半数体苔類,ジャゴケ自然集団における遺伝的多型と地理的変異

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The amount of polymorphism and genetic differentiation in natural populations of the haploid liverwort *Conocephalum conicum*

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ABSTRACT

Natural populations of diploid organisms are expected to be more polymorphic than haploid organisms under most selection models. To test this prediction and to obtain data concerning the amount of allozyme polymorphism in a haploid plant, I examined two natural populations of the haploid liverwort *Conocephalum conicum* by starch gel electrophoresis. Abundant genic variability was found within local populations in Japan: 7 of 11 loci. Average heterozygosity of these 11 loci was 0.167. This value is similar to those obtained for various kinds of diploid organisms. Gene identity (I) between the two populations was 0.994, and another measure of genetic differentiation, G_{st} was 0.018, indicating virtually no genetic differentiation between the populations. High levels of genic variability and low levels of genetic differentiation in these haploid plants are consistent with a hypothesis of selective neutrality of allozyme polymorphisms, although other possible explanations cannot be excluded.

1. INTRODUCTION

Large amounts of genic polymorphism are present in natural populations of various diploid organisms. How these genic polymorphisms are maintained has been the subject of much debate (Lewontin and Hubby 1966; Kimura 1968; Yamazaki and Maruyama 1972; Ayala *et al.* 1974; and Mukai *et al.* 1980).

Several approaches have been used to gather evidence concerning these issues: theoretical or mathematical approaches (*e.g.*, Kimura 1969), direct observation of natural selection in the laboratory using population cages or specific mating schemes (*e.g.*, Yamazaki 1971), and descriptive analysis of natural populations (*e.g.*, Ayala *et al.* 1974; Clegg and Allard 1972).

Several experimental studies have been thus far conducted on the genic polymorphisms of haploid microorganisms (Milkman 1973; Spieth 1975). These studies generally show no obvious differences between haploids and diploids. The life cycle of these microorganisms, however, is very different from higher organisms. Occasionally they escape selection by making spores. Heterokaryon, which is similar to the diploid in a sense, occupy a fairly large

proportion of life cycle. Sometimes gene migration exceeds beyond the normal taxonomic concept of species.

On the other hand *Conocephalum conicum*, a liverwort, is a eukaryotic plant, and spends most of its life history as a haploid. Thus far, only a few data are available about the genic variability of liverworts (Szweykowski and Krzakowa 1979; Krzakowa and Szweykowski 1979; Szweykowski *et al.* 1981; Yamazaki 1981).

In this study I examined 11 enzyme loci of two natural populations of *Conocephalum conicum*. The amount of heterozygosity and the degree of genetic differentiation between the populations were compared with those of diploid organisms in relation to the maintenance mechanisms of these genetic variabilities.

2. MATERIALS AND METHODS

The haploid liverwort *Conocephalum conicum* [In the previous report (Yamazaki 1981) the word "moss" was used incorrectly] was collected from two populations, Yakiyama and Kawabaru, near Fukuoka in the southern part of Japan from 1978 to 1981. These populations are about 40 km apart in different mountain ranges. A population here is defined as all liverworts growing along a single valley or a stream. All the collections within a population were done at sites less than 1 km apart.

Conocephalum conicum usually grows in small patches of less than 1 m^2, but occasionally forms much larger carpets along some mountain streams. Only one sample was collected from each continuous carpet to minimize the possibility of sampling the same clone more than once. A single individual was selected for electrophoresis of all 11 loci. Allelic frequency data were obtained from six enzyme systems (11 gene loci). Enzyme loci examined in this report include *Peroxidase-1*, *Peroxidase-2*, *Esterase-1*, *Esterase-2*, *Esterase-3*, *Esterase-4*, *Esterase-5*, *Malate dehydrogenase*, *Isocitrate dehydrogenase*, *Glutamate oxaloacetate transaminase*, and *Tetrazolium oxidase*. The difference between allelic and non-allelic isozymes was judged only from the examination of stained gels without genetic crosses. Details of electrophoretic methods and part of the data from the Yakiyama population has already been reported (Yamazaki 1981).

Heterozygosity was calculated as Het. $=1-\Sigma X_i^2$, where X_i is the frequency of ith allele at a locus. This value is the expected heterozygosity obtained when two haploid gametes unite at random. This "heterozygosity" is the same as "gene diversity" in Nei (1973).

Gene identity (I) between two populations (X, Y), was calculated by the formula $I=\Sigma X_i Y_i/\sqrt{\Sigma X_i^2 \Sigma Y_i^2}$, where X_i and Y_i are the frequency of the ith allele in X, Y populations, respectively (Nei 1972). Another measure of

genetic differentiation between populations, G_{st} (Nei 1973), was also measured. G_{st} is given by the formula $G_{st}=D_{st}/H_t$, where D_{st} is the average gene diversity between populations, including comparisons within the populations. H_t is the gene diversity in the total population. G_{st} fluctuates between 0 and 1 depending on the degree of differentiation between populations.

3. RESULTS AND DISCUSSION

Table 1 summarizes allelic frequencies and heterozygosity at 11 gene loci examined in the two natural populations from Yakiyama and Kawabaru. Also shown in Table 1 is the extent of genetic differentiation between the two populations measured by "I" and "G_{st}".

Four loci (*Mdh, Po-2, Est-1, Est-2*) out of 11 enzyme loci were monomorphic (freq. of most common allele >0.95). The other 7 loci displayed higher levels of polymorphisms. Among them, *Idh, To,* and *Got* were truly polymorphic in the sense that they had more than two different alleles. The average heterozygosities of the 11 loci examined were 0.154 at Kawabaru and 0.180 at Yakiyama. The average of the two populations was 0.167. This value is not different in magnitude from those of other diploid organisms; average heterozygosity was between 0.05–0.17 in the data for many different organisms (Lewontin 1973). Incidentally, the value we obtained using the same technique in *Drosophila melanogaster* was 0.170 (Yamazaki *et al.* 1984).

If some form of balancing selection, such as overdominance, frequency dependent selection or diversifying selection (spatial or temporal), is operating in natural populations, diploidy itself is likely to play an important role in the maintenance of these polymorphisms. Overdominance has been considered to be very effective in the maintenance of genetic variability. Under models of frequency dependent selection or diversifying selection heterozygous advantage in the strict sense is not always necessary for the maintenance of genetic variability in a population (Gillespie and Langley 1974). Under any balancing selection model, if there is a certain amount of dominance genetic variability is maintained rather easily in a population. On the other hand, diploidy itself does not play any role in the maintenance of neutral alleles in a population. Under an infinite allele model of neutral genes, the amount of heterozygosity is expected to be $4Nu/(4Nu+1)$, where N and u are population size and mutation rate, respectively (Kimura and Crow 1964). The amount of heterozygosity is the same under the assumption of neutrality if the product of population size and mutation rate is the same, regardless of the ploidy level of the organisms.

Conocephalum conicum, a liverwort, spends most of its life history as a haploid plant. Therefore, there can be no overdominance or dominance effects. Mutations should show their effects immediately after their occur-

Table 1. *Allozyme frequencies and genetic differentiation in two natural populations (Kawabaru, and Yakiyama) of Conocephalum conicum*

Locus	Allele	Kawabaru			Yakiyama		
		Sample size	Freq.	Hetero-zygosity	Sample size	Freq.	Hetero-zygosity
Isocitrate	0.99		0.040			0.020	
dehydrogenase	1.00	101	0.911	0.167	98	0.959	0.080
(Idh)	1.01		0.040			0.020	
	1.08		0.010			0	
Malate	0.92		0.010			0	
dehydrogenase	1.00	101	0.970	0.059	98	1.00	0
(Mdh)	1.05		0.010			0	
	Null		0.010			0	
Tetrazolium	0.89		0			0.010	
oxidase	0.93		0			0.010	
(To)	0.99		0.040			0.020	
	1.00	101	0.950	0.096	100	0.950	0.097
	1.01		0			0.010	
	1.00/1.10		0.010			0	
Peroxidase-1	0.86		0.010			0	
(Po-1)	0.90		0.010			0	
	1.00		0.941			0.851	
	1.30	101	0.040	0.113	94	0.011	0.262
	1.00/1.30		0			0.021	
	Null		0			0.117	
Peroxidase-2	0.97		0.030			0	
(Po-2)	1.00	101	0.960	0.077	77	1.00	0
	1.04		0.010			0	
Glutamate	0.98		0.030			0.010	
oxaloacetate	1.00		0.455			0.500	
transaminase	1.10		0.495			0.398	
(Got)	1.23	100	0	0.547	98	0.071	0.586
	1.00/1.10		0			0.010	
	Null		0.010			0.010	

(to be continued)

Table 1. (*Continued*)

Locus	Allele	Kawabaru			Yakiyama		
		Sample size	Freq.	Hetero-zygosity	Sample size	Freq.	Hetero-zygosity
Esterase-1 (Est-1)	1.00	101	0.990	0.020	88	1.00	0
	1.10		0.010			0	
Esterase-2 (Est-2)	0.98	101	0.010	0.059	88	0	0
	1.00		0.970			1.00	
	1.09		0.020			0	
Esterase-3 (Est-3)	1.00	101	0.980	0.039	88	0.920	0.147
	1.01		0.020			0.080	
Esterase-4 (Est-4)	1.00	101	0.990	0.020	88	0.795	0.326
	Null		0.010			0.205	
Esterase-5 (Est-5)	1.00	100	0.550	0.495	88	0.602	0.479
	Null		0.450			0.398	

Average heterozygosity: Kawabaru=0.154
Yakiyama=0.180
Genetic differentiation between the populations: I=0.995
G_{st}=0.018

rence, since they cannot be sheltered in the haploid. Most selection models would predict smaller amounts of genetic variability in haploid than diploid organisms. If, however, this genetic variability is neutral with respect to fitness, we would predict no differences between haploids and diploids.

No genetic differentiation was observed between the populations; Gene identity (I) was 0.994. Another measurement of genetic differentiation between populations, G_{st}, was 0.018, so that only 2 percent of total heterozygosity or gene diversity is attributable to the gene differences between populations.

Populations of *Conocephalum conicum* in Poland seem to show a different pattern, with clear differentiation between populations (Szweykowki and Krzakowa 1979; Szweykowski *et al.* 1981). Moreover, allele frequencies of several enzyme loci seem to be very different between these two studies. In this study *Po* and *Got* were very polymorphic, though little polymorphisms were found in any of 21 populations examined in their studies. It is difficult to say what is the real cause of the difference between these two studies. One possibility for the difference may be that the ecology of these two populations (European and Japanese) is quite different, or that these two populations may be biologically different species though they think the same species were studied in both studies. Little genetic differentiation of allozyme polymorphisms was generally observed between populations in many diploid organisms. (*e.g.* Prakash *et al.* 1969; Ayala *et al.* 1974; Yamazaki *et al.* 1984).

Theory predicts that genetic differentiation should increase as selection intensity increases (Yamazaki and Watanabe 1977). On the other hand, if allozyme polymorphisms are neutral with respect to fitness, genetic differentiation between populations should not increase so rapidly (Kimura and Maruyama 1971).

This study demonstrates that the amount of genic polymorphism as well as the degree of genetic differentiation between populations in a haploid plant is not different from those of diploid organisms. These two findings are difficult to explain on the basis of balancing selection such as overdominance. The interpretation of the data, of course, is not so simple, because both the amount of polymorphism and the extent of genetic differentiation depend on many factors such as selection pattern, population size, migration, mutation, and historical events. There is one more factor which makes drawing conclusions a little more complicated: the basic chromosome number of liverworts in general is 4 or 5 according to Schuster (1966), while the chromosome number of *Conocephalum conicum* is: $n=9$ (Showalter 1921). This seems to indicate that *Conocephalum conicum* is an ancient polyploid. This polyploidy, however, may not be a problem since only three individuals were apparently heterozygous among more than 2000 individuals examined (Table 1). Perhaps sufficient time has passed after polyploidization so that this plant can be considered haploid in the sense that two doses of the same genes do not exist in its genome.

We must obtain more information about these parameters of this liverwort before reaching any definite conclusions about the mechanisms of maintaining these polymorphisms. Nevertheless, considering all these factors, the assumption of the neutrality of allozyme polymorphisms is at present the simplest and easiest interpretation of the data.

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