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Genetic and Conchological Variation in *Littorina sitkana* Philippi (Mollusca, Gastropoda) on Northern Japanese Coasts

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ABSTRACT—A periwinkle, *Littorina sitkana* Philippi, 1846, does direct development and exhibits three types of shell sculpture. Lacking pelagic larval stages, this species is expected to be genetically differentiated among populations. In the present study, genetic variation of 19 populations along northern Japanese coasts was examined using protein electrophoresis. The relative abundance of shell types was also investigated at each locality. The analyzed populations were significantly differentiated genetically from one another. However, no significant genetic difference was detected between shell types within localities where two types were nearly equally frequent. When clustered genetically using UPGMA, the populations were divided into four geographic groups. The UPGMA tree also showed that the Japanese population of *L. sitkana* is clearly divided into two groups, which may have been derived from two mother populations with different genetic structures. On the other hand, allozymic and anatomical analyses of the present samples have refuted possible occurrence of sibling species of *L. sitkana* on the coasts of northern Japan.

INTRODUCTION

Littorina sitkana Philippi, 1846, an intertidal gastropod without pelagic larval stages, is widely distributed throughout the North Pacific and is highly polymorphic in shell sculpture, which has led to taxonomic confusion for a long time (Reid, 1996). Three shell types in sculpture have been recognized in this species. The first and second types have spiral ribs on the whole shell surface and only on the base of the last whorl, respectively. The third has a completely smooth surface. The third shell type is similar to the shells of four congeneric species: *L. kasatka* Reid, Zaslavskaya and Sergievsky, 1991, *L. subrotundata* (Carpenter, 1864), *L. natica* Reid, 1996, and *L. aleutica* Dall, 1872. Some of these nominal species have often been regarded as synonyms. Recently, however, all of these species were shown to be distinguishable from one another electrophoretically and anatomically in the North Pacific (Boulding *et al*., 1993; Reid and Golikov, 1991; Reid *et al*., 1991; Zaslavskaya, 1995). On the other hand, there have been only a few studies concerning genetic and/or conchological variation in *L. sitkana*.

Ohgaki (1983) found that the relative abundance of the three shell types of *L. sitkana* differed between populations in Hokkaido, and suggested their possible genetic differentiation. In the present study, I tested this possibility by allozyme analyses of snails from 19 localities on the northern coasts of Japan. I investigated possible correlation between genetic and morphological variation.

Reid (1996) and Reid *et al*. (1996) reported a sibling species *L. kasatka* from Akkeshi and Nemuro, southeastern Hokkaido. In the present study, I also electrophoretically tested whether *L. kasatka* or other sibling species inhabit northern Japan.

MATERIALS AND METHODS

Sampling and estimation of population density

Nineteen sampling localities were chosen to cover the distribution of *L. sitkana* in Japan (Fig. 1). Some localities are situated very close to each other, with distances of *ca*. 200 m between Utoro-A and -B, *ca*. 800 m between Minami-kayabe-A and -B, and *ca*. 300 m between Minami-kayabe-B and Shikabe. Sampling was carried out in 1997 at all the localities, and also in 1995 at some of them (Table 1). More than 50 individuals with the size of maturity were randomly collected from each locality. Sampled snails were kept frozen at –80°C until the analyses.

In the autumn of 1996, the population densities were also estimated at 23 localities, five of which were not electrophoretically investigated (Table 1). Only at Hamanaka could the population density not be obtained at the same place that the specimens for electrophoresis were collected. I walked around the shore at each locality for about thirty minutes to find spots with abundant snails. After selecting three or more spots of the highest densities at every locality except Wakkanai, Atsunai, and Iwanai where only one spot was examined, I set a 25×25 cm quadrat to count the number of individuals within. When a spot contained several hundred snails, a photograph was taken to estimate an approximate number within the quadrat. The population densities were estimated by calculating the average density for all the spots at 20 localities.

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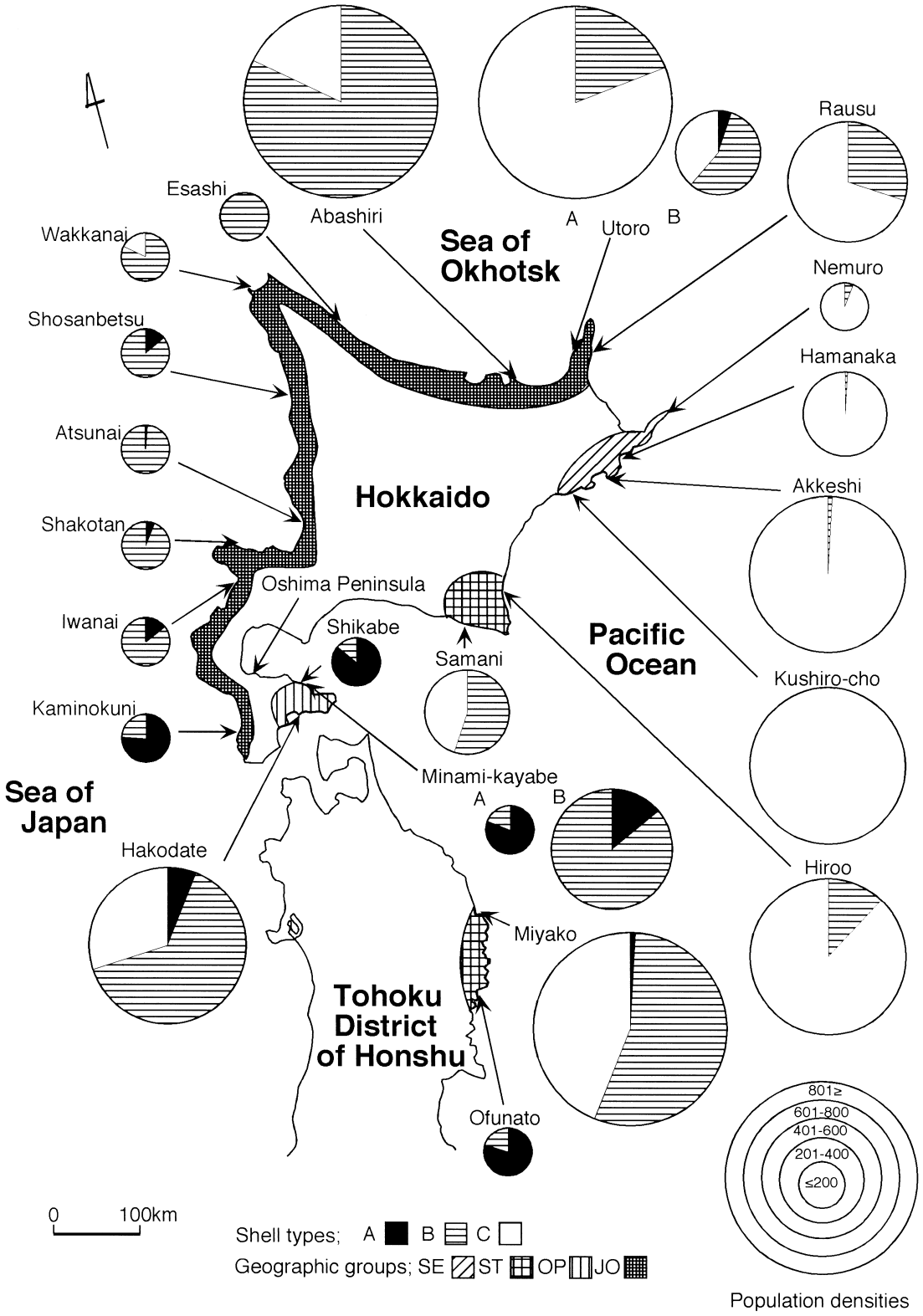

Fig. 1. Sampling localities of *L. sitkana* in Northern Japan. Circle graphs show proportions of three shell types (solid, Type A; striped, Type B; blank, Type C). Population density at each locality is represented by the size of the circle graph, as defined below with the number of individuals found per 25 × 25 cm. Localities are divided into four geographic groups (JO, SE, ST, and OP) on the basis of genetic similarity (see Fig. 2).

Identification of shell types

Samples were classified into three categories following Ohgaki's (1983) terminology. Type A has several spiral ribs on the whole shell surface of the last whorl, Type B is sculptured only at the base of the last whorl, and Type C has the smooth surface without sculpture. The relative abundances of the shell types at each locality were calculated based on collected individuals. Furthermore, field observations of relative abundance were made at several places between sampling localities around Hokkaido and along the Pacific side of the Tohoku District.

Statistical analysis

Homogeneities in allele frequency among the 19 populations were statistically examined at all the loci by the pseudo-probability test (Zaykin and Pudovkin, 1993). The same test was also employed for the two most frequent shell types at Samani, Miyako, and Utoro-B, where neither type was significantly more frequent (Table 1).

Pairwise comparisons between populations were made using Nei's (1978) genetic distance. Clustering of populations by means of UPGMA was carried out, based on Nei's genetic distances, using PHYLIP 3.5 (Felsenstein, 1993). To examine the robustness of the clusters, standard errors at the branching points (Nei, 1987) were also calculated.

Table 1. Number of analyzed snails (N), population density, and proportion (%) of shell types at each sampling locality. Geographic groups of localities are based on genetic similarity (see Figs. 1 and 2). Density is the number of individuals in a 25 × 25 cm quadrat.

Sampling locality	Geographic group	Density ± S. D.	N	Percentage of shell types			Sampling month and year
				A	B	C	
Rausu	JO	588 ± 134	71	0	30	70	Feb. 97
Utoro-A	JO	935 ± 42	65	0	19	81	Feb. 97
Utoro-B	JO	373 ± 162	64	5	56	39	Feb. 97
Abashiri	JO	912 ± 286	128	0	82	18	Mar. 95, Feb. 97
Esashi	JO	165 ± 83	58	0	100	0	Feb. 97
Shosanbetsu	JO	38 ± 23	70	14	86	0	Feb. 97
Shakotan	JO	71 ± 36	125	6	94	0	Aug. 95, Feb. 97
Kaminokuni	JO	85 ± 30	67	76	24	0	Feb. 97
Wakkanai	JO	194	50	0	82	18	-
Atsunai	JO	33	47	1	99	0	-
Iwanai	JO	16	44	14	86	0	-
Minami-kayabe-A	OP	47 ± 23	57	81	19	0	Feb. 97
Minami-kayabe-B	OP	432 ± 62	130	14	86	0	Mar. 95, Feb. 97
Shiakabe	OP	24 ± 7	128	86	14	0	Mar. 95, Feb. 97
Hakodate	OP	719 ± 115	67	6	64	30	Feb. 97
Hamanaka	SE	286 ± 151	67	0	1	99	Feb. 97
Akkeshi	SE	681 ± 219	132	0	1	99	Mar. 95, Feb. 97
Kushiro-cho	SE	771 ± 124	130	0	0	100	Mar. 95, Feb. 97
Nemuro	SE	152 ± 36	72	0	6	94	-
Hiroo	ST	688 ± 193	60	0	12	88	Feb. 97
Samani	ST	392 ± 168	71	0	55	45	Feb. 97
Miyako	ST	906 ± 208	73	1	55	44	Feb. 97
Ofunato	ST	52 ± 20	77	80	20	0	Feb. 97

Electrophoresis

Horizontal electrophoresis was carried out with 11.5% (w/v) hydrolyzed starch gel, using a continuous tris-citric acid buffer, pH 8.0, following Ward and Warwick (1980). Specimens for electrophoresis were prepared as follows. After identifying the shell type, frozen snails were thawed. A small piece of filter paper was directly touched to the soft part of each snail, soaked with its exudate for several minutes, and placed in gel.

Twenty enzyme systems were preliminarily stained according to Murphy et al. (1996); most of them were monomorphic or unclearly stained. Only five polymorphic enzyme systems were available for statistical analyses: leucine aminopeptidase (E.C. 3.4.11.1; abbreviated as LAP), mannose-6-phosphate isomerase (E.C. 5.3.1.8; MPI), peptidase (E.C. 3.4.–.–; PEP), purine-nucleoside phosphorylase (E.C. 2.4.2.1; PNP), and phosphoglucomutase (E.C. 2.7.5.1; PGM). Alleles of each locus were labeled according to their relative electrophoretic mobilities, with the slowest allele marked as the "a" allele. More than 50 individuals from each locality were analyzed for each locus.

The MDH locus was also examined because Zaslavskaya (1995) proved that *L. kasatka* or any other sibling species were distinguishable from smooth-shell individuals of *L. sitkana* by diagnostic alleles at the locus. However, the locus was not used for the statistical analyses below.

RESULTS

Dominant shell type and population density

As shown in Table 1, most populations were characterized by single dominant shell types, which nearly occupied 80% of specimens. At Samani, Miyako, and Utoro-B, Types B and C were relatively similar in relative abundance, that is, the abundance of Type C was more than a half of that of Type B. At only three localities were all the three shell types found. Table 1, as well as my field observations at several places between the sampling localities around Hokkaido, shows a general tendency of geographic variation in the relative abundance of shell types in Hokkaido. Around the Oshima Peninsula and in southwestern Hokkaido facing the Pacific Ocean, either Type A or B occurred most frequently. On the Sea of Japan and Sea of Okhotsk sides of Hokkaido, Type B was

Table 2. Allele frequencies at seven polymorphic loci of *L. sitkana* from19 localities. N is the number of examined individuals. Geographic and 2).

Geographic group	JO								OP				
Allele	Rausu	Utoro-A	Utoro-B	Abashiri	Esashi	Shosan-betsu	Shakotan	Kamino-kuni	Minami-kayabe-A	Minami-kayabe-B	Shikabe	Hakodate	Hama-naka
LAP-1													
a	.024	.132	.073	.006	.000	.000	.000	.008	.114	.104	.225	.063	.000
b	.968	.825	.879	.782	.830	.957	.898	.831	.877	.896	.761	.675	1.000
c	.008	.026	.048	.213	.170	.043	.102	.162	.009	.000	.014	.254	.000
d	.000	.018	.000	.000	.000	.000	.000	.000	.000	.000	.000	.008	.000
N	62	57	62	87	56	70	88	65	57	82	71	63	66
LAP-2													
a	.032	.250	.145	.018	.000	.000	.011	.008	.009	.056	.050	.063	.433
b	.081	.216	.258	.217	.402	.086	.247	.192	.939	.796	.879	.619	.552
c	.226	.293	.266	.464	.348	.243	.230	.454	.053	.136	.057	.167	.015
d	.024	.164	.177	.096	.054	.364	.115	.100	.000	.012	.000	.151	.000
e	.613	.078	.145	.193	.161	.293	.270	.208	.000	.000	.014	.000	.000
f	.024	.000	.008	.012	.036	.014	.126	.038	.000	.000	.000	.000	.000
N	62	28	62	83	56	70	87	65	57	81	70	63	67
MPI													
a	.937	.875	.781	.601	.702	.907	.844	.948	.000	.208	.045	.075	.652
b	.063	.125	.219	.399	.298	.093	.156	.052	1.000	.792	.955	.925	.348
N	71	64	67	84	57	70	90	67	55	77	66	67	66
PEP													
a	.000	.032	.024	.070	.000	.022	.000	.167	.045	.000	.000	.000	.000
b	.203	.563	.339	.810	.940	.674	.769	.307	.509	.087	.048	.205	.060
c	.797	.397	.637	.090	.060	.275	.192	.518	.446	.865	.952	.795	.940
d	.000	.008	.000	.030	.000	.029	.038	.009	.000	.048	.000	.000	.000
N	69	63	62	50	58	69	52	57	56	52	52	61	67
PGM-1													
a	.048	.000	.009	.031	.009	.000	.012	.000	.026	.039	.061	.018	.036
b	.738	.667	.776	.833	.673	.657	.787	.480	.509	.947	.879	.728	.848
c	.214	.333	.216	.130	.245	.231	.201	.304	.465	.013	.061	.254	.116
d	.000	.000	.000	.006	.073	.111	.000	.216	.000	.000	.000	.000	.000
N	63	57	58	81	55	54	82	51	57	76	66	57	56
PGM-2													
a	.500	.246	.213	.102	.483	.239	.044	.017	.071	.143	.134	.066	.000
b	.486	.738	.779	.898	.517	.761	.956	.983	.929	.857	.866	.934	1.000
c	.014	.016	.008	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
N	71	63	61	83	58	69	90	59	56	77	71	61	64
PNP													
a	.014	.056	.008	.006	.000	.000	.000	.000	.000	.000	.000	.000	.055
b	.014	.016	.008	.034	.019	.000	.000	.000	.009	.045	.029	.007	.000
c	.930	.929	.984	.948	.952	.993	.989	.947	.282	.149	.140	.082	.242
d	.042	.000	.000	.011	.029	.007	.011	.053	.709	.805	.831	.910	.703
N	71	63	62	87	52	70	90	66	55	77	68	67	64
MDH													
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
N	71	65	64	128	58	70	125	67	57	130	128	67	67

dominant. In southeastern Hokkaido, Type C had the highest percentage of occurrence. Along the Pacific coast of the Tohoku District, large geographic variation was detected between the two localities.

Fig. 1 and Table 1 also show a great deal of variation in population density among the localities. High population densities were generally found in the eastern half of Hokkaido. Types B or C seemed to be more frequent in dense populations than in those with low densities.

Allele frequency

As shown in Table 2, the MDH locus was fixed with the “a” allele at any populations; all the specimens lacked the diagnostic alleles of the sibling species of *L. sitkana* found by Zaslavskaya (1995).

Allele composition at every locus was more or less different among the 19 populations (Table 2). Allele frequencies were significantly heterogeneous at each of the seven loci (Table 3). Genetic differentiation was apparent from the different patterns of allele composition between populations along

groups of localities are based on genetic similarity (see Figs. 1

SE		ST			
Akkeshi	Kushiro-cho	Hiroo	Samani	Miyako	Ofunato
.005	.012	.009	.000	.059	.000
.984	.988	.955	1.000	.838	.953
.011	.000	.009	.000	.103	.047
.000	.000	.027	.000	.000	.000
92	83	55	71	68	64
.540	.451	.036	.190	.000	.100
.273	.469	.364	.183	.529	.338
.188	.080	.600	.599	.414	.454
.000	.000	.000	.021	.057	.108
.000	.000	.000	.007	.000	.000
.000	.000	.000	.000	.000	.000
88	81	55	71	70	65
.522	.625	.347	.076	.317	.514
.478	.375	.653	.924	.683	.486
91	84	59	66	71	74
.000	.000	.000	.000	.038	.007
.036	.009	.043	.045	.023	.399
.964	.991	.957	.955	.939	.594
.000	.000	.000	.000	.000	.000
55	53	58	67	66	69
.041	.042	.139	.065	.000	.053
.882	.917	.713	.759	.781	.904
.076	.042	.148	.120	.219	.044
.000	.000	.000	.056	.000	.000
85	72	54	54	57	57
.017	.080	.042	.039	.014	.029
.983	.920	.958	.953	.986	.942
.000	.000	.000	.008	.000	.029
88	81	60	64	71	69
.006	.019	.000	.000	.000	.000
.006	.019	.000	.000	.000	.000
.341	.526	.319	.476	.657	.393
.648	.435	.681	.524	.343	.607
88	77	58	63	70	70
1.000	1.000	1.000	1.000	1.000	1.000
132	130	60	71	73	77

the Pacific Ocean and those on the Sea of Japan and Sea of Okhotsk coasts of Hokkaido. For example, two alleles were roughly equally frequent at the MPI, PNP, and PEP loci along the Pacific Ocean, while only single alleles were dominant in the others (Table 2). In addition, the “e” and “f” alleles of the LAP-2 locus were frequent only in the latter.

At Samani, Miyako, and Utoro-B, where two shell types were similarly abundant, snails of different shell types showed no significant genetic differentiation at any loci (Table 3).

Table 3. Results of pseudo-probability tests for overall genetic homogeneities of all the examined localities and of the otwo most frequent shell types at three localities where two shell types were similarly frequent.

Locus	All	Samani	Miyako	Utoro-B
LAP-1	548*	–	1.34	2.44
LAP-2	2344*	4.17	3.62	3.18
MPI	1040	0.21	1.63	1.00
PEP	1170	0.01	3.47	3.74
PGM-1	576	1.53	1.55	0.69
PGM-2	512	5.88	1.71	1.67
PNP	1409	0.73	0.13	2.77

Significance level is indicated by * ($p < 0.01$)

Genetic similarity and shell variation

Table 4 shows Nei’s genetic distances between populations. The genetic distance ranging from 0.009 to 0.637 was not correlated with geographic distance (Fig. 1; Table 4). For example, Kaminokuni on the west coast of Hokkaido is more distant geographically from Rausu on the east coast than the latter is from Hamanaka on the east coast. However, the genetic distance between Kaminokuni and Rausu was smaller than that between Rausu and Hamanaka.

Using the genetic distances, the 19 populations were clustered primarily into two groups by UPGMA (Fig. 2). One group comprised the populations on the Sea of Japan and Sea of Okhotsk sides of Hokkaido (abbreviated as JO group), and the other group comprised the rest of populations. Further, the latter clustered geographically into three groups, the first consisting of the populations on the eastern half of the Oshima Peninsula (OP), the second those of southeastern Hokkaido along the Pacific coast (SE), and the third those of the Pacific coast of south Hokkaido and the Tohoku District (ST). In the UPGMA tree, standard errors at the branching points of the clusters were large, probably because of the small number of

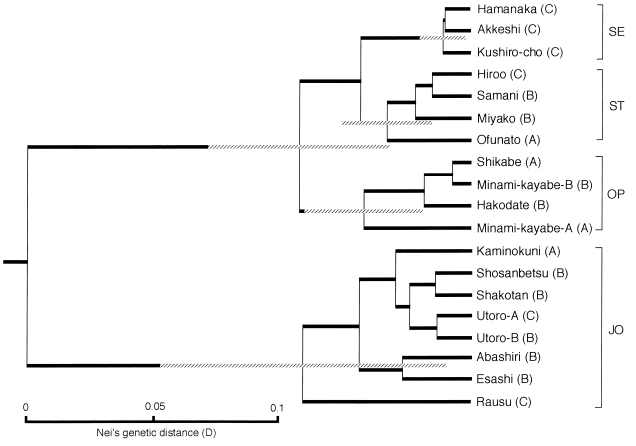

Fig. 2. Rooted tree of 19 sampling localities of *L. sitkana* by UPGMA. A, B, or C in parentheses after locality names indicate the most frequent shell type at each locality. Shaded areas of horizontal bars show ±1 standard error at the branching points. Four geographic groups are shown as SE, ST, OP, and JO.

Table 4. Nei's genetic distances between19 populations of *L. sitkana* in Northern Japan.

Sampling locality	Rausu	Utoro-A	Utoro-B	Abashiri	Esashi	Shosan-betsu	Shako-tan	Kamino-kuni	Minami-kayabe-A	Minami-kayabe-B	Shikabe	Hakodate
Utoro-A	.092											
Utoro -B	.058	.016										
Abashiri	.204	.063	.081									
Esashi	.180	.066	.109	.045								
Shosanbetsu	.094	.024	.043	.057	.061							
Shakotan	.141	.032	.051	.023	.057	.021						
Kaminokuni	.107	.047	.042	.101	.153	.055	.060					
Minami-kayabe-A	.598	.422	.381	.353	.389	.500	.402	.469				
Minami-kayabe-B	.426	.410	.302	.404	.486	.501	.432	.428	.090			
Shikabe	.533	.518	.387	.502	.596	.637	.548	.538	.080	.009		
Hakodate	.562	.484	.385	.433	.546	.572	.494	.480	.064	.033	.027	
Hamanaka	.275	.246	.051	.349	.435	.336	.297	.249	.203	.070	.113	.122
Akkeshi	.260	.325	.167	.317	.433	.327	.297	.242	.231	.083	.126	.124
Kushiro-cho	.188	.176	.107	.276	.355	.262	.238	.190	.248	.094	.141	.168
Hiroo	.301	.290	.203	.303	.422	.363	.331	.245	.176	.059	.095	.077
Samani	.340	.318	.219	.296	.442	.394	.360	.301	.163	.089	.110	.096
Miyako	.243	.209	.120	.220	.327	.280	.241	.177	.151	.078	.100	.103
Ofunato	.240	.159	.121	.144	.230	.196	.161	.169	.164	.082	.140	.112

the examined loci.

According to Figs. 1 and 2 and my field observations, the geographic groups except the ST group may be characterized by peculiar shell types. In the JO and SE groups, Types B and C were dominant, respectively. In the OP group, either Type A or B occurred most frequently, depending on the locality.

DISCUSSION

Sibling species of *L. sitkana*

Littorina sitkana and its North Pacific sibling species, *L. kasatka*, *L. subrotundata*, *L. natica*, and *L. aleutica*, are distinguishable from one another by the shape of penis and/or pallial oviduct (Reid and Golikov, 1991; Reid *et al*., 1991; Reid, 1996). In addition, it is proved that *L. sitkana* can be distinguished from *L. kasatka*, *L. subrotundata*, and *L*. sp. by diagnostic alleles at the MDH locus (Zaslavskaya, 1995).

In the present study, all the examined snails were safely assigned to *L. sitkana* because of lacking the diagnostic alleles at the MDH locus; anatomically the same is true, as will be reported elsewhere (Nohara, in preparation). Reid (1996) and Reid *et al*. (1996) found *L. kasatka* at Akkeshi, but the present Akkeshi samples did not contain this species at all. Therefore, it can be concluded that the Northern Japanese coasts are generally inhabited only very rarely, if at all, by *L. kasatka*, while *L. sitkana* is abundant.

Genetic differentiation in *L. sitkana* on the Japanese coasts

The allozymic variation of *L. sitkana* in the present study indicates that populations are significantly differentiated genetically from one another. Zaslavskaya *et al*. (1994) also found similar genetic differentiation in *L. sitkana* between two populations ca. 4 km apart in Vostok Bay, Sea of Japan. Some other *Littorina* species, which are direct developers like *L. sitkana*, have shown similar genetic phenomena (Janson and Ward, 1984; Tatarenkov and Johannesson 1994; Ward, 1990; Ward and Warwick, 1980). On the other hand, littorinid snails with planktonic larval stages are much less differentiated (Janson, 1987; Johannesson, 1992; Tatarenkov, 1995; Ward, 1990; Nohara, unpublished data). This difference is probably attributable to the restricted level of gene flow in direct developers, where genetic drift and/or natural selection may give rise to genetic differences among populations.

Genetic relationship of populations

In the present study, the 19 populations were genetically clustered by UPGMA into four geographical groups. The genetic similarities of the populations within the groups suggest that they were derived from respective common ancestors with different genetic structures. In addition, because the three southern groups on the Pacific coast (SE, ST, and OP) are genetically closer to one another than to the JO group, the JO and the southern groups are likely to be from genetically different mother populations.

Ohgaki (1983) divided populations of Hokkaido into two groups on the basis of the different proportions of the three shell types A, B, and C. One group, characterized by the dominant Type B, included populations of Northern Hokkaido along the coasts of both the Sea of Japan and the Sea of Okhotsk. The other group was recognized by the presence of all three shell types in various proportions, and comprised of populations on the southern coast of Hokkaido facing the Pacific Ocean. The JO group and the three southern groups discerned in the present study appear to correspond to Ohgaki's northern and southern groups, respectively, because the UPGMA tree topology shows the deepest branching situated between the JO group and the cluster of southern groups.

On the Far Eastern Russian coasts (Fig. 3A), Type B was predominant along the Sea of Japan, while on the coast of the Kurile Islands near eastern Hokkaido, Type C was abundant with the other types occurring sporadically (Reid, 1996; Zaslavskaya, personal communication). Further, Zaslavskaya

Hama-naka	Akkeshi	Kushiro-cho	Hiroo	Samani	Miyako
.014					
.017	.015				
.075	.052	.078			
.129	.072	.105	.023		
.095	.076	.063	.034	.039	
.067	.055	.069	.041	.072	.063

(1995) found significant genetic difference between the populations of Peter the Great Bay in the Sea of Japan and the Kurile Islands. These findings seem to be similar to the present results. In addition, in the latest Würm glacial period, Hokkaido and the Far Eastern Russian coasts were connected by land bridges between Sakhalin Island and Northern Hokkaido, and between the Kurile Islands and Eastern Hokkaido, as shown in Fig. 3B (Japan Association for Quaternary Research, 1987). These may suggest the possibility that the Russian population along the Sea of Japan is historically related to that of northern coast of Hokkaido, while the Kurile population to that of southeastern coast of Hokkaido. However, this possibility should be closely examined in terms of molecular population genetics.

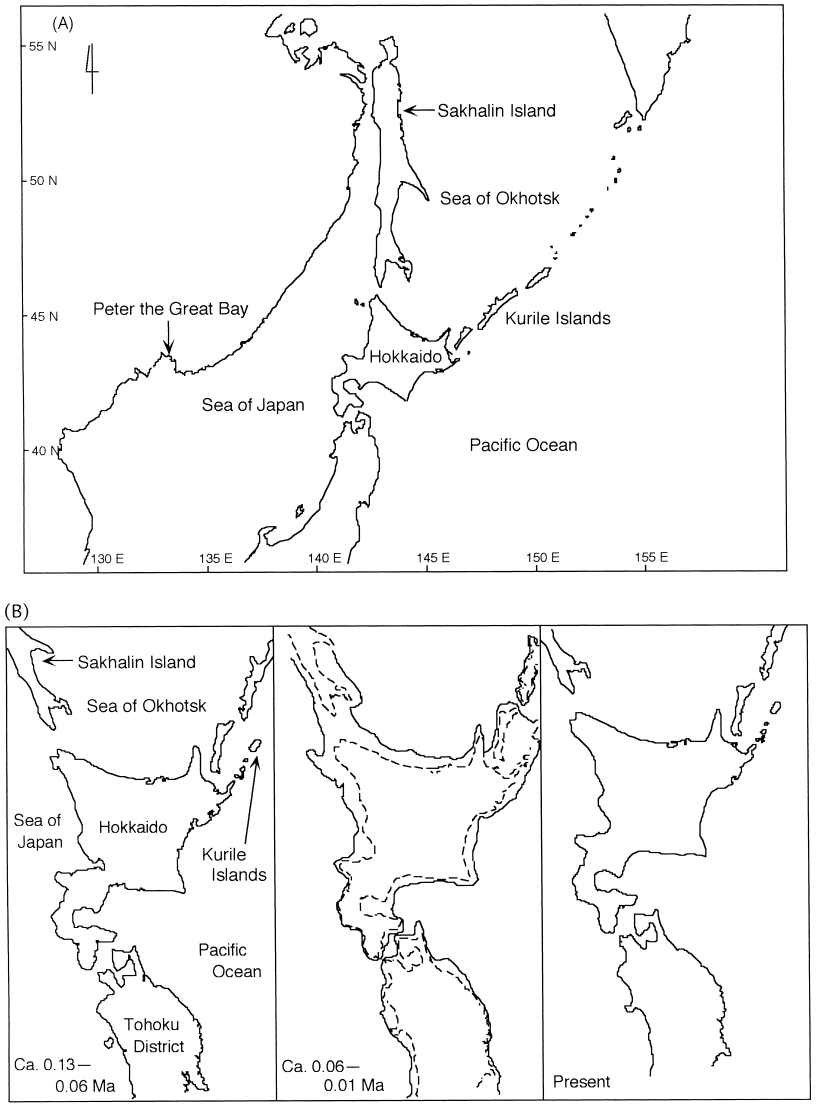

Fig. 3. (A) Present coastline around the Far East. (B) Changes of the coastline around Northern Japan (Japan Association for Quaternary Research, 1987).

Gene flow between shell types

Johannesson et al. (1993) found that, in *Littorina saxatilis* (Olivi, 1792) without pelagic stages, two forms with different shell sculptures were segregated by microhabitat on the Swedish coast, and had reduced gene flow along the same shore. In *L. sitkana*, however, at Samani, Miyako, and Utoro-B, where two shell types occurred frequently, no significant difference in allele frequency could be detected between the shell types. The different shell types in Japan inhabit the same microhabitats, and mate with one another in the aquarium with no apparent behavioral isolation (personal observation).

Variation in shell sculpture among populations: Environmentally or genetically ?

In *L. sitkana*, it has so far been claimed that the growth of shell sculpture depends mainly on environmental factors. Boulding *et al.* (1993) concluded that the variation in shell sculpture was determined by growth rate on the basis of experimental analyses of individuals from three populations on the Pacific coast of North America; shells tended to become smooth-surfaced when reared at a low density, in which a richer food supply presumably resulted in faster shell growth. Reid (1996) also attributed the predominance of the smooth-shell form in the northwestern Pacific populations with high densities, including southeastern Hokkaido ones, to a high growth rate due to a rich food supply. He regarded the high densities as a result of a large food supply.

However, it seems difficult to deduce that geographic variation of shell sculpture in the present study resulted solely from different growth rates among populations. This is because Type B was consistently dominant on the coasts of both the Sea of Japan and the Sea of Okhotsk in spite of large differences in population density between both these regions (Fig. 1). Air and water temperatures are also different between both coasts. For example, the sea-surface temperature around Shakotan on the Sea of Japan side is roughly 5°C warmer throughout the year than that of Abashiri on the Sea of Okhotsk coast; especially, the mean sea-surface temperature in February is about 6°C around the former while around the latter, covered with ice-floes in winter, drops nearly to 0°C (National Astronomical Observatory, 1996).

On the other hand, Ohgaki (1983) explained the conchological variation of *L. sitkana* in Hokkaido in terms of heat dissipation by sculptured shell, as such that, shells grow sculptured at warmer regions such as the Sea of Japan side of Hokkaido, as well as the Pacific coasts of southwest Hokkaido and the Tohoku District while shells become smooth at cooler regions such as the east coast of Hokkaido. However, Ohgaki's hypothesis cannot be applied to the difference in predominant shell type between neighbor populations such as Utoro-A (Type B) and -B (Type C) with similar air and water temperatures.

Alternatively, there is a possibility that the geographic variation in shell sculpture was genetically determined, and the differentiation among populations was caused by genetic drift or the founder effect, since the conchological variation appeared to be correlated with the genetic variation to some extent in the present study (Fig. 2). For instance, Shakotan and Abashiri mentioned above are proved to belong to the same geographic group based on the genetic similarity. And the founder effect and genetic drift are highly possible to occur in sedentary animals without pelagic stages like *L. sitkana* (Janson K and Ward RD, 1984; Jones *et al.*, 1977). Moreover, it is known in some snails that shell form is genetically controlled although this is not yet confirmed in *L. sitkana* (Boulding and Hay, 1993; Jones *et al.*, 1977; Newkirk and Doyle, 1975).

However, these considerations do not mean that the growth of shell sculpture has nothing to do with environmental factors, such as a food supply and habitat (air/sea surface) temperature. These and some unknown factors, interacting with one another complicatedly, may play a role in the shell-surface morphology. Further studies are required to conclude decisively how the growth of shell sculpture is determined by environmental and genetic factors in *L. sitkana*.

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