HOKKAIDO UNIVERSITY

Title	Genetic relations among wild populations of Saccharina japonica in the western North Pacific
Author(s)	Yotsukura, Norishige; Liu, Chang; Terai, Masakazu et al.
Citation	Regional Studies in Marine Science, 53, 102357 https://doi.org/10.1016/j.rsma.2022.102357
Issue Date	2022-04-21
Doc URL	https://hdl.handle.net/2115/92599
Rights	©2022. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	Regional Studies in Marine Science_53_102357.pdf

Hokkaido University Collection of Scholarly and Academic Papers

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Genetic relations among wild populations of *Saccharina japonica* in the western North Pacific

Norishige Yotsukura[a], Chang Liu[b], Masakazu Terai[c], Anna Klimova[d], Dmitrii Galanin[e], Nina Klochkova[4], Toshio Suzuki[c]

[a]Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, 060-0809, Japan

[b]Graduate School of Environmental Science, Hokkaido University, Sapporo, 060-0810, Japan

[c]R & D Department, Fujicco Co., Ltd., Kobe, 650-8558, Japan

[d]Department of Ecology and Nature Management, Kamchatka State Technical University, Petropavlovsk-Kamchatsky, 683003 Russia

[e]Sakhalin Branch, Russian Federal Research Institute for Fisheries & Oceanography, Yuzhno-Sakhalinsk, 693023, Russia

Corresponding author: Norishige Yotsukura

Field Science Center for Northern Biosphere, Hokkaido University, Kita 9 Nishi 9, Kita-ku, Sapporo, Hokkaido, 060-0809, Japan

e-mail: yotsukur@fsc.hokudai.ac.jp

Abstract

We performed microsatellite polymorphism analysis for wild populations of *Saccharina japonica*, a kelp species that is, among wild production areas, mainly produced in Hokkaido, is indispensable as an ingredient of Japanese food that differs in application and value depending on the origin (variety), growing in southern Sakhalin, Primorsky Krai, and Hokkaido to investigate the genetic relations among the populations. A total of 230 alleles (Polymorphic Information Content: 0.08–0.69) were detected from 18 loci. In the analysis by region, the differentiation index (F_{st}) and genetic distance (DS) were lower between the Sakhalin southwestern-end populations or the Sakhalin southern coast populations and the Hokkaido populations. However, gene structure analysis showed that the clusters dominant in the kelp in the Sakhalin or Hokkaido population group also existed in the other population group, suggesting that genes are exchanged between these regions. When analyses were performed by origin, one of the Sakhalin southwestern-end populations formed a clade with the Hokkaido populations in the phylogenetic tree, both having similar genetic structure, and the F_{st} and DS values were particularly low with the Hokkaido East populations (*S. japonica* var. *diabolica*. The F_{st} and DS values were also low between the five Sakhalin southern populations – a sister group with the Hokkaido East populations in the phylogenetic tree – and the North Hokkaido populations (*S. japonica* var. *ochotensis*), indicating that these Sakhalin southwestern-end and southern populations may be used as alternatives to Hokkaido populations for culinary purposes.

Keywords: genetic relation, Hokkaido, microsatellite polymorphism, *Saccharina japonica*, Sakhalin

1. Introduction

The kelp species *Saccharina japonica* (J.E. Areschoug) C.E. Lane, C. Mayes, L.D. Druehl & G.W. Saunders is primarily harvested in Hokkaido in Japan. It has been harvested for more than a thousand years (Oishi 1987), and is an indispensable ingredient in Japanese cuisine which has been designated in the World Intangible Cultural Heritage (e.g. Mouritsen et al. 2019). Recently, however, stock of this species has significantly decreased in Hokkaido due to changes in the marine environment, such as increased water temperature due to the increase in the force of the Tsushima Warm Current; for example, the wild catch in the Oshima Region, a major production area of this kelp species, decreased from 1,803,665 tons in fiscal 2012 to 303,874 tons in fiscal 2020 (unpublished data by Hokkaido Marine Products Grading Corporation). It is feared that the shortage of kelp products due to the decrease in the stock not only makes it difficult to secure ingredients that support the Japanese cuisine culture, but also raises the prices of kelp products in the market, consequently separating consumers from kelp foods. Recently, various efforts have been made to create colonies of this species, which may provide benefits in the future (e.g. Yotsukura et al. 2021). However, future predictions of distribution of the species associated with climate change are that its distribution in northern Japan will move northward, and its growth area will be greatly reduced in both scenarios RCP4.5 and RCP8.5 of the IPCC (Sudo et al. 2020). At present, although Japan's importation of foreign kelp is restricted by the Import Quota system, it is important to investigate whether kelp from areas other than Hokkaido could substitute for kelp from Hokkaido in order to secure stable production of this species in the future.

In the waters adjacent to Hokkaido, China has been conducting large-scale aquaculture of this species, harvesting 1,460,000 tons in 2016 (Hwang et al. 2019). However, although the kelp seedlings used in China originate from the southern Hokkaido strain, their current genetic structure differs from that of the wild strain in Hokkaido, which is thought to be the result of the founder effect, genetic drift in the introduced population, and artificial selection in aquaculture (Shan et al. 2017). Despite the main strain farmed in China is larger than the kelp from Hokkaido, its blade is thinner, and it has a low glutamic acid content (the main component of umami) although it is rich in water content (Saito et al. 2005, Yao et al. 2016, Cao et al. 2017, Jiang et al. 2018), thus characteristics as a food ingredient differ between them. Therefore, it is unlikely at present that the widely farmed Chinese kelp could substitute for the Japanese kelp. However, although there is no knowledge on the kelp varieties in southern Sakhalin and Primorsky Krai, which are also close to Hokkaido, the wild colonies of this species are abundant, the contents of minerals and amino acids are high in the kelp growing in the former, and the size of the kelp is comparable to that of the Hokkaido strain (TINRO 2020, Aminina et al. 2014, Galanin and Repnikova 2014, Kawashima 2012, Zhang et al. 2015b, Kawai et al. 2018). Shallow genealogies have been indicated in two groups corresponding to the Russian Far-East and Hokkaido's northwestern populations in the haplotype network constructed based on the sequences of mitochondrial *COI* and *trnW-trnL* (Zhang et al. 2015b). Based on these, it is expected that kelp in this region could be a substitute for that from Hokkaido.

Saccharina japonica contains four regional varieties each of which has a unique distribution range in Hokkaido (Yotsukura et al. 2008): var. *japonica*; var. *religiosa* (Miyabe) Yotsukura, Kawashima, T. Kawai, T. Abe & L.D. Druehl; var. *ochotensis*

(Miyabe) Yotsukura, Kawashima, T. Kawai, T. Abe & L.D. Druehl; and var. *diabolica* (Miyabe) Yotsukura, Kawashima, T. Kawai, T. Abe & L.D. Druehl. Among these, the varieties other than var. *religiosa* have high industrial value and are mainly used to produce broth, but their applications vary from one variety to another (Kawashima 1989). Therefore, understanding this species at the varietal level is required in order to make full use of this species in Japan.

Genetic diversity within this species is generally low; however, genetic divergence among varieties (in wild kelp), among cultivars (in farmed kelp), and among wild and farmed kelp has been detected using DNA polymorphism analysis, revealing their phylogenetic relationships and population-level genetic structures (Yotsukura et al. 2001, Wang et al. 2004, Shan et al. 2011, Liu et al. 2012, Yotsukura et al. 2016). Particularly, codominant microsatellite sequences, which are tandem repeats of several bases randomly distributed throughout the genome, can be a powerful tool for population analysis because of their high polymorphism and high information content – previous studies of samples collected mainly in Japan and China have shown their usefulness for this species (Liu et al. 2012, Yotsukura et al. 2016, Shan et al. 2017). In this study, we performed simple sequence repeat (SSR)-marker analysis on *S. japonica* growing in southern Sakhalin, Primorsky Krai, and along Hokkaido coasts to infer their genetic relations at the varietal level. The results obtained must become useful information for efficient implementation of detailed componential analysis that will be indispensable in the future.

2. Materials and methods

2.1. Sporophytes used

The wild sporophytes of *S. japonica* used in this study were collected from 10 sites in

southern part of Sakhalin: five sites along Aniwa Bay in the south, two sites in the southwestern-end and three sites on the western coast, two sites in Primorsky Krai, and four sites in Hokkaido (a total of 157 samples) (Fig. 1, Table 1).

After sample collection, approximately 15-cm^2 pieces were cut with a razor from an immature part with fewer adherents near the meristem of the thallus. The surface of the pieces was then thoroughly washed using sterile seawater and, after removal of the seawater, stored at 4°C until use in zippered plastic bags together with silica gel, middle granule (Kishida Chemical, Japan) for drying.

2.2. DNA extraction and microsatellite genotyping

The genomic DNA was extracted from each sample using a TsingKe Plant Genomic DNA Extraction Kit (TsingKe Biotechnology, China) in accordance with the product protocol.

The microsatellite loci to be compared were 21 loci that were reported in previous studies: Zspj6, Zspj9, Zspj14, Zspj17, Zspj20, Zspj26, Zspj28, Zspj39, Zspj40 in Zhang et al. (2014); MS-10, MS-11, MS-16, MS-17, MS-18, MS-24, MS-29, MS-30, MS-31, MS-32 in Maeda and Yotsukura (2013); H123 in Shi et al. (2007); SSR227 in Zhang et al. (2015a). The PCR template used for amplification of each microsatellite region consisted of 1 μl of extracted DNA, 1 μl of 10 μM fluorescent-labeled primer (forward), 1 μl of 10 μM fluorescent-unlabeled primer (reverse), and 27 μl of Super PCR Polymerase Mix (TsingKe Biotechnology, China). The PCR profiles used were as follows: (1) 2 min at 98°C, (2) 10 s at 98°C, (3) 10 s at annealing temperature for each primer (Ta value), (4) 10 s at 72°C, (5) 30 cycles of steps 2–4, and (6) 72°C for 5 min. The PCR products obtained were electrophoresed on a 1% agarose gel at 300 V to confirm the amplification of the target microsatellite regions. In doing so, the dilution

factor for the PCR products used in subsequent capillary electrophoresis was determined based on the obtained electrophoretic images. After dilution, 10 μl of GeneScan 500 LIZ (Thermo Fisher Scientific, USA) and 10 μl of prepared HiDi – obtained by adding 200 μl of 500 Liz to 25 ml of HiDi Formamide (Applied Biosystems, USA) – were added dropwise to a 96-well semi skirted plate, and then 1 μl of diluted sample was added and mixed well by being agitated. This mixed sample was kept still at 95°C for 10 min and then at 4°C for 20 min before being stored at −20°C. In use, the samples were spin down in a centrifuge and then underwent microsatellite genotyping in ABI3730XL Genetic Analyzer (Applied Biosystems, USA), and the allelic sizes were determined using Gene Mapper Software 4.0.

2.3. Data analysis

Data analysis was carried out by microsatellite locus and by sample population, and for the latter at the regional levels, namely, for three regional populations (Sakhalin southern populations, Primorsky Krai populations, and Hokkaido populations), for eight regional populations (Sakhalin southern coast populations, Sakhalin southwestern-end populations, Sakhalin western coast populations, Primorsky Krai populations, and four Hokkaido populations from different producing areas), and 16 regional populations by origin. To determine their genetic diversity, total allele count (Na), effective allele count (N_e), Shannon–Wiener index (I), observed frequency of heterozygotes (H_o), and expected frequency of heterozygotes (H_e) were determined using GenAlEx 6.502 (Peakall and Smouse 2012), and Polymorphic Information Content (PIC) was calculated using CERVUS (Kalinowski 2007). Also, inbreeding coefficient (F_{is}), degree of inbreeding that occurred at the individual level (F_{it}), differentiation index (F_{st}), and genetic distance (DS) between populations were calculated using Populations 1.2.31 (cf.

https://bioinformatics.org/populations/). In addition, a dendrogram was constructed using the neighbor joining (NJ) method and the UPGMA method using POPTREE2 (Takezaki et al. 2010). In doing so, the reproducibility of each branch was estimated based on the bootstrap value calculated from 1,000 pseudo-datasets.

The population-level genetic structure was estimated using Structure 2.3.4 (Pritchard et al. 2000). For each of the assumed numbers of ancestral populations, i.e., K = 1–16, the mean log-likelihood was calculated by running 100,000 burn-in iterations and then using 20 trial data by independently testing 10 runs of 200,000 iterations of Markov chain Monte Carlo and discarding three runs with extreme log-likelihood. Then ΔK (Evanno et al. 2005) was calculated using Structure Harvester web v0.6.93 (Earl and von Holdt 2011).

3. Results

Electrophoresis of the 21 loci yielded an extremely thin banding image for one locus (M-32) and multiple banding images for two loci (Zspj6 and M-18); therefore, these loci were excluded from subsequent analyses. As a result, a total of 230 alleles were detected from the remaining 18 loci. The mean value of the five diversity indicators, and the value of PIC and the three F-statistics (F_{is}, F_{it}, and F_{st}), for each locus over all populations are shown in Table 2.

When all samples were treated as three regional populations, unique alleles were detected for all populations. They were detected at 18 loci in the Sakhalin populations, at 11 loci in the Primorsky Krai populations, and at eight loci in the Hokkaido populations, with the mean number of alleles at each locus in each population being 4.72, 1.22, and 0.94, respectively. Also, the mean percentage of polymorphic loci was 98.15. Among the five diversity indicators, N_a was remarkably higher in the Sakhalin

populations (11.44), followed by Hokkaido (6.78) and Primorsky Krai populations (5.67). The values of the other indicators and F_{is} were highest in the Sakhalin populations (N_e = 3.36, I = 1.47, H_o = 0.45, H_e = 0.64, and F_{is} = 0.30) and lowest in the Hokkaido populations (N_e = 2.44, I = 1.00, H_o = 0.39, H_e = 0.47, and F_{is} = 0.21) (Table S1, available online in Supplementary Material). On the other hand, F_{st} and DS were highest between the Primorsky Krai and Hokkaido populations (0.13 and 0.42, respectively) and lowest between the Sakhalin and Hokkaido populations (0.05 and 0.11, respectively) (Table S2, available online in Supplementary Material). Structure analysis showed that the value of ΔK obtained from Structure Harvester resulted in an optimal K value of 3 (Fig, S1, available online in Supplementary Material), indicating the presence of three clusters: 1–3 (Fig. 2). Cluster 1 was dominant in many individuals from the Sakhalin and Primorsky Krai populations. Cluster 2 was markedly dominant in individuals from the Hokkaido populations, and cluster 3 was dominant in some individuals from the Sakhalin populations. In the constructed UPGMA tree, the Sakhalin and Hokkaido populations formed a clade, which was supported with a bootstrap value of 100%.

When all samples were treated as eight regional populations, unique alleles were detected for all populations. They were found at 14 loci in the western coast populations and at 11 loci in the Primorsky Krai populations, while they were detected at only one locus in the Hokkaido populations. For these, the mean numbers of alleles at each locus in each population were 2.72, 1.22, and 0.06, respectively. Also, the mean percentage of polymorphic loci was 95.14. Of the five diversity indicators, N_a was remarkably higher in the western coast populations (9.78), followed by the southern (5.83) and Primorsky Krai populations (5.67), with the minimum value of 3.17 found in the OD population.

The values of the other indicators and F_{is} are shown in Table S3 (available online in Supplementary Material); all were highest in the western coast populations (N_e = 5.12, I = 1.74, H_o = 0.53, H_e = 0.73, and F_{is} = 0.28) and were generally low in the Hokkaido populations – lowest: Ne = 2.11 (UJ population), I = 0.73 (OD population), H_o = 0.32 (OD population), H_e = 0.39 (OD population), and F_{is} = −0.03 (OI population). On the other hand, F_{st} and DS were highest between the Primorsky Krai and UJ population (0.17 and 0.51, respectively) and lowest between the OD and OI populations (0.04 [same value for between the OR population and the OD population or the OI population]) and 0.05, respectively) (Table S4, available online in Supplementary Material). Structure analysis showed that the value of ΔK obtained from Structure Harvester resulted in an optimal K value of 6 (Fig. S1, available online in Supplementary Material), indicating the presence of six clusters: 1–6 (Fig. 2). Cluster 1 was notably dominant in individuals from the Sakhalin populations. Cluster 2 was dominant in many individuals from the southwestern-end population and was notably dominant in individuals from the Hokkaido populations. Clusters 3 and 4 were dominant in individuals from the western populations, and clusters 5 and 6 were dominant in individuals from the Primorsky Krai populations. Cluster 6 was also dominant in individuals from the southwestern-end populations. In the constructed UPGMA tree, the southwestern-end and Hokkaido populations formed a clade, supported with a bootstrap value of 66%.

When all samples were treated as 16 regional populations, unique alleles were detected for all populations. Among them, the highest number of loci was 10 in the western coast BO population, the lowest number of loci was one in the southern AB and Hokkaido OD populations, and the average number of alleles at each locus in each

population was 1.06 and 0.06, respectively. Also, the mean percentage of polymorphic loci was 91.67. Of the five diversity indicators, N_a was highest in the western BO population (6.44) and lowest in the southwestern-end MC2 population (2.56). The values of the other indicators and F_{is} are shown in Table 3; they were highest in the western BO population for the four indicators (N_e = 4.41, I = 1.55, H_o = 0.56 [same value for the DC population], and H_e = 0.72), while F_{is} was highest in the Hokkaido OD population (0.25), and all were lowest in the southwestern MC2 population (N_e = 1.96, I = 0.59, H_o = 0.32, H_e = 0.32, and F_{is} = -0.07). On the other hand, F_{st} and DS were highest between the southwestern-end MC2 and Primorsky Krai BC populations (0.36 and 0.95, respectively) and lowest between the southern AB1 and AB3 populations (0.03 [same value for between AB5 and AB1 or AB3] and 0.04, respectively) (Figure 3, Table S5, available online in Supplementary Material). Structure analysis resulted in an optimal K value of 7 from the value of ΔK obtained from Structure Harvester (Fig. S1, available online in Supplementary Material), indicating the presence of seven clusters: 1–7 (Fig. 2). Cluster 1 was dominant in individuals from the southern AB1-5 population. Cluster 2 was dominant in many individuals from the southwestern-end MC1 and Hokkaido populations, and cluster 3 was dominant in many individuals from the Primorsky Krai DC and southwestern-end MC2 populations. Clusters 4–7 were dominant in individuals from the western BO population, the western ZY population, the western AN population and individuals from Primorsky Krai BC population, respectively. Both the constructed NJ tree and UPGMA tree showed that the southwestern-end MC1 and Hokkaido populations formed a clade, forming a sister group with the southern AB1-5 population (Fig. 4).

4. Discussion

Polymorphisms effective for analysis were detected in 18 of the 21 microsatellite loci targeted in this study. In *S. japonica*, Li et al. (2016) detected 223 alleles for 60 microsatellite loci using 15 samples from China, five from Japan, and four from Russia. The number of alleles and the mean N_a value obtained in this study were greater than those of the previous study, while the H_o and H_e values were similar. Also, the PIC value was highly informative at 13 loci and reasonably informative at four loci (cf. Botstein et al. 1980), showing the usefulness of the loci targeted in this study.

In this study, phylogenetic analysis targeting regional populations showed that the Sakhalin populations (excluding its western populations) formed a clade with the Hokkaido populations with high bootstrap values; and the Sakhalin populations, especially southern and southwestern-end populations, had smaller values of F_{st} and D_s with the Hokkaido populations. Also, the gene structure analysis in this study showed that the clusters dominant in individuals from the Hokkaido populations intermingled in many individuals in the Sakhalin populations, and the clusters dominant in individuals from the Sakhalin populations intermingled in the Hokkaido populations, although in small numbers, indicating recent gene exchanges between the population groups. Zhang et al. (2015b) stated that direct gene exchanges between the Sakhalin and Hokkaido populations are suppressed by the Soya Current and that the two population groups have complex interrelationships through post-LGM population expansion and anthropogenic interference. In this species, which travels little as drifting algae, the migration of zoospores seems to greatly affect the population gene structure, and the migration distance of zoospores in the waters west of Hokkaido is reported to be up to 1.3–1.9 km (Akino et al. 2005). Therefore, given that the narrowest part of the strait is about 42 km and that the current flows at a rate of 1.5–3 knots there throughout the year, direct gene

exchanges via zoospore swimming is unlikely. It is believed that in Dalian, China, *S. japonica* was introduced via zoospores and juvenile sporophytes attached to logs imported from Hokkaido and northern Honshu in 1927 (Tseng 2001); however, with marine transport services operating between southern Sakhalin and Hokkaido, i.e., regular ferry services between Wakkanai and Korsakoff (temporarily halted since 2019) and cargo liners operating between Wakkanai/Otaru and Korsakoff, it is likely that the genetic resources were moved by ships.

The phylogenetic analysis of regional populations by origin showed that the MC1 population of the Sakhalin's southwestern-end populations formed a clade with the Hokkaido populations in a phylogenetic tree, and its DS values were lower with the Hokkaido populations, especially the OI population (*S. japonica* var. *diabolica*). It has been reported that in waters around Cape Crillon, *Laminaria longipes* Miyabe et Tokida with large, wide, lanceolate blade and very long stipe occurs (Miyabe 1928, Miyabe 1936), but the samples used in this study exhibited the same characteristics (maximum length 491.0 cm, maximum blade width 31.8 cm, and maximum stipe length 95.0 cm), and we believe these two are the same. Nagai (1936) reported that the differences in the blade base morphology and the stipe length seen between *f. genuina* (normal type) and *f. longipes* of *L. diabolica* were due to the effects of their growth conditions, particularly tidal flow. Given the recent view that *S. longipedalis* with a similarly long stipe can be considered as *S. japonica* var. *diabolica* (Yotsukura et al. 2016b), it is presumable that the samples obtained from the MC1 population are *S. japonica* based on the genetic data obtained in this study. It is known that water temperatures around Cape Crillon, including those in coastal areas, are lower than those in surrounding waters due to cold seawater upwelling there and that the kelp vegetation in this sea area differs from that in

surrounding areas (Miyabe 1928, Kawai et al. 2014, TINRO 2020). Generally, *S. japonica* var. *diabolica* grows in waters east of mainland Hokkaido and the coasts of Kunashiri and Etorofu Islands, and the reasons for its occurrence in locations distant from these main producing areas need to be investigated in the future; however, it is considered that low water temperatures and nutrient-rich seawater environments similar to the main producing areas foster the large-sized kelp. In Japan, *S. japonica* var. *diabolica*, or "*Oni-Kombu*" in Japanese, is a key industrial kelp being a high-value source of broth and used for various processing purposes. The production volume of *S. japonica* has declined from 752,881 tons in fiscal 2012 to 526,228 tons in fiscal 2020 (unpublished data by Hokkaido Marine Products Grading Corporation), and it is necessary to secure the kelp resource to maintain the diversity of the use of *S. japonica* for the future. This study shows that the kelp from the Sakhalin southwestern-end populations is expected to be able to substitute for *S. japonica* var. *diabolica* from waters east of Hokkaido. It will be required to analyze the components of the former to investigate its appropriate use in the future.

The Sakhalin southern coast AB population formed a sister group with the Hokkaido populations in the phylogenetic analysis, although having a different genetic structure (dominant cluster) to the Hokkaido population, and the DS values were generally lower between that and the OD population (*S. japonica* var. *ochotensis*). The *S. japonica* var. *ochotensis* is thought to be distributed in Hokkaido from the vicinity of Mashike Town through to Cape Soya and the vicinity of Akaiwa district in Rausu Town, and the kelp harvested in these waters is currently treated as one industrial species "*Rishiri-Kombu*" in the market. However, Yotsukura et al. (2016a) showed that the genetic structure differed greatly between the populations from the Japan Sea coast north of Ishikari City,

including Rishiri and Rebun Islands, and the populations from the west of Wakkanai City, and Zhang et al. (2015b) reported that mtDNA haplotypes differed greatly between the populations from Aniwa Bay and those from Wakkanai City; these studies, as well as this study, support the results of Yotsukura et al. (2016a). *Rishiri-Kombu* is an indispensable ingredient for traditional Japanese foods, and it is believed that the kelp from Rishiri and Rebun Islands ("*Shimamono*") is suitable for making clear soup, and that from mainland Hokkaido ("*Jikata*") is suitable for making pot-food broth (Okui Kaiseido 2022). This study indicates that the kelp from the southern coast of Sakhalin could substitute for *Jikata*.

In this study, the H_o values, calculated for Primorsky Krai and different producing areas of Hokkaido did not differ greatly from those of a previous study (Shan et al. 2017). However, unlike the farmed populations from various parts of China described in that report, H_e was higher than H_o in most populations in this study, especially in the populations in the Japan Sea coasts of Sakhalin and Hokkaido. Similar results were reported from a population in Shiebunino, 15 km from Gornozavodsk on the western coast of Sakhalin (Zhang et al. 2017). In this study, the F_{st} values between the Sakhalin western coast populations were 0.05–0.11, indicating a clear genetic differentiation. Among them, the values between BO and ZY, ZY and AN, and BO and AN populations were 0.05, 0.09, and 0.11, respectively, and the values between the two adjacent populations increased toward the north. In southwestern Sakhalin, the Tsushima Warm Current flows northward away from the shore basically, but a branch that flows clockwise is known near the coast line in the vicinity of 47 degrees north latitude (Pishchalnik et al. 2011). Herein, it is suggested that *S. japonica* in this area (i.e. BO, ZY, and AN) has expanded its distribution range through this flow. The gene structure

analysis showed that in the MC1 southwestern-end population and the western coast BO and ZY populations, the same clusters as in their neighboring populations were dominant in some individuals. This turned out to indicate higher genetic diversity within these populations, and the BO and AN populations had greater F_{is} values, although with markedly higher genetic diversity compared to other populations. This may be because there is no genetic exchange between two subpopulations with different dominant clusters in each population, and there are some barriers to interaction between them. It is speculated that high heterozygosity in kelp seedlings has been induced in China through the use of different population strains at seedling production facilities (Shan et al. 2017); nevertheless, at production sites, the lowering of productivity and quality caused by inbreeding has become a problem (Zhao et al. 2016). Since it is also known that an increase in homozygotes due to inbreeding can result in a decrease in genetic diversity, leading to a decrease in fitness, the use of genetic diversity should be investigated in the future to promote the conservation of the wild kelp resource in the study area.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Dr. Tifeng Shan (Chinese Academy of Science) for the kind advice and useful comments. The authors also thank Mr. Anatoly Tretyakov (Sakhalin Diving Co.), Mr. Shohei Seki (Hokkaido University) and Mr. Yoshihiro Hara and Ms. Yuka Tojyo (Fujicco Co., Ltd.) for collecting materials. This research was supported by a

research project “Promoting the Establishment of Food Value Chain (fiscal 2019-2020)” from the Ministry of Agriculture, Forestry and Fisheries, Japan.

References

Akino, H., Kawai, T., Yotsukura, N., Kono, T., 2015. Transportation and spatial distribution of *Saccharina japonica* var. *religiosa* zoospores in surface water off the coast of Tomari, Hokkaido, Sea of Japan. Fish. Eng. 52, 1-9. (in Japanese).

Aminina, N.M., Vishnevskaya, T.I., Galanin, D.A., Repnikova, A.R., Guruleva, O.N., 2014. Characteristics of commercial reserves of Japanese saccharin in the Aniva Bay (Sea of Okhotsk). Izvestia TINRO 178, 116-123. (in Russian).

Botstein, D., White, R.L., Skolnick, M., Davis, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32, 314-331.

Cao, Z., Wang, W., Qu, Y., Wang, N., Liang, G., Liu, X., 2017. Analysis and evaluation of seasonal changes in amino acids from kelp “Dongfang No.7”. Fish. Sci. 36, 132-137. (in Chinese).

Earl, D.A., von Holdt, B.M., 2011. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genet. *Resour.* 4, 359-361.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of

individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611-2620.

Galanin, D., Repnikova, A., 2014. Present situation of fisheries research on kelp in south Sakhalin, Russia. Fish. Eng. 51, 65-69. (in Japanese).

Hwang, E.K., Yotsukura, N., Pang, S.J., Su, L., Shan, T.F., 2019. Seaweed breeding programs and progress in eastern Asian countries. Phycologia 58, 484-495.

Jiang, X., Liu, N., Sun, Y., Zhou, D.Q., 2018. Analysis of nutritional components of Rongcheng fresh kelp and its dry and salt products. J. Food Saf. Foof Qual. 9, 1874-1880. (in Chinese).

Kalinowski, S., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mor. Ecol. 16, 1099-1106.

Kawai, T., Galanin, D., Tskhay, Z., Latokovskaya, E., Nagai, N., Yotsukura, N., 2014. Relationship between occurrence of kelp species and water temperature in northern Hokkaido, Japan, and southern Sakhalin, Russia. Algal Resources 7, 107-116.

Kawai, T., Galanin, D., Tshay, Z., Kroupnova, T., Yotsukura, N., 2018. Present status of saccharinian kelp in southern Primorye and Sakhalin, far-east Russia, and northern Hokaido, Japan, in Sea of Japan. Algal Resources 11, 33-42.

Kawashima, S., 1989. Ilustration Book of Japanese Laminariales. Kitanihon Kaiyo Center, Sapporo. (in Japanese).

Kawashima, S., 2012. Morphology and taxonomy of the laminariaceous algae in cold water area of Japan. Seibutsu Kenkyusha, Tokyo. (in Japanese).

Li, Q., Zhang, J., Yao, J., Wang, X., Duan D., 2016. Development of *Saccharina japonica* genomic SSR markers using next-generation sequencing. J. Appl. Phycol. 28, 1387-1390.

Liu, F., Yao, J., Wang, X., Repnikova, A., Galanin, D.A., Duan, D., 2012. Genetic diversity and structure within and between wild and cultivated *Saccharina japonica* (Laminariales, Phaeophyta) revealed by SSR markers. Aquaculture 15, 139-145.

Maeda, T., Yotsukura, N., 2013. Development of microsatellite markers for *Saccharina japonica* by dual-suppression PCR. Algal Resources 6, 67-71.

Miyabe, K., 1928. On the occurrence of a certain Behring and Kurile species of Laminariaceae in a small isolated region off the southern extremity of Saghalien. Proc. 3rd Pan-Pacific Sci. Congr. (Tokyo, 1926), 954-958.

Miyabe, K., 1936. On the Laminariaceae of Hokkaido, in: Okamura, K. (Ed.), Marine algae of Japan. Uchida Rokakuho, Tokyo, pp. 283-300. (in Japanese).

Mouritsen, O.G., Rhatigan, P., Pérez-Lloréns, J.L., 2019. The rise of seaweed gastronomy: phycogastronomy. Bot. Mar. 62, 195–209.

Nagai, M., 1936. A variety of *Laminaria diabolica* and the origin. Kagaku 6, 464-466. (in Japanese).

Oishi, K., 1987. Kombu no michi. Daiichi shobo, Tokyo. (in Japanese).

Okui Kaiseido, 2022. Species and growing area of Kombu. https://www.konbu.jp/kuragakoi/kind.shtml searched on 3 Febuary 2022. (in Japanese).

Peakall, R., Smouse, P.E., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28, 2537-2539.

Pishchalnik, V. M., Leonov, A. V., Arkhipkin, V. S., Melky, V. A., 2011. Mathematical modeling of conditions for the functioning of the ecosystem of the Tatar Strait: monograph. SakhGU, Yuzhno-Sakhalinsk. (in Russian).

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945-959.

Saito, M., Hirano, E., Kunisaki, N., 2005. Free amino acid content of five Kombu species. The journal of Kagawa Nutrition University 36, 71-74. (in Japanese).

Shan, T.F., Pang, S.J., Zhang, Y.R., Yakovleva, I.M., Skriptsova, A.V., 2011. An AFLP-based survey of genetic diversity and relationships of major farmed cultivars and geographically isolated wild populations of *Saccharina japonica* (Phaeophyta) along the northwest coasts of the Pacific. J. Appl. Phycol. 23, 35-45.

Shan, T., Yotsukura, N., Pang, S., 2017. Novel implications on the genetic structure of representative populations of *Saccharina japonica* (Phaeophyceae) in the Northwest Pacific as revealed by highly polymorphic microsatellite markers. J. Appl. Phycol. 29, 631-638.

Shi, Y., Yang, G., Liu, Y., Liao, M., Li, X., Cong, Y., 2007. Development of 18 polymorphic microsatellite DNA markers of *Laminaria japonica* (Phaeophyceae). Mol. Ecol. Notes 7, 620-622.

Sudo, K., Watanabe, K., Yotsukura, N., Nakaoka, M., 2020. Predictions of kelp distribution shifts along the northern coast of Japan., Ecol. Res. 35, 47-60.

Takezaki, N., Nei, M., Tamura, K., 2010. POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with windows interface. Mol. Biol. Evol. 27, 747-752.

TINRO, 2020. Resources and rational use of seaweed and grasses of the Far Eastern seas of Russia, N. Akulin, N., (Ed.), TINRO, Vladivostok. (in Russian)

Tseng, C.K., 2001. Algal biotechnology industries and research activities in China. J. Appl. Phycol. 13, 375-380.

Wang, X.L., Yang, Y.X., Cong, Y.Z., Duan, D.L., 2004. DNA fingerprinting of selected *Laminaria* (Phaeophyta) gametophytes by RAPD markers. Aquaculture 238, 143-153.

Yao, H., Wang, F., Liu, F., Liang, Z., Wang, W., Sun, X., Li, X., 2016. Chemical analysis and nutritional assessment of new varieties of *Saccharina japonica*. Food Sci. 37, 95-98. (in Chinese).

Yotsukura, N., Kawai, T., Motomura, T., Ichimura, T., 2001. Random amplified polymorphic DNA markers for three Japanese laminarian species. Fish. Sci. 67, 857-862.

Yotsukura, N., Kawashima, S., Kawai, T., Abe, T., Druehl, L.D., 2008. A systematic re-examination of four *Laminaria* species: *L. japonica*, *L. religiosa*, *L. ochotensis* and *L. diabolica*. J. Jpn. Bot. 83, 165-176.

Yotsukura, N., Maeda, T., Abe, T., Nakaoka, M., Kawi, T., 2016a. Genetic differences among varieties of *Saccharina japonica* in northern Japan as determined by AFLP and SSR analyses. J. Appl. Phycol. 28, 3043-3055.

Yotsukura, N., Nagai, N., Kawai, T., 2016b. A taxonomic re-examination of *Saccharina longipedalis* (Laminariales, Phaeophyceae), an endemic kelp species around Lake

Akkeshi in eastern Hokkaido, Japan. Bot. Mar. 59, 205-210.

Yotsukura, N., Unjo, K., Hashimoto, Y., Kawai, T., Katsuyama, Y., 2021. Method for creating laminarialean kelp colonies using seawater that contains naturally derived polymers. Reg. Stud. Mar. Sci. 43, https://doi.org/10.1016/j.rsma.2021.101663.

Zhang, L., Peng, J., Li, X., Liu, Y., Cui, C., Wu, H., Wu, R., Tian, P., Li, Yan., 2014. Development of 27 trinucleotide microsatellite markers for *Saccharina japonica* using next generation sequencing technology. Conservation Genet. Resour. 6, 341-344.

Zhang, J., Li, W., Qu, J., Wang, X., Liu, C., Liu, T., 2015a. Development and characterization of microsatellite markers from an enriched genomic library of *Saccharina japonica*. J. Appl. Phycol. 27, 479-487.

Zhang, J., Yao, J.T., Sun, Z.M., Fu, G., Galanin, D.A., Nagasato, C., Motomura, T., Hu, Z.M., Duan, D.L., 2015b. Phylogeographic data revealed shallow genetic structure in the kelp *Saccharina japonica* (Laminariales, Phaeophyta). BMC Ecol. Evol. 15, DOI 10.1186/s12862-015-0517-8.

Zhang,J., Wang, X., Yao, J., Li, Q., Liu, F., Yotsukura, N., Krupnova, T.N., Duan, D., 2017. Effect of domestication on the genetic diversity and structure of *Saccharina japonica* populations in China. Sci. Rep. 7, DOI: 10.1038/srep42158.

Zhao, X.B., Pang, S.J., Liu, F., Shan, T.F., Li, Jing, Gao, S.Q., Kim, H.G., 2016.

Intraspecific crossing of *Saccharina japonica* using distantly related unialgal gametophytes benefits kelp farming by improving blade quality and productivity at Sanggou Bay, China. J. Appl. Phycol. 28, 449-455.

Figure legends

Figure 1 Collecting sites of the samples used in this study

Figure 2 Genetic structure of *Saccharina japonica* estimated by Bayesian clustering analysis based on microsatellite analysis. (A): samples were treated as three regional populations; (B): samples were treated as eight regional populations; (C): samples were treated as 16 regional populations. Each column corresponds to one individual. Clusters obtained are classified by color at every one. The vertical axis shows the ratio that each cluster occupies

Figure 3 The color-coded Fst values (above diagonal) and DS values (below diagonal) between two within 16 regional populations

Figure 4 The UPGMA dendrogram based on the genetic distance among 16 populations of *Saccharina japonica*. Bootstrap values greater than 50 % are indicated. The scale bar indicates the genetic distance

Figure S1 The ΔK calculations obtained from Structure Harvester. (A): samples were treated as three regional populations; (B): samples were treated as eight regional populations; (C): samples were treated as 16 regional populations.

Table 1 Collection date and sites of *Saccharina japonica* used for this study.

Collection date	Collection site			Lat / Long	Abbreviation of the population	Number of individuals	Additional statement
8 Septrember, 2019	Southern part of Sakhalin	Southern coast	Aniva Bay	46°36'36.8"N / 142°50'01.0"E	AB1	18	
25 July, 2020			Aniva Bay	46°36'41.6"N / 142°50'17.6"E	AB2	15	
8 Septrember, 2019			Aniva Bay	46°36'27.4"N / 142°57'54.6"E	AB3	13	
26 July, 2020			Aniva Bay	46°36'24.4"N / 142°57'55.3"E	AB4	15	
8 Septrember, 2019			Aniva Bay	46°35'54.1"N / 143°02'44.8"E	AB5	10	
11 September, 2019		Southwestern-end	Majdely cape	45°58'09.7"N / 141°58'54.7"E	MC1	8	large, wide, lanceolate blade and long stipe
11 September, 2019			Majdely cape	45°58'21.2"N / 141°59'24.5"E	MC2	5	
23 July, 2020		Western coast	Bogdanovich	46°32'40.5"N / 141°48'32.8"E	BO	15	
23 July, 2020			Zyryanskoe	46°54'12.6"N / 141°59'12.9"E	ZY	15	
10 August, 2020			Antonovo	47°08'38.9"N / 142°03'18.4"E	AN	15	
10 July, 2015	Primorsky Krai		Babkina Cape	42°33'10.8"N / 131°12'33.1"E	BC	10	
15 July, 2015			DeLivron Cape	42°50'42.4"N / 132°36'12.4"E	DC	12	
8 August, 2011	Hokkaido		Usujiri	41°56'06.6"N / 140°57'18.2"E	UJ	10	*Saccharina japonica* var. *japonica*
16 March, 2010			Oshoro	43°12'36.6"N / 140°51'28.0"E	OR	10	*S. japonica* var. *religiosa*
13 October, 2011			Oshidomari	45°15'11.7"N / 141°14'54.2"E	OD	10	*S. japonica* var. *ochotensis*
18 May, 2012			Ochiishi	43°11'00.5"N / 145°33'21.8"E	OI	10	*S. japonica* var. *diabolica*

Table 2 The mean valuse of the five diversity indicators, and the values of PIC and the three F-statistics (Fis, Fit, and Fst), for each locus

Locus	Na	Ne	I	Ho	He	PIC	Fis	Fit	Fst
Zspj9	5.19	3.17	1.2	0.46	0.58	1.2	0.21	0.39	0.22
Zspjl4	3.31	2.26	0.83	0.3	0.45	0.83	0.34	0.58	0.36
Zspjl7	6.38	3.86	1.47	0.65	0.68	1.47	0.05	0.23	0.18
Zspj20	1.75	1.2	0.23	0.08	0.12	0.23	0.38	0.47	0.14
Zspj26	2.94	2.05	0.77	0.3	0.44	0.77	0.32	0.53	0.31
Zspj28	2.69	2.01	0.72	0.44	0.44	0.72	-0.01	0.22	0.23
Zspj39	2.63	2.04	0.72	0.6	0.45	0.72	-0.33	-0.1	0.17
Zspj40	4.25	2.88	1.01	0.39	0.49	1.01	0.2	0.41	0.26
MS-10	2.63	1.59	0.48	0.24	0.26	0.48	0.05	0.24	0.2
MS-11	4.19	2.34	0.99	0.47	0.51	0.99	0.09	0.25	0.18
MS-16	3.81	2.55	1.04	0.57	0.59	1.04	0.03	0.14	0.12
MS-17	7.38	4.63	1.68	0.77	0.76	1.68	-0.02	0.11	0.13
MS-24	7.06	4.4	1.65	0.71	0.76	1.65	0.06	0.17	0.12
MS-29	3.44	1.89	0.67	0.3	0.34	0.67	0.12	0.51	0.45
MS-30	2.63	1.8	0.62	0.27	0.36	0.62	0.24	0.5	0.34
MS-31	4.31	2.73	1.05	0.46	0.54	1.05	0.16	0.44	0.34
H123	3.75	2.15	0.84	0.41	0.45	0.84	0.1	0.4	0.33
SSR227	2.56	1.9	0.69	0.42	0.41	0.69	-0.01	0.3	0.3
Mean							0.11	0.32	0.24

Table 3 The values of the five diversity indicators and Fis when all samples were treated as 16 regional populations

Population	Na	Ne	I	Ho	He	Fis
AB1	3.67	2.23	0.89	0.46	0.48	0.06
AB2	3.22	1.99	0.75	0.34	0.41	0.15
AB3	3.11	2.08	0.76	0.37	0.43	0.2
AB4	3.56	2.34	0.85	0.49	0.46	-0.08
AB5	3.56	2.03	0.79	0.4	0.42	0.02
MC1	3.78	2.68	0.99	0.49	0.53	0.08
MC2	2.56	1.96	0.59	0.32	0.32	-0.07
BO	6.44	4.41	1.55	0.56	0.72	0.23
ZY	6.28	4	1.47	0.55	0.69	0.21
AN	4.83	3.03	1.11	0.49	0.54	0.09
BC	2.61	1.89	0.62	0.38	0.35	-0.06
DC	4.28	2.59	1.06	0.56	0.56	0.01
UJ	3.56	2.11	0.79	0.39	0.41	0.03
OR	4.56	2.43	1.02	0.4	0.51	0.24
OD	3.17	2.22	0.73	0.32	0.39	0.25
OI	3.83	2.41	0.85	0.43	0.43	-0.03

Table S1 The values of the five diversity indicators and Fis when all samples were treated as three regional populations

Population	Na	Ne	I	Ho	He	Fis
Sakhalin	11.44	3.36	1.47	0.45	0.64	0.3
Primorsky Krai	5.67	2.98	1.18	0.45	0.58	0.24
Hokkaido	6.78	2.44	1	0.39	0.47	0.21

Table S2 Pairwise Fst values (above diagonal) and DS values (below diagonal) between two within three regional populations

Population	Sakhalin	Primorsky Krai	Hokkaido
Sakhalin	***	0.07	0.05
Primorsky Krai	0.29	***	0.13
Hokkaido	0.11	0.42	***

Table S3 The values of the five diversity indicators and Fis when all samples were treated as eight regional populations

Population	Na	Ne	I	Ho	He	Fis
Southern coast[1)]	5.83	2.25	0.94	0.41	0.47	0.14
Southwestern-end[2)]	4.33	2.87	1.03	0.42	0.53	0.16
Western coast[3)]	9.78	5.12	1.74	0.53	0.73	0.28
Primorsky Krai	5.67	2.98	1.18	0.45	0.58	0.24
UJ	3.56	2.11	0.79	0.39	0.41	0.03
OR	4.56	2.43	1.02	0.4	0.51	0.24
OD	3.17	2.22	0.73	0.32	0.39	0.25
OI	3.83	2.41	0.85	0.43	0.43	-0.03

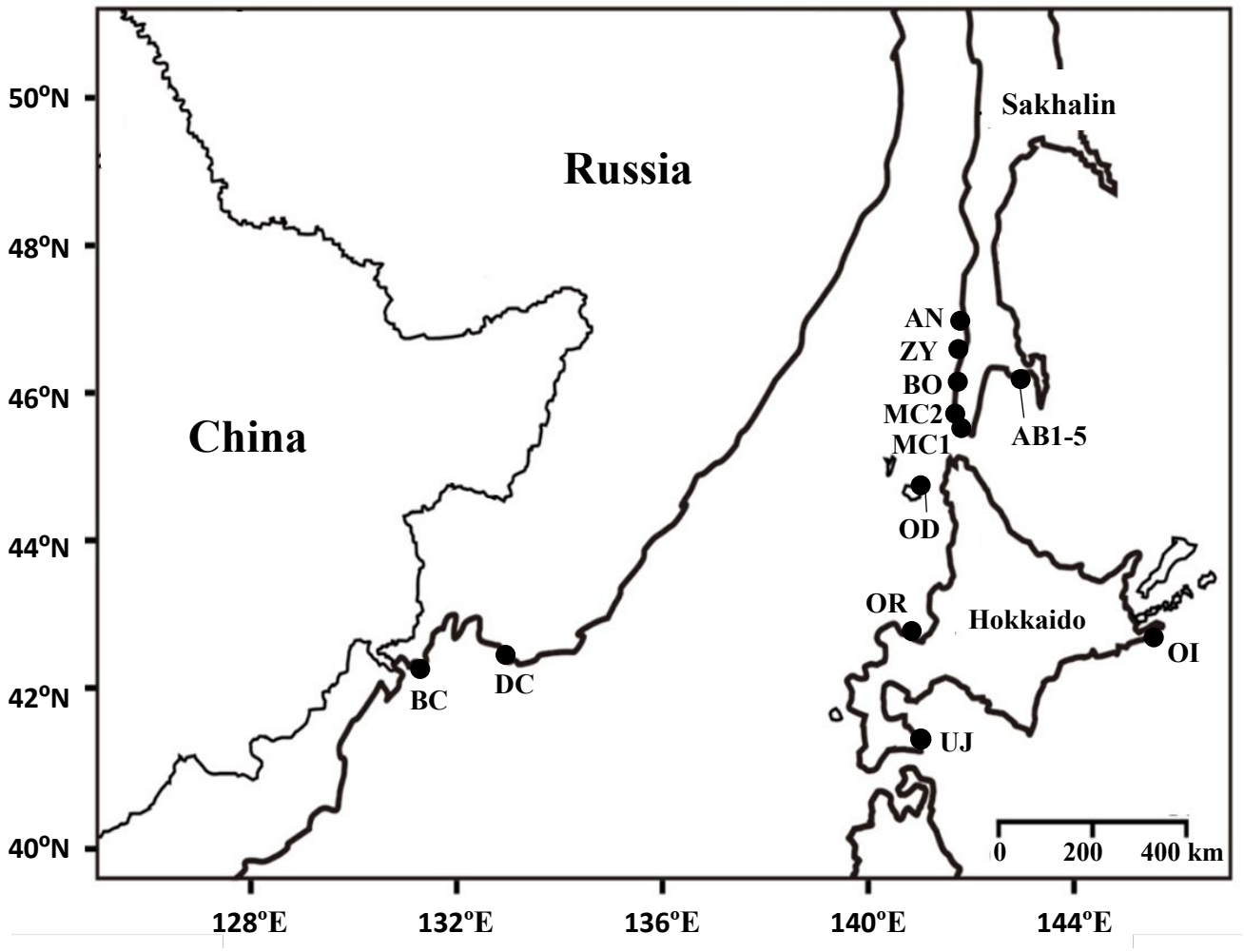
Table S4 Pairwise Fst values (above diagonal) and DS values (below diagonal) between two within eight regional populations

Population	Southern coast	Southwestern-end	Western coast	Primorsky Krai	UJ	OR	OD	OI
Southern coast[1)]	***	0.11	0.11	0.11	0.12	0.08	0.09	0.09
Southwestern-end[2)]	0.33	***	0.06	0.1	0.1	0.06	0.08	0.08
Western coast[3)]	0.38	0.2	***	0.09	0.12	0.07	0.1	0.1
Primorsky Krai	0.35	0.36	0.44	***	0.17	0.11	0.15	0.15
UJ	0.29	0.23	0.35	0.51	***	0.05	0.07	0.05
OR	0.21	0.17	0.22	0.4	0.08	***	0.04	0.04
OD	0.19	0.16	0.26	0.45	0.12	0.06	***	0.04
OI	0.21	0.16	0.28	0.45	0.08	0.07	0.05	***

Table S5 Pairwise Fst values (above diagonal) and DS values (below diagonal) between two within 16 regional populations

Population	AB1	AB2	AB3	AB4	AB5	MC1	MC2	BO	ZY	AN	BC	DC	UJ	OR	OD	OI
AB1	***	0.05	0.03	0.04	0.03	0.09	0.27	0.16	0.12	0.14	0.22	0.12	0.14	0.09	0.1	0.11
AB2	0.09	***	0.04	0.04	0.04	0.11	0.31	0.18	0.14	0.15	0.19	0.16	0.18	0.12	0.13	0.13
AB3	0.04	0.07	***	0.05	0.03	0.1	0.31	0.18	0.15	0.16	0.23	0.15	0.14	0.1	0.11	0.11
AB4	0.07	0.08	0.09	***	0.05	0.09	0.26	0.16	0.12	0.13	0.24	0.14	0.12	0.08	0.09	0.11
AB5	0.06	0.06	0.05	0.08	***	0.11	0.31	0.19	0.16	0.16	0.23	0.16	0.14	0.11	0.12	0.11
MC1	0.25	0.28	0.24	0.21	0.26	***	0.17	0.11	0.1	0.07	0.22	0.11	0.07	0.04	0.05	0.04
MC2	0.73	0.8	0.82	0.61	0.82	0.36	***	0.2	0.19	0.17	0.36	0.14	0.28	0.2	0.25	0.26
BO	0.73	0.74	0.82	0.65	0.86	0.5	0.58	***	0.05	0.11	0.22	0.13	0.17	0.12	0.16	0.15
ZY	0.45	0.47	0.53	0.41	0.59	0.39	0.56	0.33	***	0.09	0.19	0.12	0.17	0.1	0.14	0.14
AN	0.47	0.44	0.46	0.36	0.49	0.21	0.4	0.54	0.38	***	0.2	0.13	0.13	0.08	0.11	0.1
BC	0.6	0.43	0.56	0.56	0.58	0.64	0.95	0.89	0.69	0.59	***	0.23	0.26	0.2	0.25	0.26
DC	0.42	0.49	0.43	0.45	0.49	0.35	0.28	0.72	0.6	0.49	0.74	***	0.18	0.13	0.17	0.16
UJ	0.33	0.39	0.3	0.26	0.32	0.14	0.6	0.62	0.57	0.31	0.64	0.53	***	0.05	0.07	0.05
OR	0.24	0.29	0.23	0.18	0.28	0.11	0.47	0.52	0.37	0.22	0.52	0.44	0.08	***	0.04	0.04
OD	0.21	0.26	0.22	0.17	0.25	0.08	0.51	0.55	0.43	0.25	0.58	0.49	0.12	0.06	***	0.04
OI	0.24	0.28	0.23	0.21	0.23	0.06	0.55	0.58	0.48	0.25	0.6	0.46	0.08	0.07	0.05	***

Figure 1


50°N
48°N
46°N
44°N
42°N
40°N
128°E
132°E
136°E
140°E
144°E
Russia
China
Sakhalin
Hokkaido
AN
ZY
BO
MC2
MC1
AB1-5
OD
OR
OI
UJ
BC
DC
0
200
400 km

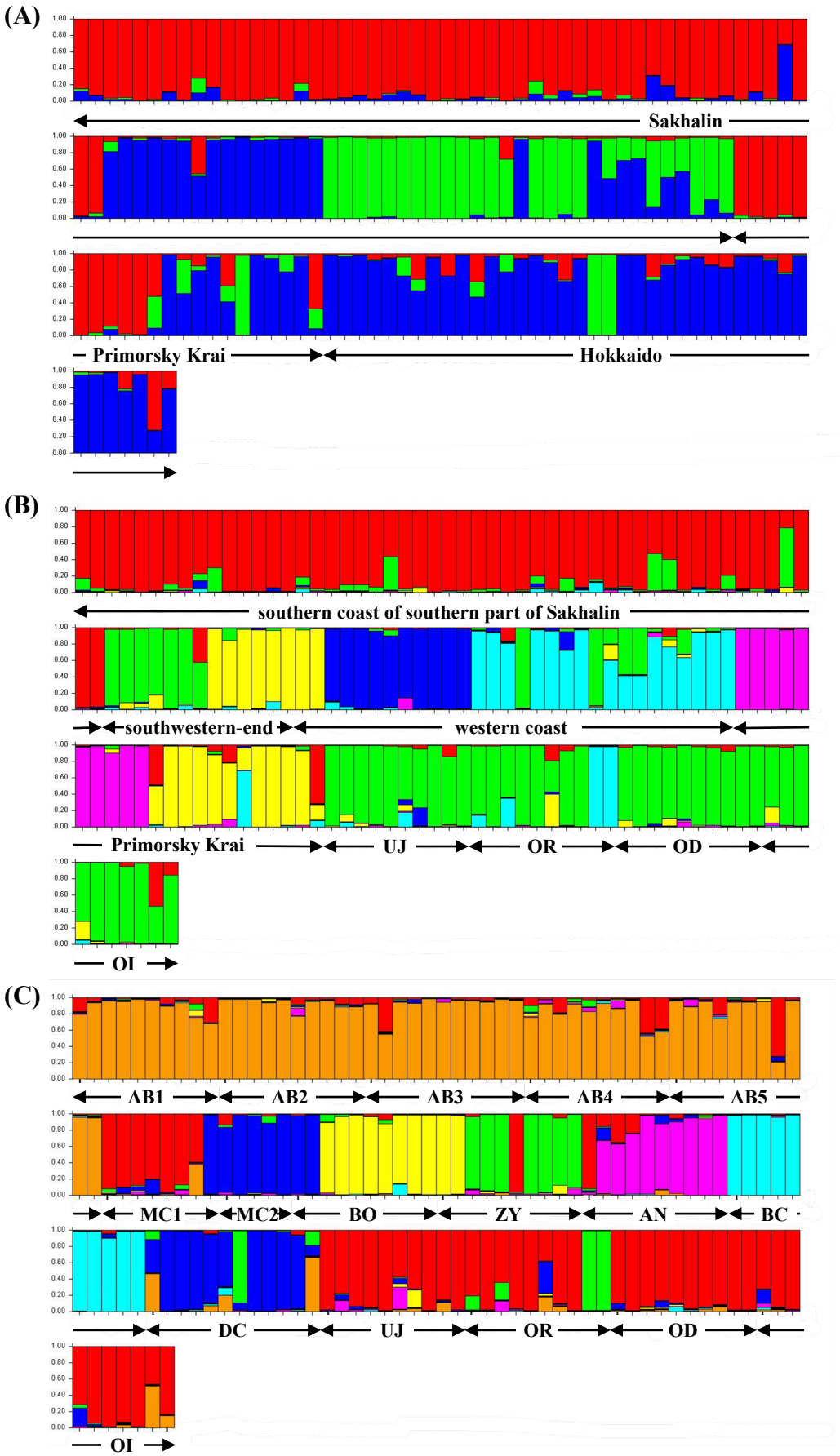

(A)
Sakhalin
Primorsky Krai
Hokkaido
(B)
southern coast of southern part of Sakhalin
southwestern-end
western coast
Primorsky Krai
UJ
OR
OD
OI
(C)
AB1
AB2
AB3
AB4
AB5
MC1
MC2
BO
ZY
AN
BC
DC
UJ
OR
OD
OI

Figure 3

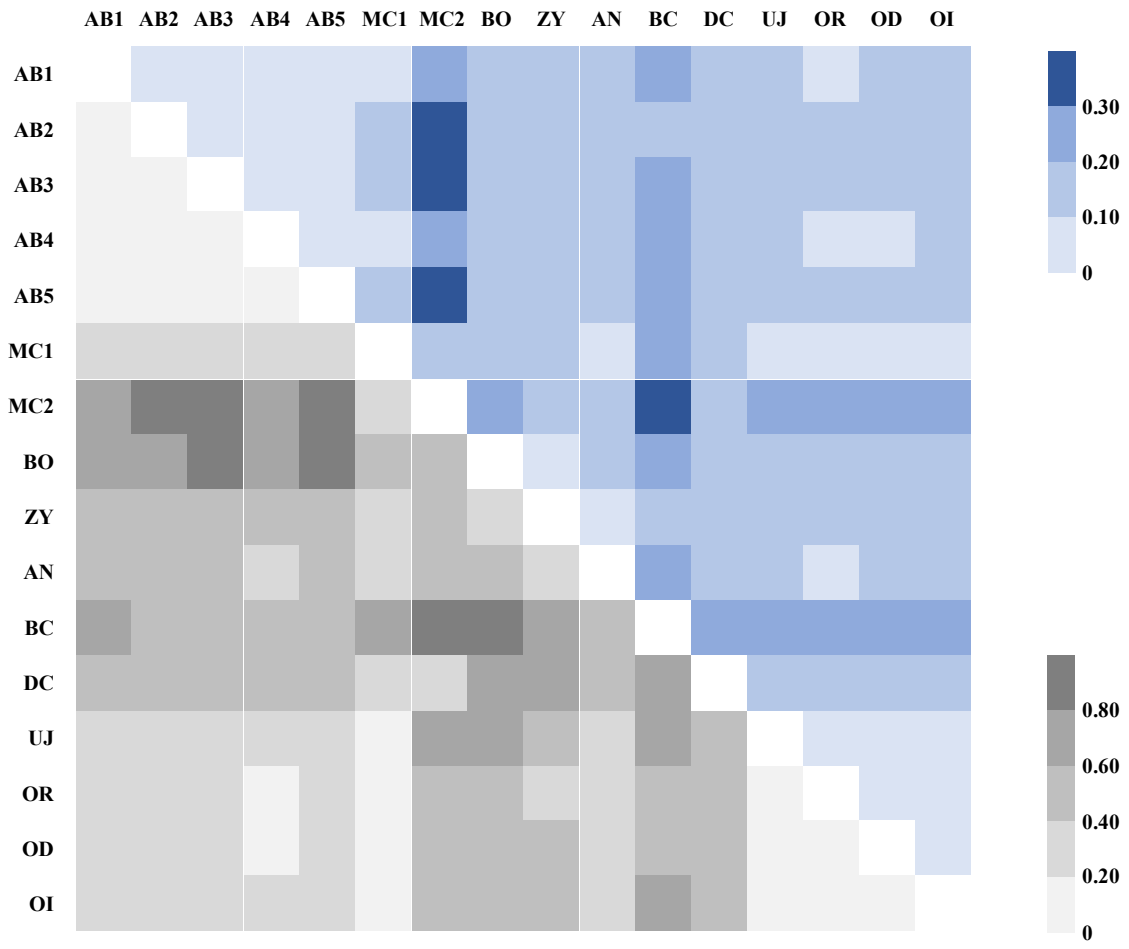
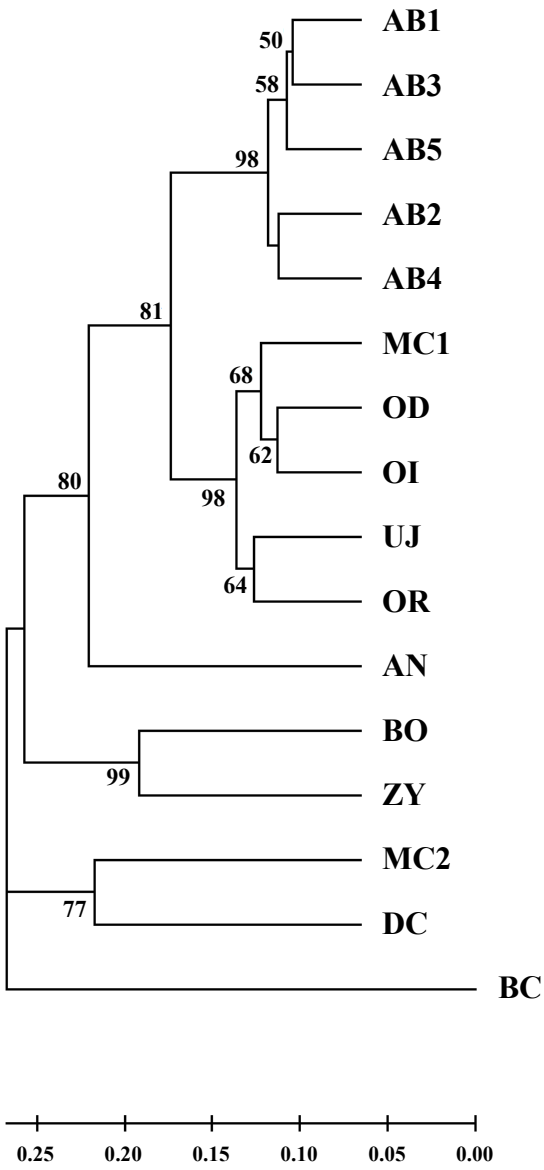
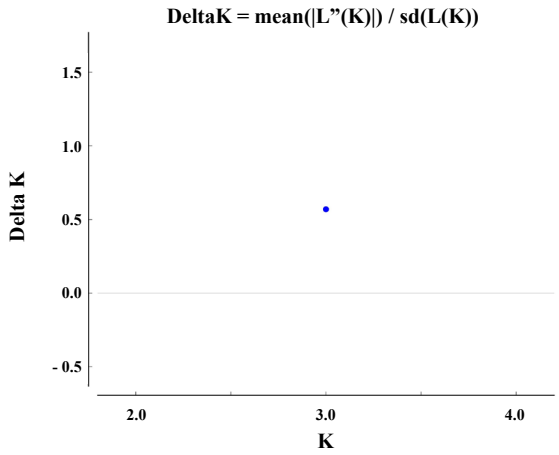

AB1
AB2
AB3
AB4
AB5
MC1
MC2
BO
ZY
AN
BC
DC
UJ
OR
OD
OI
0.30
0.20
0.10
0
0.80
0.60
0.40
0.20
0

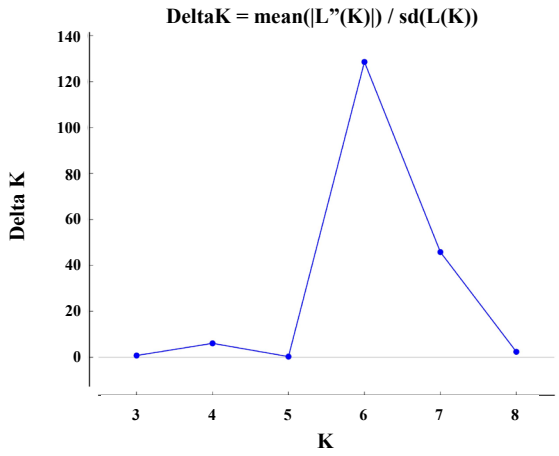
Figure 4


AB1
AB3
AB5
AB2
AB4
MC1
OD
OI
UJ
OR
AN
BO
ZY
MC2
DC
BC
50
58
98
81
68
62
80
98
64
99
77
0.25
0.20
0.15
0.10
0.05
0.00

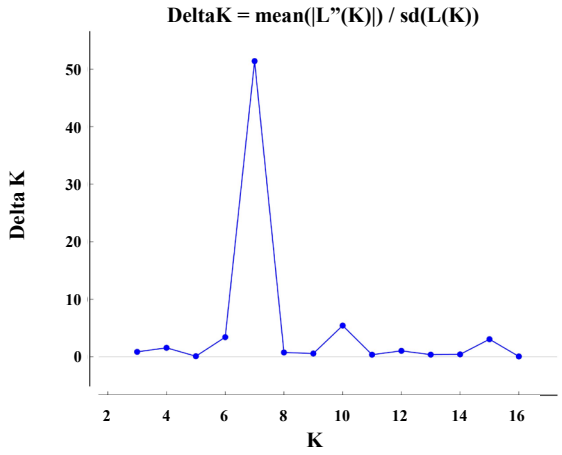
(A)


DeltaK = mean(|L"(K)|) / sd(L(K))
Delta K
1.5
1.0
0.5
0.0
- 0.5
2.0
3.0
4.0
K

(B)


DeltaK = mean(|L"(K)|) / sd(L(K))
Delta K
140
120
100
80
60
40
20
0
3
4
5
6
7
8
K

(C)


DeltaK = mean(|L"(K)|) / sd(L(K))
Delta K
50
40
30
20
10
0
2
4
6
8
10
12
14
16
K