sea surface temperature (SST) statistics, and published genetic data, namely, 53 single nucleotide polymorphisms (SNPs) from 114 locations, lactate dehydrogenase (LDH) and tripeptide aminopeptidase (PEPB) isozymes from 81 locations, and mitochondrial DNA (mtDNA) D-loop haplotypes from 48 locations, in the distribution range (n = 27,170). SST in the summer, when juveniles inhabit Japanese coasts, was found to be negatively correlated with adult return rates 4 years later ($r = -0.62$). According to SNP-based population-specific F_{ST} values, the species may have originated in western Alaska and expanded its distribution southward. At the southern edge of the Asian distribution, the population structure of chum salmon has been rearranged by hatchery operations. Key functions of nine genes characterizing the distinct nature of the Japanese populations are reproduction, regulation of circadian rhythm, bacteria/parasite defense, DNA damage repair, growth and energy metabolism. Thermally adapted, derived alleles of LDH-A1*100, predominantly expressed in skeletal muscle, have often been replaced by ancestral alleles, while the ancestral mtDNA-B3 haplotype is significantly rarer. This genetic replacement might result in lower metabolic efficiencies in skeletal muscle and mitochondria at higher temperatures, thereby leading to the survival decline of juveniles in a warming climate.

Keywords: Evolution, fitness, global warming, Pacific salmon, thermal adaptation

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1 | INTRODUCTION

Hatchery propagation is one of the most popular tools for fishery management (Laikre, Schwartz, Waples, & Ryman, 2010). More than 26 billion juveniles of 180 marine species are released into the wild in globally diverse environments annually (Kitada, 2018). Among target species, Pacific salmon has the longest history of hatchery propagation, and its management constitutes the world's largest set of artificial propagation programs (Amoroso, Tillotson, & Hilborn, 2017). Currently, Canada, Korea, Russia, the United States and Japan operate Pacific salmon hatcheries. Hatchery release was originally designed for augmentation of fishery production but is now often used for conservation purposes. Several populations of Pacific salmon are listed as a species requiring conservation under the Endangered Species Act in the United States, where hatchery release has been used as a conservation tool (Hard, Jone, Delarm, & Waples, 1992). Despite extensive, longstanding efforts to enhance and/or recover salmonid populations, however, the long-term usefulness of Pacific salmon hatcheries for fisheries and conservation purposes is unclear (Naish et al., 2007). Hatchery propagation programs require the involvement of politicians, scientists, government agencies and the public, and scientific analyses are needed to inform decisions on future strategies (Ruckelshaus, Levin, Johnson, & Kareiva, 2002). To achieve these objectives, the evaluation of large-scale releases is indispensable, as pilot-scale releases cannot measure the long-term effects of hatchery releases on stocked populations (Hilborn, 2004). Hatchery release of chum salmon (*Oncorhynchus keta*, Salmonidae) is the world's largest marine stock enhancement and sea-ranching program (Amoroso, Tillotson, & Hilborn, 2017; Kitada, 2018). According to North Pacific Anadromous Fish Commission statistics (NPAFC, 2019), the total number of chum salmon released into the North Pacific during six decades (1952–2018) was 133 billion, of which Japan released 88 billion juveniles (66%). Approximately 60% of chum salmon are of hatchery origin (Ruggerone, & Irvine, 2018). The biomass of chum salmon is at a historical maximum in the North Pacific, whereas the proportion in Japan has steeply decreased (Figure S1).

Japan runs the world's largest chum salmon hatchery release program (*Supplemental Note*). At present, 262 salmon hatcheries operate in Japan (Kitada, 2020). Releases of chum salmon juveniles from Japan have increased remarkably since the 1970s—to ~1.5 billion in 2018 (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). Nevertheless, the mean body weight of chum salmon returning to Japan during this time significantly decreased, thus suggesting intra- and inter-species density-dependent effects in the North Pacific (Ishida, Ito, Kaeriyama, McKinnell, & Nagasawa, 1993; Kaeriyama, 1998). 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 (Figure S2). The 1993 closure of high-sea salmon fisheries, which harvested a mixed stock of North American and Asian chum salmon populations (Myers, Klovach, Gritsenko, Urawa, & Royer, 2007; Urawa et al., 2009), 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 S2), 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), is 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.

The increase in the sea surface temperature (SST) around Japanese coasts is negatively correlated with the decreased number of chum salmon returning to Japan (Kaeriyama, & Urabe, 2018; Kitada, 2018; Wagawa et al., 2016). Almost all chum salmon returning to Japan are hatchery-released fish (Kaeriyama, 1999) or possibly wild-born hatchery descendants with substantial gene flow between rivers (Kitada, 2020). The stray rate was very high, ~50%, in rivers in Hokkaido (Kitada, 2020); this is consistent with the results of an otolith thermal-marking study of chum salmon in Alaska that found that 51% of fish were hatchery-origin strays (McConnell et al. 2018). The serious decline in chum salmon returns may be due to the 130-year long-term hatchery operation (Kitada, 2020).

Genetic effects of hatchery-reared animals on wild populations are of major concern (e.g., Laikre, Schwartz, Waples, & Ryman, 2010; Waples, 1991, 1999; Waples, & Drake, 2004). Evidence exists that hatchery-reared salmonid breeding in the wild has reduced survival rates (Reisenbichler & McIntyre, 1977; Reisenbichler & Rubin, 1999) and reproductive success (Araki, Cooper, & Blouin, 2007, 2009; Fleming et al., 2000; McGinnity et al., 2003). High survival rates during hatchery rearing might relax natural selection and reduce the fitness of hatchery-reared salmonids in the wild (Araki, Cooper, & Blouin, 2007; Christie, Ford, & Blouin, 2014; Ford, 2002). Genetic adaptation to hatchery environments is also known to occur (Christie, Marine, French, & Blouin, 2012; Christie, Marine, Fox, French, & Blouin, 2016; Le Luyer et al., 2017). Hatchery environments can favor certain genotypes associated with domestication when genes from farmed fish are substantially introgressed into hatchery broodstock (Hagen et al., 2019). Moreover, artificial selection in hatcheries may drive maladaptation to climate change and act in a direction opposite to that of natural selection (Tillotson, Barnett, Bhuthimethee, Koehler, & Quinn, 2019). Enhanced growth of salmon juveniles due to the warming climate has enabled earlier migration to the ocean from freshwater, which can increase competition between wild and hatchery-released salmon (Cline, Ohlberger, & Schindler, 2019). An increased abundance of hatchery fish may cause genetic changes and reduce the resilience of salmon populations to changes in their habitat induced by a warming climate (Beamish, 2017).

To understand the cause of the decline in Japanese chum salmon returns in an evolutionary context, we made inferences about environmental and anthropological effects on Japanese populations using published genetic data sets of adult chum salmon collected from the species' distribution range. The data comprised single nucleotide polymorphisms (SNPs), mitochondrial DNA (mtDNA) haplotypes and isozyme allele frequencies. The SNP data set included annotated loci, which were expected to be useful for inferring the effects of environmental adaptation and anthropological selection on chum salmon physiology. We focused on lactate dehydrogenase (LDH) as an isozyme marker, which is potentially 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). In various fish species, *LDH-A* and *LDH-B* genes are predominantly expressed in skeletal muscle and the heart (Powers, Lauerman, Crawford, & DiMichele, 1991; Somero, 2004, 2010), 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, 2010). For comparative purposes, we used PEPB (tripeptide aminopeptidase), a digestive enzyme of fish (Govoni, Boehlert, & Watanabe, 1986), because of its possible selection pressure in hatcheries. We also focused on haplotype frequencies of the mtDNA D-loop region because recent studies

have inferred that this region functions in dNTP metabolism (Nicholls, & Minczuk, 2014), cold-temperature adaptation and energy metabolism (Nishimura, Motoi, Niri, Hoshi, Kondo, & Watanuki, 2012) and oxygen consumption (Kong et al., 2020).

We estimated the evolutionary history of chum salmon throughout its distribution range by comparing genome-wide population-specific F_{ST} values based on SNP genotypes among local populations on western and eastern sides of the North Pacific. We performed a meta-analysis of combined allele/haplotype frequencies, which allowed us to look for a correlation between significant geographical clines in allele frequencies and changes in the distribution range. In addition, we used the meta-analysis to make inferences about the selection and adaptation of chum salmon physiology in a warming climate.

2 | CHUM SALMON BIOMASS IN THE NORTH PACIFIC

To begin, we reviewed changes in regional catch statistics in the North Pacific and the relationship between SSTs of Japanese coasts and the number of returned fish. Chum salmon catch and release data were retrieved from NPAFC statistics (NPAFC, 2019). As revealed by changes in regional catches in the North Pacific, the number of hatchery-originated Japanese chum salmon, which occupied the bulk of the highest-ever recorded catch, 117 million fish in 1996, had drastically shrunk by 2018 (Table S1, Figure 1). In contrast, the total catch was relatively stable in Alaska, with some regional variation, while slight decreases were observed in southeastern Alaska (SEA), British Columbia (BC) and Washington (WA). We analyzed trends in catches in Russian areas and Japan and Korea (1971–2018) (Figure S3). A Mann-Kendall trend test confirmed that Russian catch trends after 1996 were all significantly positive, thereby identifying a remarkable increase in the catch, as follows: eastern Kamchatka ($\tau = 0.68$, $p = 0.00001$), Kuriles ($\tau = 0.70$, $p = 0.00000$), western Kamchatka ($\tau = 0.74$, $p = 0.00000$), Continental Coast ($\tau = 0.36$, $p = 0.01620$), Sakhalin Coast ($\tau = 0.74$, $p = 0.00000$), Amur River Basin ($\tau = 0.79$, $p = 0.00000$), Primorye ($\tau = 0.58$, $p = 0.00014$) and southwestern Sakhalin ($\tau = 0.32$, $p = 0.03462$). Despite having the highest number of released fish, Japan was the only country in Asia exhibiting a significant decrease ($\tau = -0.61$, $p = 0.0003$). Korea also experienced a decreased catch, but this decrease was insignificant ($\tau = -0.01$, $p = 0.9579$). Chum salmon catches significantly increased in all areas of Russia since 1996, while Korean catches remained very small. According to long-term trends in the catch of chum salmon in Asia, catches have significantly increased in all areas of Russia since 1996, with a substantial drop observed only in Japan.

We next organized the number of released and returning chum salmon by area in Japan. The largest numbers of returning chum salmon were caught in the Sea of Okhotsk and on the Pacific coast of northern Japan, the latter affected by the cold-water Oyashio current. The catch in Iwate Prefecture was particularly notable in 1996, when the total catch in Japan was at a historical maximum of 81 million (Table S2, Figure 2a). 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 S4). 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 2a).

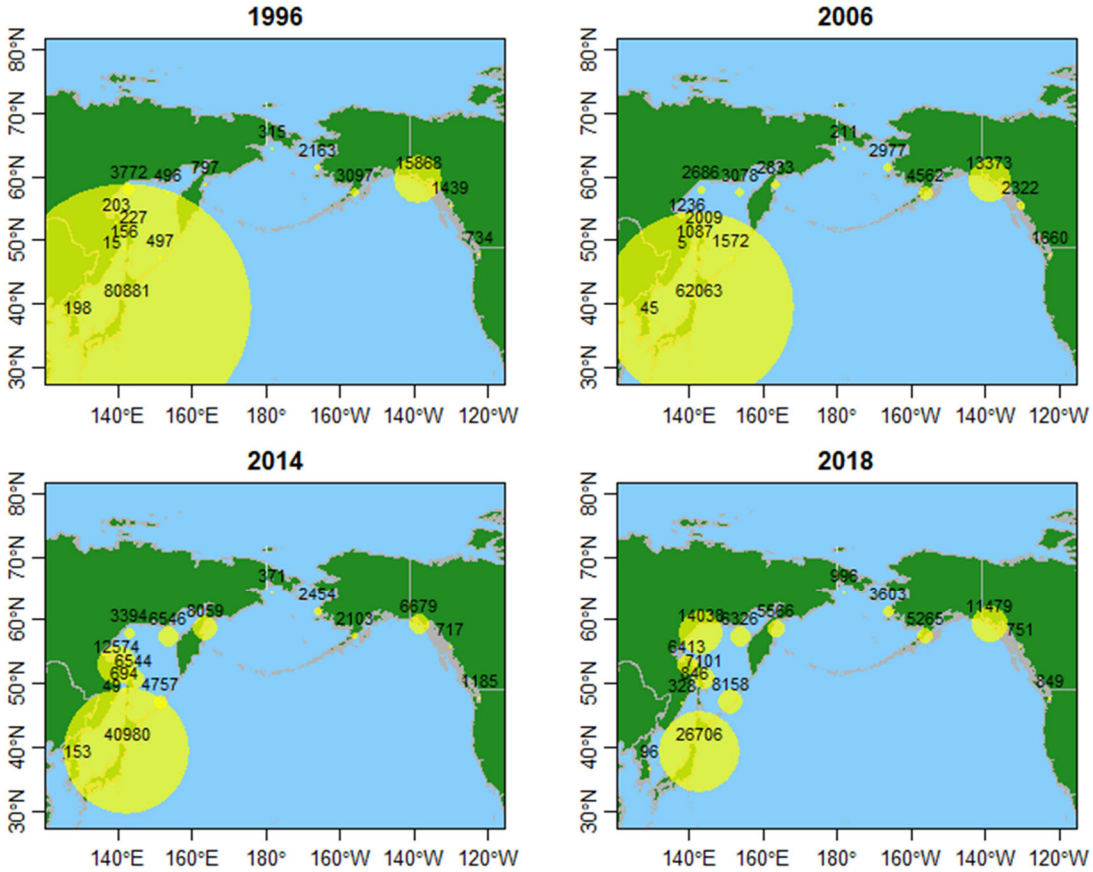

FIGURE 1 Changes in the chum salmon catch in the North Pacific over recent decades. Numbers (in thousands) are shown for 1996—the year of historical maxima in the North Pacific (117 million fish) and Japan (81 million fish). Circle sizes are proportional to the number of salmon. Data are from the North Pacific Anadromous Fish Commission (www.npafc.org, accessed January 2020; NPAFC, 2019) and given in Table S1.

We added new data on SSTs and the number of returning chum salmon in 2017 and 2018 to the information from our previous study (Kitada, 2018) and extended our analysis by seasons using the data summarized for spring (April–June), summer (July–September), autumn (October–December) and winter (January–March). SST data for four areas—Kushiro-Oki (Hokkaido Pacific coast), Hokkaido Sea of Japan coasts, Sanriku-Oki (Honshu Pacific coast) and Honshu Sea of Japan coasts—were retrieved from the Japan Meteorological Agency (http://www.data.jma.go.jp; accessed August, 2019). We averaged SSTs over the four areas for each season and for spring and summer. The linear regression between the number of chum salmon returning to Japan and SST anomalies (Table S3) was examined using the lm function in R. Mean SST in spring (April–June) and summer (July–September) was negatively correlated with catch but not correlated in autumn (October–December) or winter (January–March) (Table S4). Almost all fish returning to Japan were 3 to 5 years old (Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013); therefore, average SST values 3 to 5 years before the return year might be a reasonable explanatory variable corresponding to chum salmon juvenile stages. The average summer SST 3 to 5 years before return was significantly negatively correlated with catch ($r = -0.62$) and had the

highest coefficient of determination (Table S4, Figure 2b). Homing chum salmon frequently descended into deep water (>100 m) off the Sanriku coast, Honshu, from early October to December, which suggests that this behavior avoided high SST and helped minimize metabolic energy costs (Tanaka, Takagi, & Naito, 2000). The results of our regression analysis suggest that warming SSTs reduced juvenile survival rates during summer in coastal waters, whereas SSTs in autumn and winter had no effect on survival rates of homing adults.

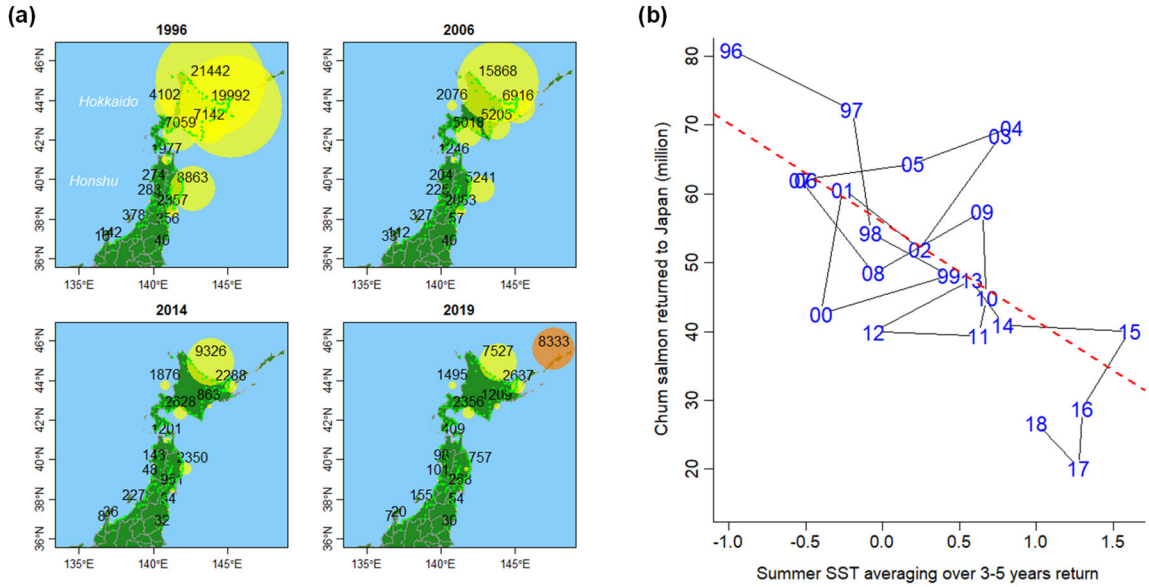

FIGURE 2 Changes in the number of chum salmon returning to Japan and sea surface temperatures (SSTs). (a) Numbers (in thousands) are shown for 1996 (the historical maximum catch year,) 2006, 2014 and 2019 (including the catch on Iturup Island, Russia). Circle sizes are proportional to the number of salmon. Green dots show salmon hatcheries. Data are given in Table S2. (B) Mean SST anomalies in summer in Hokkaido and northern Honshu vs. the number of adult fish returning to Japan ($r = -0.62, t = -3.61, \mathrm{df} = 21, p = 0.0016$). The dashed line shows the estimated regression line. Data are given in Table S3.

3 | HISTORY AND POPULATION STRUCTURE

3.1 SNP data and methods

SNP genotypes retrieved from the Dryad data repository included 58 SNPs from samples throughout the whole distribution range (Seeb et al, 2011). We excluded five loci following the original study, namely, *Oke_U401-220*, *Oke_GHII-2943*, *Oke_IL8r-272*, *Oke_U507-87* and a mtDNA-combined locus, thus leaving 53 SNPs in our analysis (n = 10,458). To infer the evolutionary history of chum salmon, we used population-specific F_{ST}, defined as $\frac{H_{\mathrm{B}}-H_i}{H_{\mathrm{B}}}$, where H_{B} and H_i are respectively between-population heterozygosity and the heterozygosity of population i. Because newly derived populations have had fewer opportunities for mutations to appear after their foundation, they have smaller values of population-specific F_{ST}. We used the bias-corrected moment estimator of population-specific F_{ST} (Weir, & Goudet, 2017), a combined ratio estimator (Cochran, 1977) often called as "ratio of averages" estimator expected to be unbiased when the number of markers is large (Weir, & Cockerham, 1984; Weir, & Hill, 2002; Bhatia, Patterson, Sankararaman, & Price, 2013). For inferring population structure, we used the bias-

corrected G_{ST} moment estimator to compute pairwise F_{ST} (Nei, & Chesser, 1983), which gives unbiased pairwise F_{ST} values when the number of markers is large (Kitada, Nakamichi, & Kishino, 2017). After converting the SNP genotype data to Genepop format (Rousset, 2008), we computed averaged population-specific F_{ST} values over loci using the pop_specificFST function and pairwise F_{ST} values using the pop_pairwiseFST function in FinePop2_ver.0.4 on CRAN. The mean expected heterozygosity (*He*) of each population was also computed using the read.GENEPOP function. A neighbor-joining (NJ) tree (Saitou, & Nei, 1987) of the pairwise F_{ST} distance matrix was inferred using the nj function in the R package ape. A linear regression between mean expected heterozygosity and mean WG population-specific F_{ST} values was performed using the lm function in R.

3.2 Evolutionary radiation out of Alaska and current population structure

Population-specific F_{ST} values of 53 SNPs from 114 locations (Figure S5) averaged 0.101 ± 0.074 (SD) based on the sample genotypes. The Stuyahok River in Alaska had the lowest population-specific F_{ST} value, while the Sturgeon River on Kodiak Island had the highest (Table S5, Figure S6). The northernmost Alaskan populations generally had small population-specific F_{ST} and higher *He* values. Population-specific F_{ST} values were higher for southern populations from Russia, the Alaskan Peninsula, SEA/BC and WA. Six out of 17 Japanese populations and a Korean one had similar F_{ST} values and heterozygosity as those from SEA/BC and WA. Since population-specific F_{ST} was nearly zero in Alaska, chum salmon originated in this area.

Populations in western Alaska had the smallest population-specific F_{ST} values (shown in red in Figure 3a), while those in more southern areas had larger F_{ST} values (in blue). Using the criterion of pairwise $F_{ST} < 0.02$ (Table S6), many populations with high gene flow were identified (connected by yellow lines in Figure 3). Populations in Japan/Korea were isolated from other populations, even at a threshold of $F_{ST} < 0.05$ (Figure S7), thus demonstrating that gene flow between Russia and Japan/Korea was interrupted. Taking into account population-specific F_{ST} values combined with the topology of the NJ tree of pairwise F_{ST} values (Figure 3b), chum salmon originated in western Alaska and expanded to the Chukchi Sea, Yukon, Alaskan Peninsula, and SEA/BC and WA. The Sturgeon River population became isolated on Kodiak Island. The northernmost Russian population, on Anadyr, originated in Alaska and expanded to Kamchatka and Amur. Japanese populations branched off from Anadyr separately from those of Kamchatka and Amur, which are phylogenetically more closely related to Alaskan populations.

Population-specific F_{ST} values indicate that chum salmon originated in western Alaska and then expanded mainly southward along North Pacific coastal lines (Figure 3a). As demonstrated by the fossil record and molecular data, Atlantic salmon (*Salmo salar*) and Pacific salmon (*Oncorhynchus*) had diverged by the early Miocene (15–20 million years ago [mya]), and three most closely related species—pink, chum and sockeye salmon—can be distinguished in the fossil record by 6 mya (McPhail, 1997; Montgomery, 2000; Waples, Pess, & Beechie, 2008). Our results suggest that chum salmon originated in western Alaska, with the species range then expanding mainly to the south. The Sturgeon River had the largest population-specific F_{ST} value and the smallest *He*, in agreement with previous research implying that the population is genetically isolated and that an insufficient number of generations has likely passed since the beginning of gene flow to adjacent populations (Petrou et al., 2014; Seeb, & Crane, 1999). Another possibility is that a bottleneck has occurred in the population.

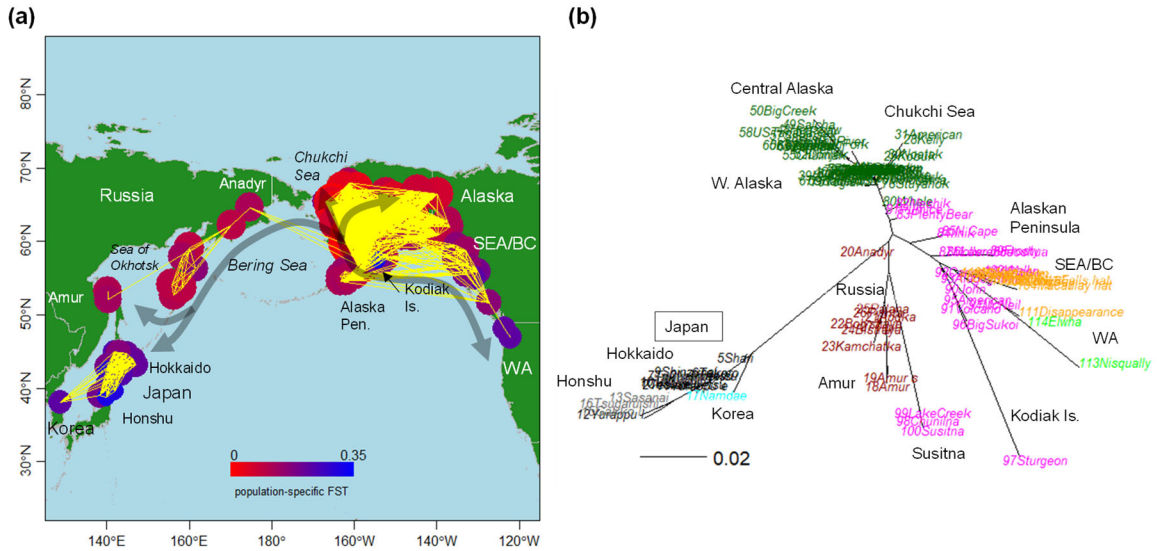

FIGURE 3 Out-of-Alaska population expansion and current population structure of chum salmon. (a) SNP population-specific F_{ST} visualization of populations, with gene flow estimated from pairwise F_{ST} values based on 53 SNPs of 114 populations (n = 10,458) (Seeb et al., 2011). Populations connected by yellow lines are those with pairwise F_{ST} < 0.02. The radius of each sampling point is proportional to the level of H_e as visualized by H_e^{70}. Gray lines show inferred population history. (b) Neighbor-joining tree of the pairwise F_{ST} matrix. Detailed sampling locations are shown in Figure S5. Data are given in Tables S5 and S6.

No population with a larger population-specific F_{ST} value than the Sturgeon River population was identified; this was even true for Japanese and Korean populations at the southern margin of the species range, which were expected to be younger and thus have a larger population-specific F_{ST} and lower *He*. According to the population-specific F_{ST} and pairwise F_{ST} values calculated in this study, many extant Japanese and Korean populations are closely related to SEA/BC and WA populations, and almost all Japanese populations located around the Chitose River group with the Namdae River, Korea, population (Figure S6). The Chitose Hatchery, established in 1888, is the oldest national hatchery in Japan (Kobayashi, 1980). This hatchery has played a central role in the Japanese salmon hatchery program and is the major source of transplanted eggs and juveniles in Japan (Kaeriyama and Qin, 2014). Huge numbers of chum salmon juveniles were transplanted into the Chitose River from all areas of Hokkaido during the 1960s and 1980s, and massive numbers of juveniles were then transferred from the Chitose Hatchery to almost all hatcheries in Japan (Kaeriyama and Qin, 2014). We produced a statistical summary and found that 1.24 billion eggs were transplanted from hatcheries in Hokkaido to rivers in Honshu over 60 years between 1928 and 1985; the highest intensity was in the 1980s, when the Chitose Hatchery was a major source of transplantation (Table S7, Figure S8). In 1985, when the first Korean chum salmon hatchery was constructed, 100,000 eggs were transferred to Korea from the Chitose Hatchery. Our results suggest that the effects of artificial selection—cumulating in the oldest hatchery, which has played a central role in Japanese chum salmon propagation—have spread over Japan and Korea with the transplantation of juveniles.

4 | SYNTHESIS OF ALLELE FREQUENCIES

4.1 Data sets

We used genotypes of the 53 SNPs analyzed in Section 3 (Seeb et al, 2011), which were obtained from samples collected between 1989 and 2003 and used for an allozyme analysis in earlier research. The data set therefore included samples used in our isozyme analysis (this paper) that were collected between 1987 and 1994. We used the minor alleles as the first alleles of all SNPs. For the data sets used in our meta-analysis, we recorded approximate longitudes and latitudes of the sampling sites based on the names of rivers and/or areas and maps of the original studies using Google Maps.

We organized available allele frequencies of *LDH-A*, *LDH-B* and *PEPB-1* isozymes in the Pacific Rim, namely, those collected in Japan (Kijima, & Fujio, 1979; Okazaki, 1982), Japan and Russia (Winans, Aebersold, Urawa, & Varnavskaya, 1994), and Alaska, the Alaskan Peninsula, SEA/BC and WA (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). These data were also used in another study (Seeb, & Crane, 1999) that confirmed the presence of equal allele frequencies in the same rivers in Japan and Russia. All Japanese samples were caught in weirs in hatchery-enhanced rivers and hatcheries and were therefore hatchery-reared fish and/or hatchery descendants. In contrast, Russian samples were collected from natural streams; these fish were assumed to be wild because most Russian chum salmon were naturally maintained (Winans, Aebersold, Urawa, & Varnavskaya, 1994). Samples collected from three hatcheries were excluded from the North American samples (Seeb, Crane, & Gates, 1995). We obtained allele frequencies of the three isozyme loci in 81 chum salmon populations in the distribution range (n = 14,550) (Table S8, Figure S9). Four alleles were detected 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 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 of allele frequencies.

We also used published haplotype counts of the mtDNA D-loop region collected from 48 chum salmon populations in the distribution range (n = 2,162) (Sato et al, 2004). Haplotype frequencies of the mtDNA D-loop region were computed from 48 chum salmon populations in the distribution range (Figure S10a). Among 30 haplotypes, the major ones were A1, B3, B13 and C1, with the B3 haplotype characterizing Alaska and Russia, B13 characterizing SEA/BC and WA, and C1 and A1 characterizing Asian populations (Table S9, Figure S10b). All populations except for two Japanese populations possessed the B3 haplotype; this suggests that B3 was the ancestral, cold-adapted haplotype, with the other haplotypes derived by thermal adaptation.

4.2 Meta-analysis of allele frequencies

We classified the allele/haplotype frequencies of the 53 SNPs, the mtDNA D-loop region and the three isozyme loci into six geographical groups (Japan/Korea, Russia, Alaska, Alaskan Peninsula, SEA/BC and WA). Distributions of allele/haplotype frequencies in the six geographical groups exhibited different patterns (Figure S11). To detect significant geographical variations in allele frequencies in the distribution range, we conducted a principal component analysis (PCA) using the prcomp function in R (Table S10). Because

the PCA identified differences between Japan/Korea and the other areas as a primary component, we performed a two-sample *t*-test of allele frequencies between Japanese/Korean and Russian/American samples for each marker using the t.test function. The second component corresponded to a latitudinal cline among Russian/American samples. We tested the significance of the correlation between allele/haplotype frequencies and latitudes within Russian/American samples using the cor.test function for each marker. Population-specific F_{ST} values and heterozygosity were plotted on global maps using the R package sf. We also added allele frequencies to sampling locations and superimposed 95% confidence ellipses for data with significant correlations using the R package ellipse. All analyses in this study were performed using R statistical software (https://www.r-project.org/).

4.3 Genes exhibiting latitudinal clines

The first principal component (PC1) characterized the distinctiveness of Japanese/Korean populations, while the second component (PC2) located American and Russian populations in a latitudinal cline (Figure 4a). In contrast, PC3 highlighted the difference between Alaska and Russia (Figure 4b). PC1, PC2 and PC3 collectively accounted for 88% of the variance, respectively explaining 47%, 27% and 14%. PC1 comprised *Oke_U502-241**, *Oke_GnRH-373*, *Oke_TCP1-78*, *Oke_IL8r-406*, *Oke_ras1-249*, *Oke_GHII-3129*, *Oke_u217-172*, *Oke_U505-112*, *Oke_DM20-548*, *Oke_U506-110**, *Oke_u200-385* (U22), *Oke_u1-519*, *Oke_Tf-278** and mtDNA_B3 (Table S11, Figures 4a, S12). In this list, locus names followed by an asterisk indicate five outlier SNPs found in the original study, while *U* and *u* designate unknown SNPs (Seeb et al., 2011). PC2 was formed by *Oke_MARCKS-362*, *Oke_PP2A-635* (CTS1627), *Oke_copa-211**, *Oke_RFC2-618**, *Oke_GPH-78*, *Oke_u1-519*, *Oke_FARSLA-242*, *Oke_Tf-278** and *Oke_U502-241**, which were highly correlated with latitude. Although isozyme loci *PEPB-1* and *LDH-A1* had slightly smaller loadings for PC2, the correlation with latitude in American and Russian samples was highly significant. Finally, *Oke_U504-228*, *Oke_U502-241**, *Oke_u200-385* (U22), *Oke_RFC2-618**, *Oke_serpin-140*, *Oke_copa-211** and *Oke_U506-110** had large loadings for PC3 (Table S11, Figures 4b, S12).

Consistent with our interpretation of the biplot, the allele frequencies of the top eight genes constituting PC1 (excluding unknown SNPs) in the Japanese/Korean populations were significantly different from those of populations in Russia and North America (Figure 5). Except for *Oke_IL8r-406* and *Oke_DM20-548* (Table S11), their clinal variation with latitude was also significant. More precisely, six of the eight genes (mtDNA_B3, *Oke_GnRH-37, Oke_TCP1-78*, *Oke_IL8r-406*, *Oke_ras1-249* and *Oke_DM20-548*) differentiated the Japanese/Korean populations, while the other two (*Oke_Tf-278** and *Oke_GHII-3129*) distinguished two groups: Japan/Korea and Alaska (Figure 5).

The allele frequencies of the top eight genes contributing to PC2 generally displayed a clear latitudinal clinal pattern in Russia, Alaska and the western coast of North America, although this clinal variation was insignificant in the case of *Oke_GPH78* and *Oke_HP-182* (Figure S13). *Oke_PP2A-635*, *Oke_RFC2-618** and *LDH-A1* allele frequencies were fixed at or near 0 or 1.0 in southern areas (SEA/BC, WA and Japan/Korea), whereas those of *LDH-A1* in Japan/Korea were similar to those of Alaska.

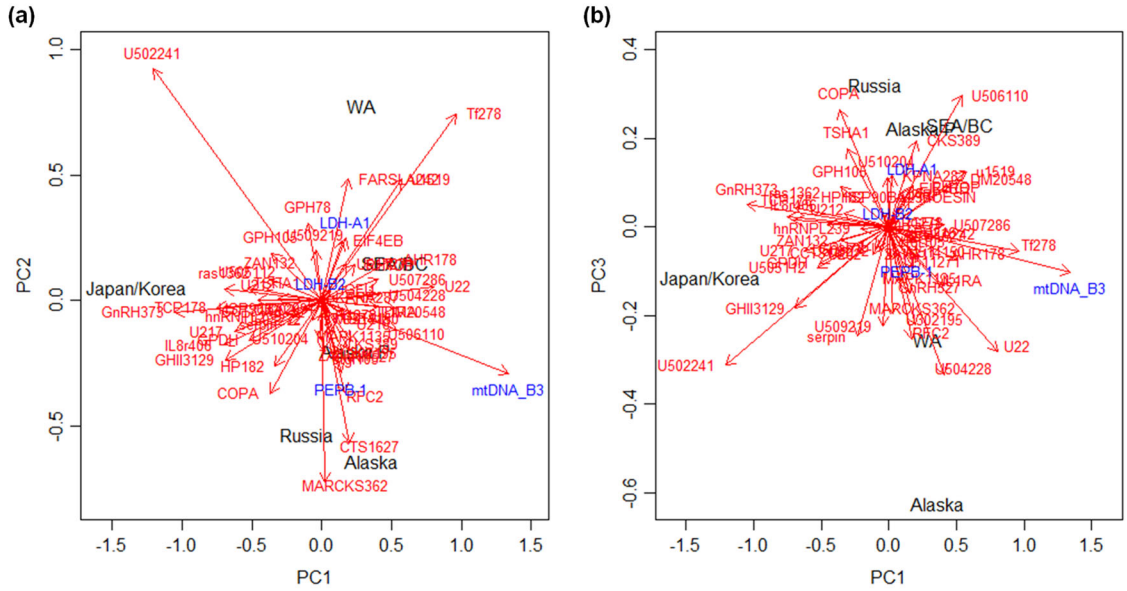

FIGURE 4 Principal component analysis biplots of allele/haplotype frequencies. (a) PC1 vs. PC2; (b) PC1 vs. PC3. Cumulative proportions are as follows: 47% for PC1, 74% for PC2 and 88% for PC3. Data are given in Table S10.

In regards to the top three genes constituting PC3, the allele frequencies of *Oke_RFC2-618** was high in Alaska, while a similar situation was observed for *Oke_copa-211** in Russia and *Oke_serpin-140* in Alaska and Japan (Figure S14). Geographical distributions of allele frequencies of the other 39 loci varied among the different markers (Figure S15). With respect to the five outlier SNPs found in the original study (Seeb et al, 2011), *Oke_U502-241** was much more frequent in WA and Japan/Korea, while the frequency of *Oke_U506-110** was higher on the Alaskan Peninsula and in SEA/BC, with the highest value, 0.92, observed in the Sturgeon River. In contrast, the frequency of LDH-B2*100 was fixed at or near 1.0 in all populations.

By inspecting allele frequencies with reference to latitude, we identified nine genes that differentiated Japanese/Korean populations from those of other areas (Figure 6). With respect to mtDNA_B3, *Oke_GnRH-373, Oke_TCP1-78*, *Oke_IL8r-406* and *Oke_ras1-249* latitudinal clines, Japan/Korea were isolated. In contrast, allele frequencies of *Oke_Tf-278**, *Oke_GHII-3129*, *Oke_serpin-140* and *LDH-A1* in Japan/Korea increased from south to north and became similar to those of Alaskan samples. All Japanese samples, which were collected from hatchery-enhanced rivers, were hatchery-reared fish and/or possibly hatchery descendants (Kitada, 2020), and the contribution of natural spawning (Iida et al., 2018; Kitada, 2014; Miyakoshi et al., 2012; Morita et al., 2013) was not substantial. The altered allele frequencies could therefore have been caused by hatchery rearing repeated for more than 26 generations (assuming a generation time of 5 years for 130 years). The key functions of the nine genes were divided into four biological process categories, namely, reproduction, immune system response, growth and energy metabolism, and analyzed.

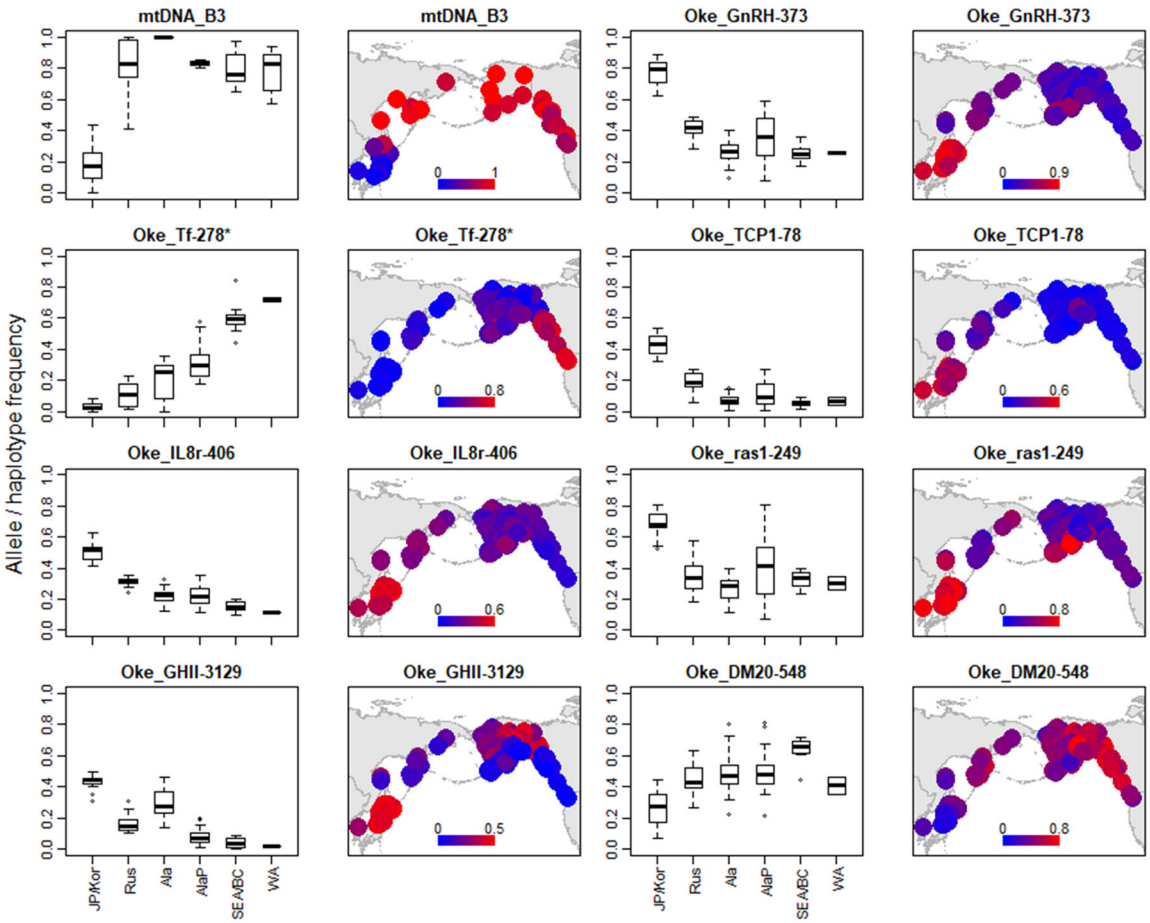

FIGURE 5 Geographical distribution of the top eight genes contributing to PC1 that differentiate Japanese and Korean chum salmon populations from other populations in the distribution range. Unknown SNPs were excluded. The plots are ordered according to the absolute eigenvectors of PC1, as shown in Figure S11 and Table S11.

5 | SIGNATURE OF SELECTION IN JAPANESE POPULATIONS

5.1 Reproduction and circadian rhythm

Two genes related to reproduction were detected, namely, *Oke_GnRH-37* (gonadotropin-releasing hormone) and *Oke_TCP1-78* (T-Complex 1). Their allele frequencies were distributed along a north-to-south gradient (Figure 6). GnRH is a key regulator of vertebrate reproduction, including that of salmonids (Khakoo, Bhatia, Gedamu, & Habibi, 1994). In chum salmon, this gene is involved in gonadal maturation during the early and final phases of upstream migration (Kudo et al., 1996). TCP1 is related to sperm–zona-pellucida interaction and spermatozoan fertilization ability (Dun et al., 2011). Interestingly, the allele frequencies of the two genes were highly correlated ($r = 0.80$) (Figure S16a). In Japan, eggs and sperm are artificially taken from returning fish collected from enhanced rivers. Such artificial fertilization methods might select individuals better fit for artificial fertilization. Although the allele frequencies of these two genes were greatly different in Japanese/Korean populations, they exhibited north-to-south clinal variation. This result suggests that these reproduction-related genes have been adapted to hatchery environment.

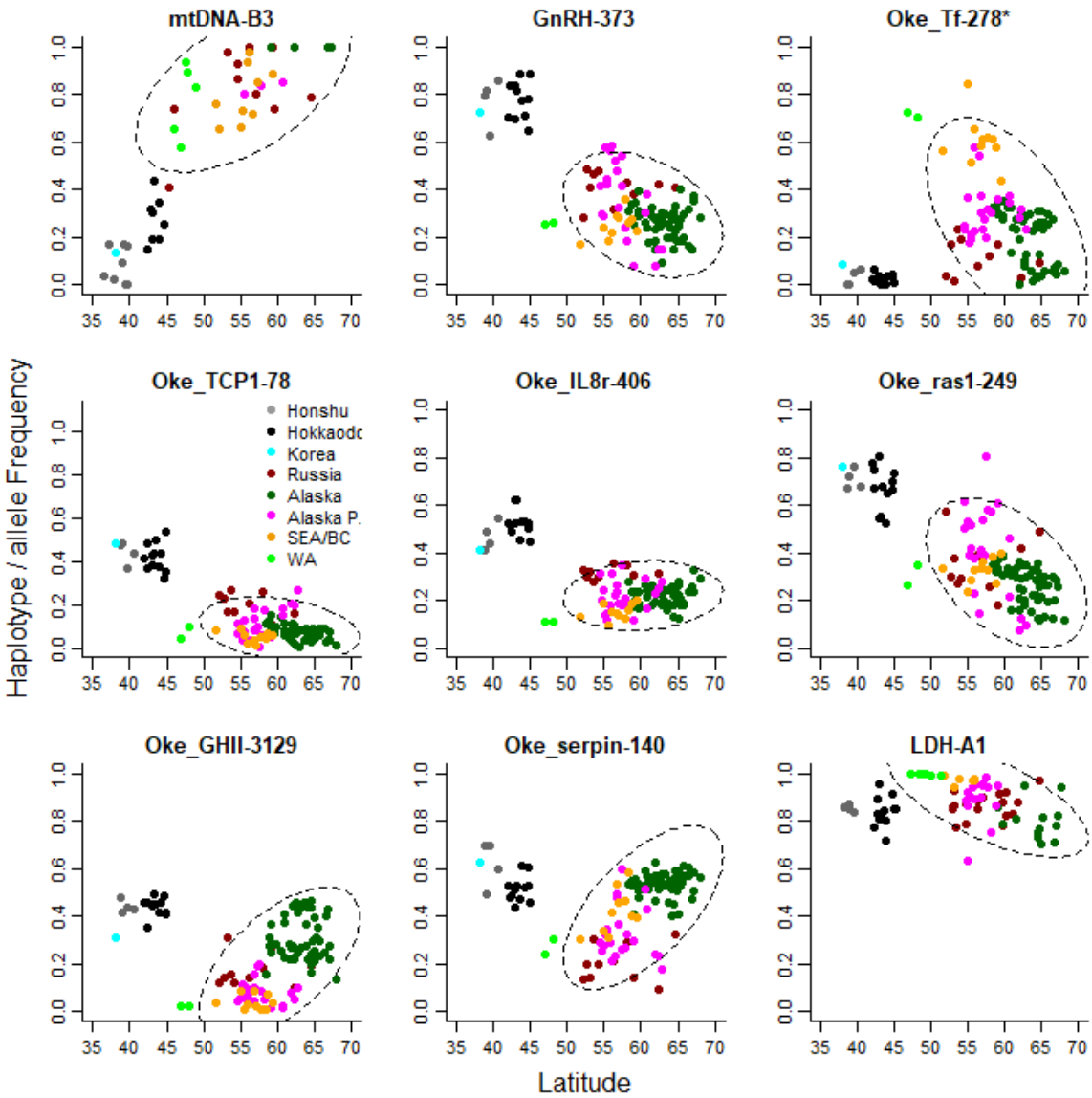

FIGURE 6 Nine genes characterizing the distinctiveness of Japanese/Korean chum salmon. The dotted circles show 95% confidence ellipses for the latitudinal cline exhibited by American and Russian samples. Colors of points in the panels correspond to the sampling locations indicated in Figures S5, S8 and S9. Gene functions and *p*-values for statistical tests are given in Table S11.

GnRH may also play an important function in triggering the shift from feeding migration to homing in salmon populations. In addition, the pulsatile secretion of GnRH is controlled by a clock gene, which may be a response of the circadian timing system to light (O'Malley, Camara, & Banks, 2007). The aryl hydrocarbon receptor (AhR) may modulate light-dependent regulation of circadian rhythm (Mukai, & Tischkau, 2007). In fact, the allele frequencies of *Oke_AhR1-78* were negatively correlated with those of *Oke_GnRH-37* ($r = -0.67$) (Figure S16b). In hatcheries, fertilized eggs are kept undisturbed for ~2 months; the hatched larvae are then reared for ~2 months, generally in indoor rearing ponds. This darker environment for egg emergence compared with natural streams may affect the circadian rhythm of chum salmon. In addition, efforts to enhance early-run

populations since the early 1980s changed the run timing of Hokkaido chum salmon, with late-run populations having almost disappeared by the late 1990s (Miyakoshi, Nagata, Kitada, & Kaeriyama, 2013). This shift may have also affected *Oke_AhR1-78* allele frequencies in Japanese chum salmon populations.

5.2 Immune system

In contrast to the above-described north-to-south clines, allele frequencies of *Oke_Tf-278** (transferrin), *Oke_IL8r-406* (interleukin) and *Oke_serpin-140* (serine protease inhibitors) increased in the opposite direction (Figure 6). Transferrin is related to iron storage and resistance to bacterial disease in Pacific salmon (Ford, 2001; Seeb et al., 2011). Interleukins are a subgroup of cytokines that play a major role in the intercellular regulation of the fish immune system (Secombes, Wang, & Bird, 2011). In Japanese salmon hatcheries, fish are often infected with bacterial kidney disease, infectious pancreatic necrosis and bacterial cold water disease (BCWD). In river surveys in Hokkaido, the causal bacteria of BCWD was found in the body cavity fluid of 85%–98% of females and the sperm of 78%–100% of males. Such bacterial infection rates may have altered the allele frequencies of bacterial disease resistance genes. Importantly, bacterial diseases are directly passed from parents to fertilized eggs (Urawa et al., 2013). Allele frequencies of *Oke_IL8r-406* were highly correlated with those of *Oke_GnRH-37* ($r = 0.80$) and *Oke_TCP1-78* ($r = 0.84$) (Figure S17), which suggests that genes associated with gamete production have developed resistance to bacterial disease.

Serpins are related to immune defense response against salmon louse in host fish (Maldonado-Aguayo, & Gallardo-Escárate, 2014). Japanese hatcheries have suffered from several parasites, such as an ectoparasitic flagellate (*Ichthyobodo necato*, Bodonidae), trichodinids (*Trichodina truttae,* Trichodinidae) and *Chilodonella piscicola* (Chilodonellidae). In particular, *Ichthyobodo necator*, whose parasitization of the body surface reduces the adaptability of juvenile chum salmon to seawater, has an occurrence rate of 40% in Japanese hatcheries (Urawa et al., 2013). Although selection on *Oke_serpin-140* to defend against these parasites might change the allele frequencies of this gene, this does not explain why its allele frequencies in Japan/Korea are similar to those of Alaskan populations (Figure 6). A serpin family gene (serpin B5) has a pathway related to DNA damage response and tumor suppressor (GeneCards). The mean egg-to-release survival rate of chum salmon has continued to increase since approximately 1990 and is currently ~90% (*Supplemental Note*). Such high egg-to-juvenile survival rates might relax natural selection, with less DNA repair required. In addition, mutation rates of genes might be lower in colder environments (Balloux, Handley, Jombart, Liu, & Manica, 2009; Koyano, & Kishino, 2010). In such cold environments, DNA damage at early life stages might be lowered, again reducing the necessity of DNA repair. These phenomena might explain the similarity of *Oke_serpin-140* allele frequencies between Japanese/Korean and Alaskan populations. If this is the case, deleterious genes could remain unselected.

5.3 Growth

Oke_ras1-249 (ras) allele frequencies followed a north-south latitudinal gradient, while those of *Oke_GHII-3129* (growth hormone 2) were distributed in the opposite direction, thus becoming similar to those of Alaskan populations (Figure 6). The *ras* gene has a central role in cell growth (Rotchell, Lee, Chipman, & Ostrander, 2001), while GH is an important regulator of somatic growth in salmonids (Björnsson, 1997). Sizes of chum salmon at release have been increasing in Japan (Miyakoshi, Nagata, Kitada, & Kaeriyama,

2013), which could lead to the selection of individuals with higher growth rates and altered *Oke_GHII-3129* allele frequencies. GH also increases swimming activity and feeding behavior while diminishing the anti-predator behavior of juvenile salmonids (Björnsson, 1997). An ecological trade-off between high growth and longevity may be a cause of normal growth of natural populations at sub-maximal rates, and selection on GH may affect long-term survival of released juveniles (Björnsson, 1997). In contrast, the north-to-south gradient of PEPB-1*-100 allele frequencies (Figure S13) suggests that hatchery rearing has not compromised digestive ability, which is consistent with the enhanced growth of hatchery fish fed copious amounts of food every day.

5.4 Energy metabolism and thermal adaptation

5.4.1 mtDNA D-loop region

Japanese/Korean populations had significantly smaller frequencies of the mtDNA D-loop B3 haplotype. The distribution of this haplotype followed a latitudinal gradient (Figure 6), suggesting adaptation to warmer environments. The water temperature (~8°C) widely used for egg hatching and fry rearing in Japanese chum salmon hatcheries is in fact generally higher than that of natural streams in winter; for example, the temperature of the tributary of the Ishikari River, Hokkaido, is 1.1–2.8°C (Ando et al., 2014). The high egg-to-release survival rate (~90%) in hatcheries could relax natural selection in winter. Human populations living in colder regions have lower mitochondrial diversity (Balloux, Handley, Jombart, Liu, & Manica, 2009), with the lowest mtDNA 16S rRNA diversity found in an environmental sample from Antarctica (Koyano, & Kishino, 2010). We computed haplotype diversity (Nei, 1973) based on the 30 haplotypes of the mtDNA D-loop region. We found that diversity was lowest in Alaska and was negatively correlated with latitude ($r = -0.68$) (Figure S18), which suggests that the chum salmon mtDNA D-loop region is thermal-adaptive.

Given that the mtDNA D-loop region may have functions in dNTP metabolism (Nicholls, & Minczuk, 2014), cold temperature adaptation and energy metabolism (Nishimura, Motoi, Niri, Hoshi, Kondo, & Watanuki, 2012) and oxygen consumption (Kong et al., 2020), relaxed selection pressures on this region might affect energy metabolism and oxygen consumption of chum salmon. In rearing experiments involving embryos and alevin of brook trout (*Salvelinus fontinalis*, Salmonidae), a cold-adapted species, fry acclimated to 9°C were found to have a higher Michaelis–Menten constant of pyruvate (K_m^{PYR}) (lower substrate affinity = lower catalytic efficiency) than those acclimated at 5°C (Cook, Wilson, & Burness, 2018). Japan chum salmon reared at a higher water temperature than that of the natural environment might have a higher K_m^{PYR}, resulting in lower mitochondrial metabolic efficiency and oxygen consumption compared with native individuals that lived there in the past.

5.4.2 *LDH-A1* and *LDH-B2*

LDH-A1*100 allele frequencies were distributed along a south-to-north gradient. Despite the significant latitudinal cline observed among Russian and American samples, the frequency in Japan was similar to that of Alaskan samples (Figure 6). LDH-A1*100 allele frequencies were higher in warmer areas and lower in colder environments (Figure S13). Exceptionally, LDH-A1*100 allele frequencies in Japan were significantly lower than in most American samples from SEA/BC and WA. Japan is located south of the other sampling locations, and a high LDH-A1*100 allele frequency, close to 1.0—the value for SEA/BC and WA—was therefore expected.

In fathead minnow (*Pimephales promelas*, Cyprinidae), a temperate freshwater fish, the *LDH-A* locus has two codominant alleles: LDH-A^a and LDH-A^b (Merritt, 1972). A north-south cline in LDA-A allele frequencies is seen in this species, with higher frequencies of the southern-adaptive allele LDH-A^a and the northern-adaptive allele LDH-A^b in the south and north, respectively. At or above 25°C, the homozygote of the southern-adaptive LDH-A^{aa} of fathead minnow has been found to have a significantly lower K_m^{PYR} (higher catalytic efficiency) than that of the northern-adaptive LDH-A^{bb} and the heterozygote LDH-A^{ab} (Merritt, 1972). Kilka (*Clupeonella cultriventris*, Clupeidae), a small fish inhabiting brackish and fresh waters, also has two codominant alleles; LDH-A*100 frequencies are highest in its southernmost population and exhibit a clear south-to-north clinal pattern in its distribution range between 43° N to 59° N (Karabanov, & Kodukhova, 2018) (Figure S19). Consistently, the LDH-A1*100 allele frequencies of chum salmon were fixed at or near 1.0 in SEA/BC and WA, thus suggesting that the LDH-A1*100 allele of chum salmon is southern-adaptive. Individuals homozygous for the LDH-A1*100 allele may have been expected in native chum salmon populations in Japan, as they would have had a lower K_m^{PYR} (higher catalytic efficiency) in warmer environments than heterozygotes and those homozygous for the other allele. Chum salmon currently inhabiting Japan and Korea may therefore have a higher K_m^{PYR} (lower catalytic efficiency) compared with native fish that lived there in the past.

A single amino-acid substitution in LDH-A isozymes is sufficient for adaptation of species to a new thermal range (Fields, & Somero, 1998; Somero, 2004). Repeated hatchery rearing in higher water temperature may have led to such substitutions in the LDH-A1 isozyme. Lowered LDH-A1*100 allele frequencies could reduce the percentage of homozygotes and might result in higher K_m^{PYR} (lower catalytic efficiencies) at higher temperatures as demonstrated in the experiments of fathead minnow (Merritt, 1972), leading in turn to lowered athletic performance in skeletal muscle. Indeed, Japanese hatchery-reared chum salmon fry have lower athletic performances (Kobayashi, & Ohkuma, 1983) and metabolic efficiencies (Shimizu *et al.*, 2016), thus requiring more energy in the wild than wild-reared fry (*Supplemental Note*). The athletic ability in returned chum salmon also looked low (*Supplemental Note*). Such changes in phenotypes should reduce survival rates of juveniles remaining in Japanese coastal waters in the face of warming SSTs. Reduced athletic ability in the Sea of Okhotsk, the common feeding area of juvenile chum salmon from Japan and Russia (Beamish, 2017; Mayama, & Ishida, 2003), might also reduce survival rates of juvenile Japanese chum salmon, where competition for food should be high with substantially increased Russian chum salmon abundance. In contrast, LDH-B2*100 allele frequencies are fixed at or near 1.0 in all populations (Figures S10, S14). *LDH-B2* is related to heart activity (Powers, Lauerman, Crawford, & DiMichele, 1991; Somero, 2004) and may therefore be conserved in this species.

6 | CONCLUSIONS

This study has produced the first evidence of altered allele frequencies in chum salmon populations at their southern margin, the location of the world's largest hatchery release program. According to population-specific F_{ST} values, chum salmon originated in western Alaska and then expanded southward along North Pacific coastal lines. This evolutionary history suggests that chum salmon have adapted to higher water temperatures, particularly in spawning grounds, and then expanded their distribution range. Our synthesis revealed that allele frequencies of many genes follow a latitudinal gradient, while Japanese/ Korean

populations have become isolated with limited gene flow. Our analysis detected nine genes characterizing the distinctiveness of the Japanese/Korean populations that might be altered by long-term hatchery operations in Japan. The functions of these genes include reproduction, circadian rhythm regulation, bacterial and parasite defense, DNA damage response, growth and energy metabolism. Hatchery fish exposed to higher temperatures are expected to exhibit lower athletic ability as well as reduced skeletal muscle and mitochondrial metabolic efficiency. Such physiological changes might reduce survival rates of hatchery-born juveniles on Japanese coasts in the face of warming SSTs and in the Sea of Okhotsk, where competition for food is expected to be high because of substantially increased Russian chum salmon abundance. Although presently undetected, selective pressure on DNA damage repair may be serious enough to cause unexpected deleterious genetic effects. Almost all chum salmon returning to Japan are hatchery-released fish or possibly wild-born hatchery descendants. Japanese chum salmon populations may thus continue to decline under current hatchery practices, as fitness reduction in hatchery-reared fish is cohort-specific and constant over time within a cohort (Kitada et al., 2019).

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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.

ORCID
Shuichi Kitada: http://orcid.org/0000-0001-5838-0374

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SUPPORTING INFORMATION

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