

ミズナラとミヤマナラのアロザイム変異

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**Allozyme Variation in *Quercus crispula* var.
crispula and var. *horikawae***

谷本晋一郎*・井上 健**・柴田 治* :
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Abstract

Allozyme variation in *Quercus crispula* var. *crispula* (*crispula*) and var. *horikawae* (*horikawae*) was investigated to clarify the degree of genetic differentiation between the two varieties and the amount of genetic diversity within populations. Although Nei's genetic distance between the two varieties was only 0.011, sound differentiation among populations ($G_{ST}=0.039$) was observed. This result indicates the existence of both genetic differentiation and gene flow between *crispula* and *horikawae*, suggesting that *horikawae* populations are monophyletic origin. Genetic diversities within populations are relatively high (ranging from 0.16 to 0.24), and at the similar order of other anemophilous tree species.

Key Words : Allozyme variation — Genetic differentiation — *Quercus crispula* — Var. *horikawae*

Enzyme electrophoresis is a useful tool to study the genetic variation within a population or the genetic differentiation among populations or species (HAMRICK *et al.*, 1979; LOVELESS and HAMRICK, 1984; CRAWFORD, 1985), and has been applied to over 700 plant species (HAMRICK and GODT, 1989). Here, we apply allozyme technique to clarify the relationship between *Quercus crispula* var. *crispula* and *Q. crispula* var. *horikawae*.

Quercus crispula Blume is a common species in broad-leaved deciduous forests in cool temperate Japan. Although typical plants of *Q. crispula* var. *crispula* (hereafter abbreviated as *crispula*) are tall trees, a race named as var. *horikawae* H. Ohba (abbreviated as *horikawae*) is distinct in its shrubby habit and hairy leaves. It grows in subalpine dwarf forests developed under heavy snow fall of the Japan Sea side, and NOSHIRO (1984) concluded the both types are morphologically distinct and are considered to be varieties. If NOSHIRO's view is correct, it is expected that genetic distance between *crispula* and *horikawae* is relatively large. His view is,

however, based on solely morphological observations from one mountain region. There are another possibility that *horikawae* is an ecotype adapted to heavy-snow environment and has a polyphyletic taxa which has originated more than once at different mountains under the similar selection pressures. To determine which of these alternative views is correct, we examined allozyme variation between two varieties. The result is reported here with additional evidence on variations of two varieties within a population.

Materials and Methods

Winter buds used for electrophoresis were collected from the populations of Mt. Makihata and Mt. Chokai. Collection data are presented in Table 1.

Polyacrylamide gel electrophoresis was used according to the method of TSUMURA *et al.* (1990). The following twelve enzymes were investigated: aconitase (ACO), glutamate dehydrogenase (GDH), glutamate-oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehy-

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Table 1. Collection data and number of plants analyzed (in parentheses) for population samples of *Quercus crispula*.

variety/ population	Collection data
<i>crispula</i>	
CR-MA	Niigata Pref., Mt. Makihata, alt. 700-1100 m (32).
CR-CH	Akita Pref., Mt. Chokai, alt. 450-750 m (32).
<i>horikawae</i>	
HO-MA	Niigata Pref., Mt. Makihata, alt. 1400-1750 m (48).
HO-CH	Akita Pref., Mt. Chokai, alt. 1100-1300 m (57).

drogenase (MDH), malic enzyme (ME), menadion reductase (MNR), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucose mutase (PGM), phosphoglucose isomerase (PGI), and shikimate dehydrogenase (SDH).

Genetic interpretations of banding patterns were made based on the localization and subunit structure of the enzymes (GOTTLIEB, 1982). Genetic variations within one population are described by the four parameters: the proportion of polymorphic loci (P), the mean number of alleles per locus (k), mean observed heterozygosity (H_o), and Hardy-Weinberg expected heterozygosity (H_E). Apportionment of genetic variation was calculated by the formula by NEI (1973), $H_T = H_S + D_{ST}$, where H_T is total expected heterozygosity, H_S is mean expected heterozygosity over populations, and D_{ST} is that between populations. Differentiation among populations was measured by $G_{ST} = D_{ST}/H_T$. NEI's (1972) genetic identity and distance indices were used as measures of genetic similarity or difference between populations or varieties. Computer programs BIOSYS-1 (SWOFFORD and SELANDER, 1981) were used for the calculation of genetic diversity within populations and NEI's genetic distance.

Results

Of the twelve enzymes investigated, five enzymes (IDH, ME, MNR, PGM, SDH) were omitted from the analysis because of poor resolution or inconsistent activity. Allele frequency of seven putative loci from the remaining seven enzymes are presented in Table 2. All of the major alleles of seven loci are in common between

Table 2. Allele frequencies for the four populations of *Quercus crispula*

Locus	Allele	variety/population				
		<i>crispula</i>		<i>horikawae</i>		
		CR-MA	CR-CH	HO-MA	HO-CH	
Aco	a	0.984	0.906	0.979	0.904	
	b	0.016	0.078	0.021	0.018	
	c	0.000	0.016	0.000	0.079	
Gdh	a	1.000	1.000	1.000	1.000	
	Got	a	0.016	0.032	0.000	0.000
	b	0.813	0.790	0.823	0.807	
	c	0.172	0.177	0.177	0.193	
	Lap	a	0.031	0.016	0.000	0.079
	b	0.625	0.609	0.813	0.702	
	c	0.328	0.250	0.125	0.053	
	d	0.016	0.125	0.052	0.096	
	e	0.000	0.000	0.010	0.000	
	Mdh	a	0.016	0.000	0.000	0.000
	b	0.984	1.000	0.979	1.000	
	c	0.000	0.000	0.021	0.000	
	6Pgd	a	0.016	0.016	0.011	0.000
	b	0.984	0.984	0.989	0.991	
	c	0.000	0.000	0.000	0.009	
	Pgi	a	0.063	0.141	0.177	0.289
	b	0.859	0.719	0.781	0.482	
	c	0.078	0.109	0.042	0.158	
	d	0.000	0.031	0.000	0.070	

the two varieties.

Indices of genetic diversity within populations are summarized in Table 3. Proportions of polymorphic loci (P) are 57.1% at the populations of Mt. Chokai and 42.9% at those of Mt. Makihata, and the mean numbers of alleles per locus (k) are at the range of 2.3 to 2.6. Expected values for mean heterozygosity in each population are at the range of 0.155-0.237, and observed values are at the range of 0.143-0.234. No deviation from Hardy-Weinberg equilibria were detected.

Apportionment of gene diversity is presented in Table 4. Although almost all (96.1%) of gene diversity exists within populations in the lumped case of two varieties, the estimated value of $G_{ST} = 0.039$ is sound. This suggests there are some amount of differentiation among populations.

The unbiased estimates of NEI's genetic identity and distance values are presented in Table 5. The genetic identity values are at the range of 0.976 to 0.999, and the genetic similarities between each pair of the populations are high. Clustering

Table 3. Proportion of polymorphic loci (P), mean number of alleles per locus (k), mean observed heterozygosity (H_o), and mean expected heterozygosity (H_e).

variety/ population	P	k	H_o	H_e
<i>crispula</i>				
CR-MA	42.9	2.4	0.143	0.168
CR-CH	57.1	2.6	0.234	0.225
<i>horikawae</i>				
HO-MA	42.9	2.3	0.161	0.155
HO-CH	57.1	2.6	0.213	0.237

Table 5. Nei's genetic distance values (above diagonal), and Nei's genetic identity values (below diagonal), for pair-wise comparison of populations of *Q. crispula*.

Population	CR-MA	CR-CH	HO-MA	HO-CH
CR-MA	—	0.001	0.006	0.025
CR-CH	0.999	—	0.003	0.010
HO-MA	0.994	0.997	—	0.011
HO-CH	0.976	0.990	0.989	—

of genetic distance is represented in Fig. 1. Populations of *crispula* form a close cluster, and Mt. Chokai population of *horikawae* is somewhat differentiated from the other three populations.

Discussion

Genetic differentiation between the two varieties. It is reported that mean genetic distance among conspecific varieties is at the order of 0.05 (GOTTLIEB, 1981; CRAWFORD, 1985). Mean genetic distance value of NEI between *crispula* and *horikawae* is only 0.011, and this value indicates that *horikawae* is little genetically differentiated from *crispula*. However, this is resulted from substantial gene flow between two 'varieties'. Gene flow can be estimated as $Nm = (1/G_{ST} - 1)/4$ under the island model (cf. SLATKIN, 1985). As $G_{ST} =$ approximately 0.04 in our study, it gives $Nm = 6$. This result indicates that morphological differentiation between two 'varieties' are maintained under natural selection strong enough to purge migrating genes of tall trees through high level of gene flow.

Considering the relatively high value of gene

Table 4. Mean values for total gene diversity (H_T), gene diversity within populations (H_S), gene diversity among populations (D_{ST}), and differentiation among populations ($G_{ST} = D_{ST}/H_T$) for seven loci among populations in *Q. crispula* var. *crispula* and var. *horikawae*.

	H_T	H_S	D_{ST}	G_{ST}
<i>crispula</i>	0.196	0.193	0.003	0.015
<i>horikawae</i>	0.203	0.198	0.005	0.025
<i>Q. crispula</i>	0.204	0.196	0.008	0.039

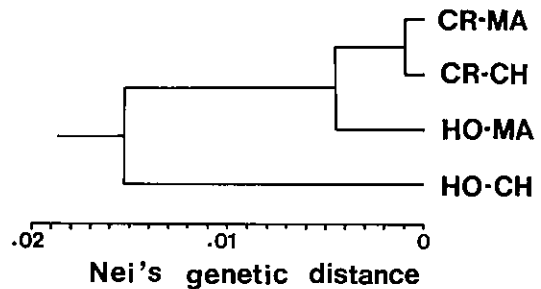


Fig. 1. Phenogram (UPGMA) of four populations of *Quercus crispula* based on NEI's genetic distance values. Genetic distance values are those presented above diagonal in Table 5.

flow among populations estimated in the above, *horikawae* seems genetically somewhat differentiated from *crispula*. Gene flows between *horikawae* and *crispula* populations at Mt. Makihata may have decreased the genetic differentiation between the two varieties. Although the obtained data suggest the monophyly of *horikawae*, more data are needed to support this conclusion.

Genetic Diversity Within and Among Populations. Japanese *Quercus crispula* populations including *horikawae* have most of the genetic diversity within each population (H_S) and populational differentiation (D_{ST}) little contribute to total diversity. This result well corresponds with those reported from other anemophilous tree species including American *Quercus* (SCHNABEL and HAMRICK, 1990). The level of genetic diversity within each population is at the similar order of other anemophilous tree species. Anemophilous allogamous tree species such as *Quercus* may tend to have the same amount of genetic diversity within populations.

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摘 要

ミズナラとミヤマナラの遺伝的な近縁度、ミズナラとミヤマナラ集団内の遺伝的多様性の程度の推定をアロザイムを用いて行った。ミズナラとミヤマナラを合わせた集団全体にはかなりの遺伝的分化が認められたが、ミヤマナラとミズナラの根井の遺伝距離は0.011と小さかった。これらの事は、ミヤマナラの集団はミズナラの集団からある程度の遺伝子の流入を受け、酵素レベルでの分化が阻害されていることを示している。ミヤマナラは単系統であることが一応支持されたが、最終的な結論には、より多くのデータが必要である。

ミズナラとミヤマナラの集団内の遺伝的多様性(平均ヘテロ接合体頻度)は0.16から0.24と比較的高く、他の風媒の樹種の報告と同程度であった。

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○ 堀田 満・井上民二・小山直樹(編) 赤道直下, 森と火山の島 スマトラの自然と人々 八坂書房(〒101 東京都千代田区神田神保町1-56), 1992年3月25日発行。B5判。175頁。定価4,800円。

これは、1980~1989年の10年間に及ぶ編者らを中心としたスマトラの自然研究の成果の一般向けの解説書である。本書は、1, スマトラ自然研究, 2, 森と植物達, 3, 多彩な昆虫達の生活, 4, サル達の暮らし, 5, 創り出された環境の4章から成り、巻末には文献解題・術語解説・写真と図のデータがある。本文中に随所にカラー写真や白黒写真がはさまれていて理解を助けてくれる

植物関係は 第2章 堀田満氏の「まだ知られないスマトラの植物達」と甲山隆司氏の「動いているスマトラの森」の記事がある。スマトラといえばすぐ思い出すのはラフレシアだが、第2章はラフレシア物語から始まり、サトイモ科の新属フルタドア発見のいきさつ、スマトラに多い溪流型植物の話、葉が1生に1枚しかでない石灰岩植物のモノフィレア、ツリフネソウ属15種の生育地や交配様式の観察など、読み易い文章で語られている。海外学術の成果としてもユニークな試みといえよう。(清水建美)