

短 報 (Note)

Microsatellite polymorphism of Siebold's beech (*Fagus crenata* Blume) at five National Forest Reserves in the Oshima Peninsula, southern Hokkaido.

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Abstract

Siebold's beech (*Fagus crenata*) is a dominant tree species of cool temperate forest ecosystems in southern Hokkaido, and is one of the most important timber species in the Oshima Peninsula. Several beech forests have been designated as forest reserves in the Oshima Peninsula, however the genetic diversity of these forest reserves has not yet been evaluated. We evaluated the genetic diversity of Siebold's beech using 12 microsatellite loci in five National Forest Reserves in southern Hokkaido: Utsai Forest Reserve (UT), Karibayama Genetic Resource Forest Reserve (KR), Okushiri Island Forest Reserve (OK), Chirichiri River Forest Reserve (CR), and Gartner Forest Reserve (GT). Expected heterozygosity (H_e) of populations in the Oshima Peninsula ranged from 0.749 in OK to 0.782 in KR. Allelic richness (R_s) based on 47 diploid individuals ranged from 12.6 in GT to 14.4 in KR. The fixation index (F_{is}) showed significant positive values from Hardy-Weinberg expectations at CR and UT ($p < 0.01$). The F_{is} of UT and CR deviated significantly and positively from Hardy-Weinberg expectations. This suggests that the influence of small population size affected the F_{is} value since the reserved areas of UT and CR are the smallest natural beech forest reserves. The forests at KR and OK had relatively large numbers of unique alleles compared with several other beech forests in the Oshima Peninsula, and the *Fs1-03* and *Fs4-46* loci in OK had the highest values among the five forests. However, the *mfc12* locus at OK had a low heterozygosity compared to other natural beech forests in the Oshima Peninsula. The forest at OK had unique genetic characteristic from other forests of the Oshima Peninsula while OK is an island population.

Key words : *Fagus crenata*, SSR, northern limit, National Forest Reserve, genetic diversity

Introduction

Siebold's beech (*Fagus crenata* Blume) is a widely distributed canopy tree species in cool temperate forests of Japan. The beech is also a typical dominant tree species of the forest ecosystem in the mountain zone within the Oshima Peninsula in Hokkaido, where it reaches its northern distribution limit at the Kuromatsunai Depression (Tatewaki, 1958). The beech forests in the Oshima Peninsula are regarded as valuable forest ecosystems at the northern distribution limit of the species because of their conservation and ecological importance, although the stand volume of beech forest has decreased through cutting during expansive afforestation programs in the past several decades (e.g., Tsunemoto, 2008). Beech forest management has now been implemented after the expansive afforestation period in the 1970s, under a policy of sustainable maintenance and expansion of broadleaf resources in the Oshima Peninsula and in Japan (e.g., Nakashizuka, 2004; Tsunemoto, 2008). Several studies on beech forest management, such as prediction of mast years

for seeds (e.g., Yasaka et al., 2001; Koyama et al., 2007), long-term storage of seeds (e.g., Koyama et al., 1997), timing and effectiveness of surface scarification (e.g., Koyama et al., 2000; Tsunemoto, 2008), and growth and survival of planted beech trees (Nagasaka et al., 2006), have been reported for this major forestry species in the Oshima Peninsula.

Several beech forests have been designated as forest reserves in the Oshima Peninsula (Hokkaido Forest Office, 2000); however, the genetic diversity of these forest reserves has not yet been evaluated. Information on genetic diversity of forest reserves provides important background knowledge for forest management; such information includes the genetic diversity of mother trees and basic information for avoiding genetic pollution in natural and artificial regeneration projects. In this report, we evaluated the beech genetic diversity using microsatellite markers in five National Forest Reserves in the Oshima Peninsula.

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Materials and Methods

The five National Forest Reserves in the Oshima Peninsula in Hokkaido (Fig.1) were designated as study sites. A description of each study site is shown in Table 1.

Leaves and winter buds were collected randomly from canopy trees in each study site in 2007. We analyzed 12 microsatellite (SSR) loci (Tanaka et al.,1999; Pastorelli et al.,2003; Asuka et al.,2004a) according to Numano et al. (2005).

The genetic parameters of expected heterozygosity (H_e ; Nei, 1987), allelic richness (R_s ; Mousadik & Petit, 1996), the fixation index (F_{is} ; Wright, 1965), and population differentiation was estimated by F -statistics (F_{st} ; Weir & Cockerham, 1984) using the computer program FSAT version 2.9 (Goudet, 2002) for each population. Since GT is an artificial beech forest, we excluded GT from calculation of F_{st} , simply to evaluate the genetic differentiation between natural beech forests.

We also compared genetic diversity in beech population using SSR between the Hokkaido populations of this study and the Honshu populations (Tanaka et al.,1999; Takahashi et al.,2003; Asuka et al.,2004b; Numano et al.,2005) and overall distributional range (Asuka et al.,2004a) from previous studies. The locations of Honshu populations are Mt. Kurikoma, Miyagi Prefecture (KK), Mt. Takahara, Tochigi Prefecture (TK1 and TK2) and Mt. Daisen, Tottori Prefecture (DS). Overall distributional range (JP) indicates average of 17 beech forests including Hokkaido populations.

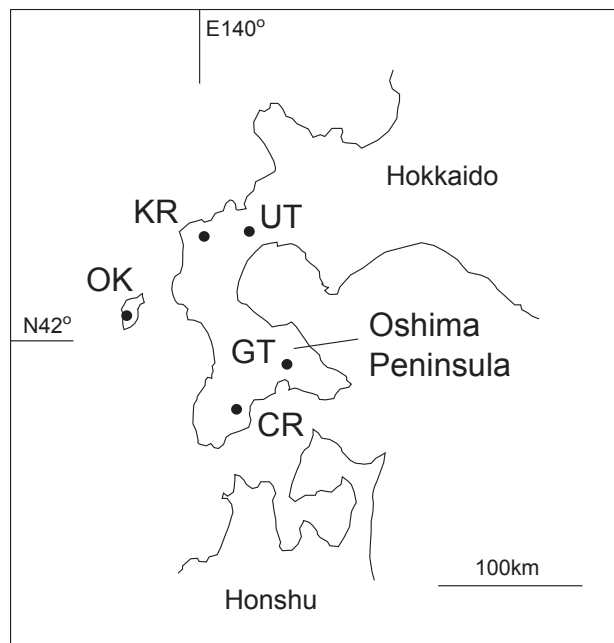


Fig. 1. Locations of the beech forest reserves in the Oshima Peninsula.

UT: Utasai Forest Reserve. KR: Karibayama Genetic Resource Forest Reserve. OK: Okushiri Island Forest Reserve.

GT: Gartner Forest Reserve. CR: Chirichiri River Forest Reserve.

Results and Discussion

There were no fixed loci among the 12 SSR loci analyzed. In total, 163 alleles were found among 12 loci. Calculated genetic parameters (H_e , R_s and F_{is}) for each study site are shown in Table 2. Expected heterozygosity (H_e) for each population in the Oshima Peninsula ranged from 0.749 in OK to 0.782 in KR. The overall estimate of H_e in the five forests was 0.778. Allelic richness (R_s) based on 47 diploid individuals ranged from 12.6 in GT to 14.4 in KR. The fixation index (F_{is}) showed significantly positive values from Hardy-Weinberg expectations at CR and UT ($p < 0.01$). The overall estimate of F_{is} in the five forests was 0.051, and deviated significantly from Hardy-Weinberg expectations ($p < 0.01$).

The numbers of unique alleles, which were only found in a single population, are listed in Table 3. The largest number of unique alleles was 15, found in KR. In particular, for *Fs4-46*, five unique alleles were found at this study site. Twelve unique alleles were found in OK, but only five unique alleles were found in CR, the smallest number among five study sites.

The H_e for each locus in each population is shown in Table 4. Three study sites, CR, OK, and KR, had the highest values at two loci (CR; *mfc12* and *sfc0018*, OK; *Fs1-03* and *Fs4-46*, KR; *mfc12* and *sfc1143*) in the five forests. GT had a high H_e only at locus *sfc0007*. Our results using SSR showed that the values of H_e in the Oshima Peninsula were slightly lower than those found in other studies in Honshu populations (Table 4). We also compared the average values of H_e in six common loci (*sfc0018*, *sfc0036*, *sfc0378*, *sfc1063*, *sfc1105* and *sfc1143*) between Hokkaido and Honshu population (KK), except for TK1, TK2 and DS, and overall distributional range (JP). This values showed lower values in Hokkaido populations (UT=0.853, KR=0.814, OK=0.809, CR=0.839, GT=0.809 and Overall in Hokkaido=0.825) than Honshu population (KK=0.852) and overall distributional range (JP=0.863).

The average H_e for each locus in the Oshima Peninsula was lower than that in Honshu populations, other than *mfc2* in TK2. In particular, H_e in *mfc12* was lower than the Honshu populations. In this locus, the smallest and the largest values were observed in OK and KR, respectively.

The F_{st} among four natural beech forests except for GT was 0.011. This indicated small population differentiation according to Hartl & Clark (1997). Tomaru et al. (1998) investigated mitochondrial (mt) DNA variation in 17 beech populations distributed throughout the species' range, and showed northeastern populations including Hokkaido was low differentiation in phylogenetic tree. The close relationship among the northeastern populations in mtDNA variation was also evident in allozyme variation (Takahashi et al.,1994; Tomaru et al.,1997). The value of F_{st} in the Oshima Peninsula also supported the results of these previous studies.

In general, populations on islands tend to have a lower level of genetic diversity than continental populations (Frankham, 1997). The *He* in OK was 0.749, which was the smallest among the five populations. However, OK maintained more unique alleles than the other beech populations in Oshima Peninsula, where *Fs1-03* and *Fs4-46* had the highest *He* values among the five forests. This suggests that beech forest in Okushiri Island currently retains conventional genetic variation. In particular, this forest has different genetic characteristics from those of beech forests in the Oshima Peninsula. We also observed lower genetic polymorphism in *mfc12* in this area, which can be attributed to the effect of genetic drift through isolation by the sea. It may be also explained linkage with adaptive gene for island habitat.

The KR forest had the highest level of genetic diversity among the five forests, and the largest number of unique alleles, while the fixation index (*Fis*) was not significantly different from Hardy-Weinberg expectations. Beech is estimated to have reached this area earlier than the other surrounding beech forests (Kito, 2003). This may imply that certain stretches of old beech forest have existed for 3000 years. This longevity of the stable forest may have resulted in retention of genetic polymorphism.

The GT has been reported to be formed by wilding from neighboring region by Gartner in 1870, and this forest is one of the oldest artificial beech forest in Japan. The *Rs* in GT was

12.6, which was the smallest level of *Rs* among the five forests. Although GT is only artificial forest among five forests, *He* was comparable with other forests (Table 2). This result might suggest that beginning of this forest originates from the seedlings with a high genetic diversity from many mother trees.

The *Fis* of UT and CR had significant positive deviation from Hardy-Weinberg Equilibrium. These positive values may have resulted from inbreeding and/or genetic substructuring (Wright, 1969). Beech is an anemophilous and obligate out-crossing species in which the effects of inbreeding are rare among mature trees. The result might be associated with the effect of the small population size and, therefore, by limitations in mating, since the population sizes of UT and CR are smaller than the other study forests. Although the *Fis* values of UT and CR are positively significant, the *He* was generally the same as other study sites. If mating limitation occurs repeatedly in these forests, then genetic variation will make them vulnerable in the future. Our results suggest that by expanding the size of the forest reserve could secure and sustain sound genetic variation in UT and CR.

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Table 1. Description of five study sites.

	Site				
	UT	KR	OK	CR	GT
Name	Utasai Forest Reserve	Karibayama Genetic Resource Forest Reserve	Okushiri Island Forest Reserve	Chirichiri River Forest Reserve	Gartner Forest Reserve
Location	Kuromatsunai-cho, Shiribeshi	Shimamaki-mura, Shiribeshi	Okushiri-cho, Hiyama	Shiriuchi-cho, Hiyama	Nanae-cho, Oshima
Area (ha)	92.43	154.32	258.68	12.68	0.38
Altitude (m)	40-160	480-780	320-560	140-220	30
Forest type	Natural	Natural	Natural	Natural	Artificial

Table 2. The number of samples and the three genetic parameters at each study site.

	UT	KR	OK	CR	GT	Overall
<i>N</i>	52	51	47	50	48	248
<i>He</i>	0.776	0.782	0.749	0.775	0.765	0.778
<i>Rs</i>	13.4	14.4	12.8	13.5	12.6	-
<i>Fis</i>	0.056**	0.022	0.024	0.084**	0.013	0.051**

N: The number of samples. *He*: Expected heterozygosity. *Rs*: Allelic richness. *Fis*: Wright's fixation index, ** $p < 0.01$.

Table 3. The number of unique alleles for each locus at each study site.

Locus	Site				
	UT	KR	OK	CR	GT
<i>Fs1-03</i>	1				1
<i>Fs4-46</i>		5	1		
<i>mfc12</i>		1	1		1
<i>mfc2</i>	2	2	1	3	1
<i>sfc0007</i>		2	1		
<i>sfc0018</i>			1		1
<i>sfc0036</i>		1	1		1
<i>sfc0161</i>	2	2	3	1	
<i>sfc0378</i>		1			
<i>sfc1063</i>			1		1
<i>sfc1105</i>	1		1	1	
<i>sfc1143</i>	2	1	1		
Total	8	15	12	5	6

These loci were developed by Tanaka et al. (1999) (*mfc2* and *mfc12*), Pastorelli et al. (2003) (*Fs1-03* and *Fs4-46*) and Asuka et al. (2004a) (*sfc0007*, *sfc0018*, *sfc0036*, *sfc0161*, *sfc0378*, *sfc1063*, *sfc1105* and *sfc1143*).

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Table 4. Comparison of heterogeneity (H_e) for each locus in Hokkaido and Honshu.

Locus	Populations in Hokkaido						Populations in Honshu				Overall distributional range
	UT	KR	OK	CR	GT	average	KK	TK1	TK2	DS	JP
<i>Fs1-03</i>	0.815	0.804	0.828	0.788	0.807	0.808	0.832	-	-	-	-
<i>Fs4-46</i>	0.658	0.801	0.803	0.797	0.744	0.761	0.763	-	-	-	-
<i>mfc12</i>	0.233	0.517	0.163	0.323	0.452	0.338	-	0.784	0.799	-	-
<i>mfc2</i>	0.937	0.952	0.932	0.957	0.921	0.940	-	0.962	0.925	0.951	-
<i>sfc0007</i>	0.592	0.474	0.472	0.468	0.488	0.499	-	-	-	-	-
<i>sfc0018</i>	0.788	0.744	0.752	0.845	0.802	0.786	0.799	-	-	-	0.80
<i>sfc0036</i>	0.908	0.867	0.903	0.856	0.891	0.885	0.904	-	-	-	0.91
<i>sfc0161</i>	0.960	0.954	0.934	0.934	0.919	0.940	-	-	-	-	0.98
<i>sfc0378</i>	0.873	0.866	0.821	0.840	0.834	0.847	0.887	-	-	-	0.89
<i>sfc1063</i>	0.876	0.854	0.778	0.861	0.861	0.846	0.856	-	-	-	0.86
<i>sfc1105</i>	0.809	0.685	0.732	0.766	0.634	0.725	0.770	-	-	-	0.79
<i>sfc1143</i>	0.866	0.870	0.868	0.863	0.832	0.860	0.894	-	-	-	0.93

KK: Mt. Kurikoma, Miyagi Prefecture (Numano et al., 2005). TK1, TK2: Mt. Takahara, Tochigi Prefecture (Takahashi et al., 2003; Tanaka et al., 1999). DS: Mt. Daisen, Tottori Prefecture (Asuka et al., 2004b). JP: Average for 17 beech forests in Japan (Asuka et al., 2004a).

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北海道渡島半島の国有林におけるブナ保護林のマイクロサテライト多型

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要 旨

北海道渡島半島国有林内のブナ保護林（歌オブナ保護林（UT）、狩場山ブナ遺伝子保護林（KR）、奥尻島ブナ保護林（OK）、チリチリ川ブナ保護林（CR）、ガルトネルブナ保護林（GT））5 地点において、マイクロサテライトマーカー（SSR）を用いた遺伝的多様性の評価を行った。ヘテロ接合度の期待値（ H_e ）およびアレリックリッチネス（ R_s ）は KR で最も高い値を示した。近交係数（ F_{is} ）は全ての集団で正の値を示し、UT と CR でハーディ・ワインバーグ平衡から正に有意にずれていた（ $p < 0.01$ ）。また、その集団のみで出現した固有対立遺伝子数は CR や UT では 5、および 8 個だったのに対し、KR では 15 個、OK では 12 個が観察された。SSR を使用した既存研究との比較から、北海道のブナ集団の保有する遺伝的多様性（ H_e ）は、本州集団と比べ同程度あるいは若干低い傾向が見られた。しかしながら、最も北限に近い UT や島嶼性集団である OK においても、遺伝的多様性の極端な低下は見られなかった。また、OK は多くの固有対立遺伝子を保有し、渡島半島のブナ林とは異なる遺伝的特徴を有する固有性の高いブナ林であることが明らかになった。また、UT や CR は他のブナ天然林に比べ集団サイズが小さいことにより、繁殖機会における交配の制限が起こっている可能性が示唆された。

キーワード：ブナ、マイクロサテライト、遺伝的多様性、国有林、分布北限域

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