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論　文（Original article）

Preliminary results for genetic variation and populational differentiation of Japanese alder (*Alnus japonica*), a pioneering tree species in the Kushiro Marshland, Hokkaido

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Abstract

In recent years, an *Alnus japonica* (Japanese alder) forest has been expanding into the Kushiro Marshland, bringing conspicuous changes to its ecosystem and landscape. To prevent these rapid changes in wetland ecosystems, we sought to clarify the genetic variation and structure of 23 populations for *A. japonica* using isozyme analysis, which might provide basic information for estimating the distribution expansion mechanism of *A. japonica* in the Kushiro Marshland. Our results show significantly high expected heterozygosity (*He*) for populations in upstream regions than in downstream regions. Other genetic parameters show no significant differences, such as the mean number of alleles per locus (*Na*), effective number of alleles per locus (*Ne*), observed heterozygosity (*Ho*), and the coefficient that measures excesses of homozygotes relative to panmictic expectations within respective populations (F_{IS}). The F_{ST} value is high (0.183), indicating differences in allele frequencies among populations. Significant clinal differences between populations in the upstream and downstream regions might be partly attributable to (i) the founder effect, a fluctuation of genetic diversity during foundation, or (ii) natural selection to certain alleles to/against different environments. The high F_{ST} value among populations might be partly attributable to the founder effect as a pioneer species and one with rapid expansion during establishment. This result might relate to the characteristic to the pioneering tree species, which establishes suitable sites by chance.

Key words : founder effect, isozyme, Japanese alder (*Alnus japonica*), the Kushiro Marshland

Introduction

The Japanese alder (*Alnus japonica*) is a deciduous broad-leaved tree species that grows in wet lowlands such as open swamps and fallow fields with wet and open environments. The tree reaches a maximum diameter of 60 cm and 20 m height. This species is distributed widely from Ussuri in China, to Japan, Korea, and Taiwan. The growth form of the *A. japonica* tree changes according to local environmental conditions: it has a tall single trunk in fertile lands, but it is shrubby with high levels of groundwater (Fujita, 2002; Nakamura et al., 2002). *Alnus japonica* is wind pollinated and has wind-dispersal seeds (Suzuki, 1975); it is an intolerant tree species (Koike, 1991). Therefore, *A. japonica* might be a pioneer tree species.

In the last 50 years, the expanded distribution of *A. japonica* has made them increasingly common in the Kushiro Marshland. Fens that had been covered with Japanese reeds (*Phragmites communis*) are changing to swamp forests of *A. japonica*. Moreover, *A. japonica* is invading into the core of the marshland, where it had only grown at the edges before. Rapid change of the Kushiro Marshland landscape might affect its ecosystem. It is necessary to prevent the rapid expansion of *A. japonica* forest. Edaphic dryness of the marshland is inferred to be a major factor for the expanded distribution of *A. japonica* forest. Nakamura et al. (2002) reported that housing and farmland development causes soil and sand inflow to the marshland, which engenders edaphic dryness.

Genetic variation and structure of populations might provide basic information for clarifying the distribution expansion process of present *A. japonica* establishment sites. For that reason, we studied genetic variation and structure of *A. japonica* populations over the Kushiro Marshland using to obtain basic information for controlling the rapid expansion of *A. japonica*.

Materials and Methods

Study site

We sampled 23 populations of *A. japonica* in the Kushiro Marshland, eastern Hokkaido (Fig. 1; Table 2). All populations are located in wetlands of spring-water swamps in foot hills, creek floodplains, and marshland edges. The populations are often mixed with ash (*Fraxinus mandshurica*) and elm (*Ulmus japonica*).

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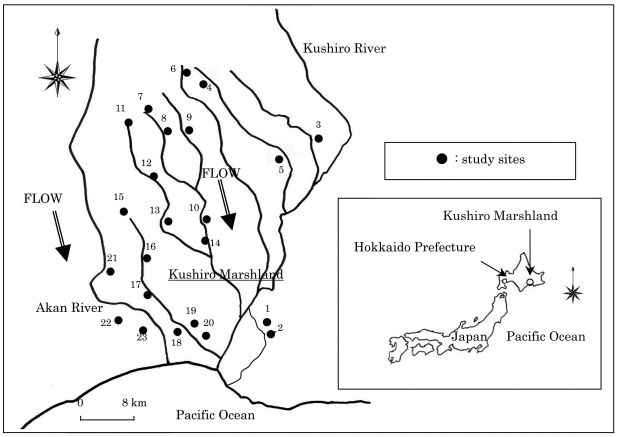

Fig. 1. Study sites for *Alnus japonica* in the Kushiro Marshland. Numbers indicate respective populations. See Table 2.

Enzyme electrophoresis

Ten plants were collected randomly from each population. Leaves were collected from each tree during June–September 2003 and were stored at -30°C until enzymes were extracted. Isozyme analyses were carried out according to Shiraishi (1988). First, 100 µg of leaf tissue was homogenized with liquid nitrogen and 1.0 mL of extract buffer with 80 mg polyvinylpolypyrrolidone. Of the resulting supernatant, 17 µL was loaded on a polyacrylamide vertical slab gel after centrifugation (20000 *g*, 20 min). Electrophoresis was conducted at 2°C, 10 mA/cm^2 for 150 min. Gels were stained for the following 12 enzyme systems: alcohol dehydrogenase (ADH; EC 1.1.1.1), amylase (AMY; EC 3.2.1.1), diaphorase (DIA; EC 1.6.4.3), glutamate dehydrogenase (GDH; EC 1.4.1.2), glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), leucine aminopeptidase (LAP; EC 3.4.11.1), malate dehydrogenase (MDH; EC 1.1.1.37), 6-phosphogluconate dehydrogenase (6 PGD; EC 1.1.1.44), phosphoglucoisomerase (PGI; EC 5.3.1.9), phosphoglucomutase (PGM; EC 2.7.5.1), and shikimate dehydrogenase (ShDH; EC 1.1.1.25) (Table 1).

Table 1. List of enzyme systems employed.

Enzyme	E.C. No.	No. of loci scored
ADH	1.1.1.1	-
AMY	3.2.1.1	2
DIA	1.6.4.3	1
GDH	1.4.1.2	2
GOT	2.6.1.1	-
G6P	1.1.1.49	1
LAP	3.4.11.1	1
MDH	1.1.1.37	1
6PGD	1.1.1.44	-
PGI	5.3.1.9	1
PGM	2.7.5.1	-
ShDH	1.1.1.25	1

- indicates that the locus was not included for scoring because of the lack of enzyme activity in certain individuals tested or because of smeared banding patterns.

Data analysis

Phenotypes were interpreted into genotypes, and allele frequencies of respective loci were calculated. The following genetic parameters were calculated for all populations, the mean number of alleles per locus (*N*a), the effective number of alleles per locus (*N*e), observed heterozygosity (*H*o), and expected heterozygosity (*H*e) using the POPGENE computer program (Yeh et al., 1997) for loci with at least two alleles.

The genetic structure within and among populations was estimated using Wright's (1965) *F*-statistics: a coefficient represents excesses of homozygotes relative to panmictic expectations within respective populations (F_{IS}); another coefficient measures excesses of homozygotes relative to panmictic expectations over all populations (F_{IT}); and another coefficient estimates relative population differentiation (F_{ST}).

We compared genetic parameters (*N*a, *N*e, *H*o, *H*e, F_{IS}) for populations according to the stream length from the river mouth to respective populations (parametric test – Pearson correlation coefficient; non-parametric test – Kendall's rank correlation coefficient); tests of significance were also conducted (Student's t-test).

Results

Genetic diversity

This study scored 16 loci from 12 enzyme systems. All 16 studied loci showed detectable polymorphisms. No locus showed monomorphism. Among these loci, we used 10 loci (*Amy-1, Amy-2, Dia, Gdh-1, Gdh-2, G6p, Lap, Mdh, Pgi, Shdh*) with a clear banding pattern for further analyses (Table 1).

The mean number of alleles per locus (*N*a) was 2.81 within populations, ranging 2.00–3.20. The effective number of alleles per locus (*N*e) was 2.13 within populations, ranging 1.65–2.53. The observed heterozygosity (*H*o) was 0.421 within populations, ranging 0.233–0.567. The expected heterozygosity (*He*) was 0.513 within populations, ranging 0.285–0.588 (Table 2). Most populations show high genetic variations (e.g. *Shdh* locus (Fig. 3)).

Most populations show that the expected heterozygosity was higher than the observed heterozygosity, suggesting an excess of homozygotes. The homozygote excess is also reflected in a population mean of 0.106 for Wright's F_{IS} (Table 3); Wright's F_{IT} value was 0.270 (Table 3).

We also analyzed relationships between respective genetic parameters for each population and the stream length from the river mouth to each population. Significant correlation was found between the expected heterozygosity (*He*) and the stream length from the river mouth (parametric test – r =0.439, $p < 0.05$;

Table 2. Calculated genetic parameters for 23 populations for *A. japonica*. Standard deviations are shown in parentheses. Sample size (*N*), mean number of alleles per locus (*N*a), effective number of alleles per locus (*N*e), observed heterozygosity (*H*o), expected heterozygosity (*H*e) and a coefficient measures excesses of homozygotes relative to panmictic expectations within respective populations (F_{IS}).

No.	River	Population	Stream length(km)*	*N*	*Na*	*Ne*	*Ho*	*He*	F_{IS}
1	Kushiro	Toya1	9.50	10	3.00	2.19	0.494(0.158)	0.534(0.152)	0.073
2		Toya2	7.37	10	3.20	2.53	0.546(0.223)	0.575(0.185)	0.051
3	Osobetsu	Oso1	53.64	10	3.00	2.32	0.459(0.199)	0.571(0.126)	0.195
4	Numaoro	Numa1	54.79	10	3.20	2.45	0.494(0.153)	0.565(0.184)	0.126
5		Numa2	38.49	10	2.90	2.13	0.437(0.241)	0.516(0.156)	0.153
6	Kuchoro	Kucho	48.02	10	2.80	1.99	0.415(0.305)	0.530(0.234)	0.217
7	Setsuri	Setsu1	45.45	10	3.00	2.35	0.418(0.251)	0.545(0.200)	0.232
8		M_setsu	43.10	10	2.70	1.97	0.409(0.288)	0.526(0.263)	0.222
9		S_setsu	42.43	10	2.44	2.01	0.330(0.248)	0.502(0.235)	0.344
10		Setsu2	23.26	10	2.70	1.87	0.416(0.213)	0.483(0.111)	0.140
11	Hororo	Horo2	47.63	10	3.10	2.23	0.567(0.256)	0.542(0.167)	-0.047
12		Horo1	38.19	10	3.00	2.26	0.493(0.237)	0.588(0.067)	0.163
13		N_horo	27.66	10	2.56	1.94	0.418(0.234)	0.477(0.218)	0.124
14		S_horo	22.00	10	3.00	2.09	0.289(0.259)	0.498(0.205)	0.420
15	Ninishibetsu	Nini	29.35	10	2.90	2.25	0.567(0.231)	0.556(0.165)	-0.020
16		M_nini	21.59	10	2.78	2.05	0.303(0.234)	0.488(0.210)	0.380
17		D_nini	15.65	10	2.70	2.11	0.406(0.325)	0.479(0.249)	0.153
18		L_nini	8.65	10	2.00	1.65	0.233(0.251)	0.285(0.301)	0.183
19		N_nini	6.52	10	2.60	2.07	0.378(0.263)	0.516(0.196)	0.266
20		K_nini	5.07	10	2.90	2.07	0.304(0.215)	0.487(0.210)	0.376
21	Akan	Aka1	14.98	10	2.90	2.38	0.427(0.293)	0.553(0.213)	0.229
22		Tsu_aka	12.83	10	2.30	1.93	0.433(0.296)	0.457(0.233)	0.053
23		Aka2	27.07	10	3.00	2.20	0.449(0.202)	0.525(0.170)	0.146
	means				2.81	2.13	0.421(0.087)	0.513(0.061)	0.182
	Species				-	-	-	0.463(0.138)	-

* Stream length is the distance (km) from the river mouth to the population along the river.

Table 3. A coefficient measures excesses of homozygotes relative to panmictic expectations within respective populations (F_{IS}) and all over populations (F_{IT}), and relative population differentiation (F_{ST}) for *A. japonica*.

Locus	F_{IS}	F_{IT}	F_{ST}
Amy-1	0.049	0.400	0.369
Amy-2	0.007	0.126	0.120
Dia	-0.183	-0.099	0.071
G6p-1	0.041	0.317	0.288
Gdh-1	0.592	0.678	0.210
Gdh-2	0.253	0.435	0.244
Lap	-0.016	0.119	0.133
Mdh	-0.146	-0.060	0.076
Pgi	0.034	0.193	0.165
Shdh	0.255	0.330	0.102
Mean	0.106	0.270	0.183

non-parametric test – $r = 0.341$, $p < 0.05$) was observed (Fig. 2). The correlation was still significant in the non-parametric test except for the No. 18 population, with the lowest *He* value (parametric test – $r = 0.409$; not significant; non-parametric test – $r = 0.304$, $p < 0.05$) (Fig. 2). No other significant correlation was observed between respective genetic parameters (*N*a, *N*e, *H*o and F_{IS}) and the stream length from the river mouth to the respective populations (Table 4).

Most loci show disparity in allele frequencies among populations (e.g. *G6p* locus (Fig. 3)). Wright's F_{ST} value was 0.183, indicating that about 18% of total allozyme variation was obtained among populations (Table 3).

Discussion

Alnus japonica in the Kushiro Marshland had a higher level of genetic variation (*N*a = 2.81, *N*e = 2.13, *H*e = 0.513) than general long-lived woody species (*N*a = 2.22, *N*e = 1.24, *H*e = 0.177; Hamrick et al., 1992). The expected heterozygosity (*H*e) value is higher than that among the Korean populations (0.235; Huh, 1999) and *A. trabeculosa* (0.222; Miyamoto, 2004). Hamrick et al. (1992) reported that widespread species have high genetic variation. The distribution of *A. japonica* is more widespread than *A. trabeculosa*. Therefore, *Alnus japonica* in the Kushiro Marshland had a higher level of genetic variation than that of *A. trabeculosa*. Moreover, the difference of genetic variation between the Korean population and populations in the Kushiro Marshland might be related to their respective distribution histories.

Significant correlation was found between the *H*e values and the stream length from the river mouth. The *H*e values

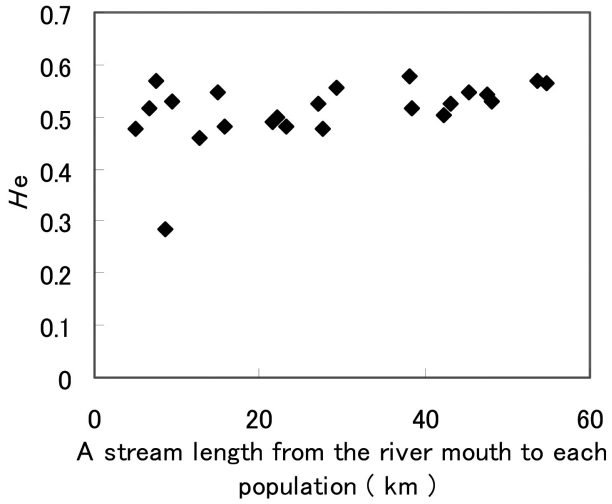

Fig. 2. Relationships between expected heterozygosity (*He*) and stream lengths from the river mouth to each population.
Significant correlation between expected *He* values and the stream length from the river mouth (parametric test: r =0.439, $p < 0.05$, non-parametric test: $r = 0.341$, $p < 0.05$) was observed. Significant correlation was observed in the non-parametric test even if it calculated except No. 18 population in which the lowest value was shown (parametric test: r =0.409, not significant, non-parametric test: $r = 0.304$, $p < 0.05$).

Table 4. Relationships between genetic parameters and stream lengths from the river mouth for all populations of *A. japonica*.

・Parametric tests

	*N*a	*N*e	*H*o	*H*e	F_{IS}
A stream length from the river mouth (km)	0.328	0.251	0.318	0.439*	-0.096

* $P < 0.05$

・Non-parametric tests

	*N*a	*N*e	*H*o	*H*e	F_{IS}
A stream length from the river mouth (km)	0.233	0.182	0.223	0.341*	-0.055

* $P < 0.05$

for populations in the upstream region were higher than those in the downstream region. This difference suggests that the genetic parameter value differs spatially between the upstream to downstream regions, thereby indicating that a certain genetic structure must exist along the streamline.

According to sea-level fluctuation recorded by the Kushiro city compilation secretariat (1988), the upstream region (mountainous and hilly regions) has probably remained stable for a long time. The downstream region was created after the glacial period. This report indicates an accumulation of genetic diversity for populations in the upstream region. However, populations in the downstream region must not have sufficient time to maintain or attain substantial genetic diversity because they were established later than in the upstream region.

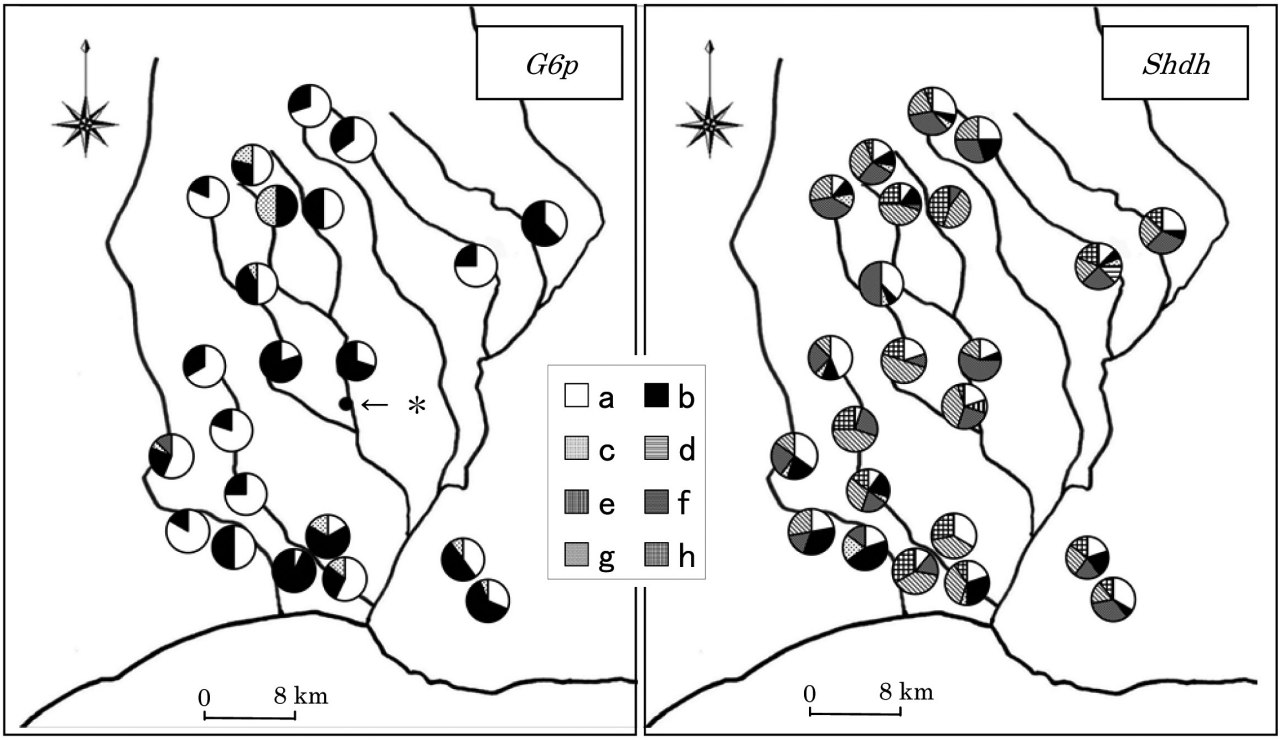

Fig. 3. Allele frequency of *Alnus japonica* for *G6p* locus and *Shdh* locus.
* *G6p* locus for this population had no clear banding pattern.

Environmental factors might have caused natural selection. Generally speaking, allozyme has been considered as neutral to selection. However, some studies suggest that remarkable natural selection occurred along environment gradients in several enzymes (Kitamura and Kawano, 1996; Prentice et al., 2000). At study sites, we assume that the ground water level is high and fluctuates in the downstream region. Lower *H*e values for populations in the downstream region might be associated with these severe environmental factors because certain allozyme variations might be influenced by natural selection in the harsh environment.

Remarkable differences in allele frequencies among 23 populations in the Kushiro Marshland were observed in this study (Fig. 3). We collected ten plants from respective populations in this research, fewer than similar reports. Therefore, the F_{ST} value might be overestimated. Consequently, the F_{ST} value is comparatively high (mean F_{ST} value =0.183, Table 3) compared to the former study of Korean populations (mean G_{ST} value = 0.095, Huh, 1999). Miyamoto (2004) also reported a high G_{ST} value (mean G_{ST} value = 0.146) among *A. trabeculosa* populations as a pioneer species in an unstable establishment site.

Alnus japonica is an intolerant tree species (Koike, 1991) that establishes on sites with disturbance and low plant coverage (Fujita, 2002). Therefore, *A. japonica* expands its establishment sites in early successional stages in the Kushiro Marshland. Practically, these suitable establishment sites for *A.japonica* (ecological disturbance and/or gap formation) occur by chance and small spatial scales, which bring in a small founder population during the establishment process. The population expanded rapidly after foundation. Because of intolerant tree species, very little recruitment for individuals with new genetic variations exists under the mature alder trees. Several studies have suggested that genetic variation in one species is influenced by evolutionary history, especially the population size history (Hamrick & Godt, 1989; Hamrick, et al., 1992). Our result of significant difference of genetic components and high F_{ST} value must reflect a strong founder effect in the course of population history of this species (Miyamoto et al., 2001).

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北海道釧路湿原における先駆性木本種ハンノキ (*Alnus japonica*) を対象とした遺伝的変異と集団分化に関する予報

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要 旨

近年、釧路湿原でハンノキ林が分布拡大してきたと言われており、生態系や景観への影響が危惧されている。湿原生態系の急激な変化を防ぐためには、ハンノキの分布拡大プロセスを明らかにすることは急務である。本研究においては、ハンノキの分布拡大プロセスの基礎データを得るために、釧路湿原のハンノキ 23 集団の遺伝的変異と遺伝構造をアイソザイム分析で明らかにすることを目的とした。分析及び解析の結果、釧路湿原一帯においては下流集団よりも上流集団のヘテロ接合体率期待値の方が高い値を示していた。しかし他の遺伝的パラメータ（*Na*、*Ne*、*Ho*、F_{IS}）についての顕著な傾向は認められなかった。また集団分化の程度を表す F_{ST} は比較的大きな値（F_{ST} =0.183）を示しており、集団間の対立遺伝子頻度の違いが示唆された。これらの結果から、下流集団と上流集団との間のヘテロ接合体率期待値のクラインは、1）集団成立初期の創始者効果と浮動、または 2）立地環境の相違による自然選択、の 2 点によって形成される可能性が示唆された。また F_{ST} 値が比較的大きいことは創始者効果が原因である可能性について示唆された。このことは好適生育サイトの発生が偶発的である先駆性木本種としての特性と関連があるものと考えられる。

キーワード：アイソザイム、釧路湿原、創始者効果、ハンノキ（*Alnus japonica*）

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