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Genetic Diversity and Population Structure of *Dioscorea tokoro* Makino, a Dioecious Climber

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Abstract *Dioscorea tokoro* Makino is a herbaceous climber species widespread in East Asia. Genetic structure of a natural population of *D. tokoro* was examined employing starch gel electrophoresis of allozymes. Genotypes of seven loci were studied for 1,128 individuals. Twenty-six populations located mainly in the Kinki district of Japan were subgrouped into four large clusters by the geographical distribution of alleles. The *D. tokoro* population was revealed to contain greater total genetic diversity (H_T=0.282) and higher intrapopulational genetic diversity (H_S=0.258) than other outcrossing species for which data are available. On the other hand, interpopulational differentiation (G_{ST}=0.096) was smaller than in other outcrossers. For the heterozygosity deficiency observed (F_{IT}=0.125), population subdivision (F_{ST}=0.096) and inbreeding within the population (F_{IS}=0.067) were revealed to contribute to the same extent. From these *F*-statistics, the migration rate among subpopulations and the rate of between-relative matings were estimated. Overall results on the genetic structure of the *D. tokoro* population indicated a high gene flow among its subpopulations, and this may be the consequence of its life form as a climber and its habitat in a disturbed environment. During the study, the geographical cline of *Pgi* allele frequencies was observed. This finding was supposed to be the result of the selection imposed on *Pgi* by the temperature differences between localities.

Key words: *Dioscorea tokoro*, allozyme, population genetic structure, dioecy, vine, *Pgi* cline.

Vine species elongate their stems by climbing trees of various species. At the same time, favorable light conditions are required for their initial growth to attain the height of the canopy. These two ecological constraints limit the habitat of vine species to the gaps and fringes of forests. Reflecting this peculiarity of the niche, vine populations are expected to have a unique genetic structure not found among populations of herb and tree species. But so far, genetic studies on the plant population structure have been confined to these latter species (reviewed by Hamrick et al., 1979; Loveless and Hamrick, 1984). The present study aims to assess the genetic population structure of vine species for the first time using *D. tokoro* Makino, a dioecious perennial climber, as the material.

Dioscorea is a large genus of herbaceous climbers of the family Dioscoreaceae, which is found throughout moist tropics and extending into temperate regions. Many *Dioscorea* species have come into cultivation, and they are collectively called "yams". All species are dioecious with *XY* type sex determination (Martin, 1966). *D. tokoro* is a diploid (2n=2x=20) species belonging to the section Stenophora Uline having sinistrorse (left-handed) stems, alternate leaves and horizontal rhizomes. It is distributed in Japan, Korea and Northeastern China. *D. tokoro* populations are usually found along the fringes of forests forming communities with other vining species, but they seldom occur in stable habitats inside the forest. The sex ratio of *D. tokoro* in a natural population is 1.16 : 1.00 with a slight excess of males (Terauchi, 1990). The dispersal of *D. tokoro* depends exclusively on seeds.

Materials and Methods

1. *Plant Materials*

Plant materials of 1,128 individuals from 26 populations of Japan were examined (Fig. 1), 24 populations located in the Kinki District, one in Nagano Prefecture, and one in Fukuoka Prefecture. Samples collected consisted of a single leaf

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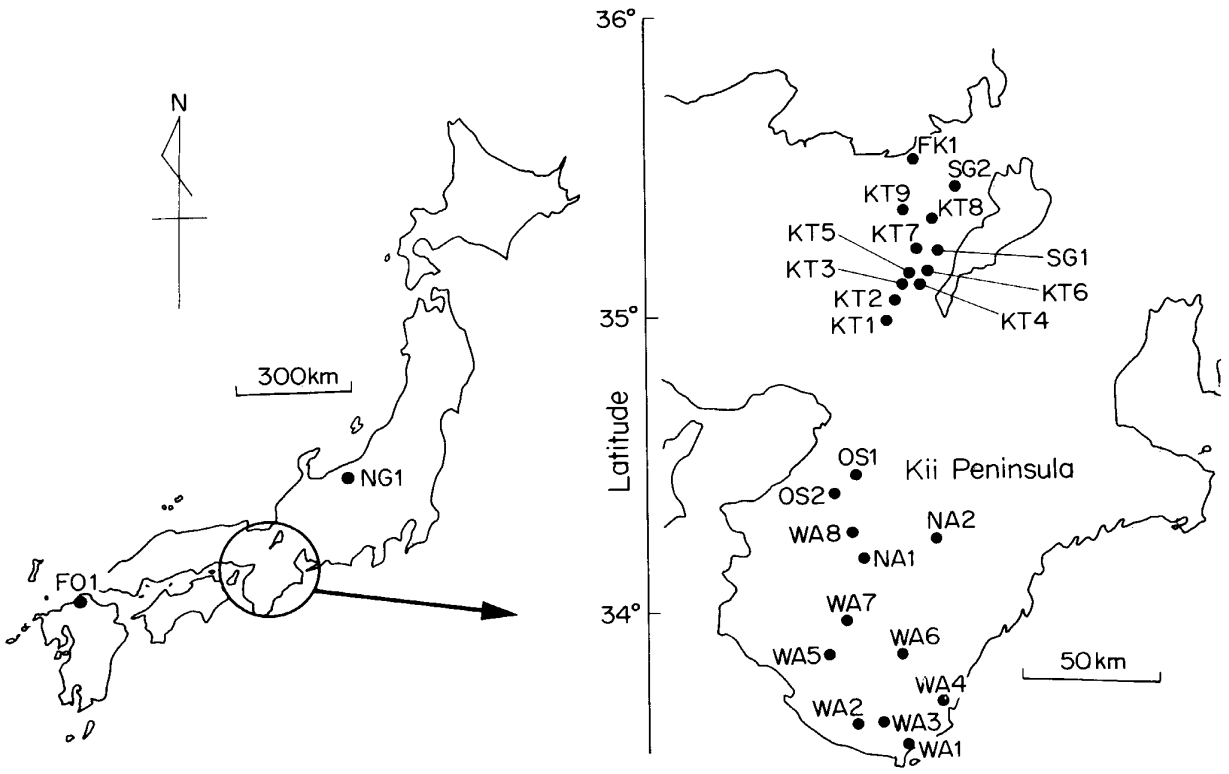

Fig. 1. Localities of 26 populations of *D. tokoro* examined in the present study.

from each matured individual which either actually bore inflorescence or was judged to have attained the dimension able to florify. The sample size of each population ranged from 10 to 209, with a mean of 43.4. Leaves were brought back to Kyoto and stored under refrigeration at 5°C until the experiment.

2. *Electrophoresis*

Little enzyme activity was detected by standard extraction and electrophoretic techniques due to a high concentration of polyphenolic substances in *D. tokoro*; therefore, the extraction buffer of Yeh and O'Mally (1980) (13.5 mM Tris, 4.3 mM citric acid, 0.75 mM NAD, 0.15 mM NADP, 1.0 mM ascorbic acid, 1.0 mM EDTA, 1.0 mM $MgCl_2$, 1.4 M mercaptoethanol, 0.1% BSA w/v, 10% PVPP w/v and 10% sucrose w/v; pH 7.4) was adopted. About 200 mg of leaf tissue was ground in a plastic reaction board with a ground glass rod in 0.1 ml of the extraction buffer. The resulting slurry was absorbed into a filter paper strip, which was inserted into the starch gel for electrophoresis. Horizontal starch gel was prepared following the standard method as described by Shield et al. (1983). Six enzyme systems were assayed—esterase (EST), glutamate-oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6PGDH), phospho glucoisomerase (PGI) and shikimate dehydrogenase (SKDH)—by the staining recipes given by Shaw and Prasad (1970). In addition, nine other enzyme systems were preliminarily surveyed (acid phosphatase, alkaline phosphatase, alcohol dehydrogenase, aminopeptidase, fumarase, hexokinase, malate dehydrogenase, malic enzyme, tetrazolium oxydase); but as banding patterns of these enzymes could not be adequately resolved, they were not included in the present study. Two buffer systems were employed for the electrophoreses depending on the enzymes analyzed—that is, 0.08 M Tris-borate buffer with EDTA (electrode buffer: Tris 9.8 g, boric acid 0.8 g, EDTA Na salt 0.6 g, H_2O 1,000 ml, pH 8.9, gel buffer: the same as electrode buffer) was used to examine EST, PGI and GOT, whereas 0.2 M Tris-citrate buffer with EDTA (electrode buffer: Tris 9.8 g, boric acid 0.8 g, EDTA Na salt 0.8 g, H_2O 1,000 ml, pH 8.0, gel buffer: H_2O 900 ml, electrode buffer 100 cc) was used to examine IDH, 6PGDH and SKDH. Electrophoresis was carried out at 150 V for approximately five hours at 2°C, until the tracking dye migrated 8 cm from the origin.

Results

1. *Electrophoretic Patterns*

Examples of electrophoretic banding patterns obtained for EST and PGI are shown in Fig. 2. EST contained two electrophoretic bands. The anodally more rapidly migrating band and the more slowly migrating one were named band **a** and band **b**, respectively. Based on the presence or absence of band **a** and band **b**, individual electrophoretic phenotypes were classified into three types: type 1 only with the band **a**, type 2 with both band **a** and **b**, and type 3 only with band **b**. In Fig. 2, PGI was revealed to contain three allelic products, bands **a**, **b** and **c**. Individual PGI phenotypes were thus classified into six types according to the presence or absence of these three bands. In the same manner, electrophoretic bands and phenotypes were named and classified for four other enzyme systems. Only one locus is assumed for each enzyme system except GOT, which was treated as containing two discrete loci, *Got1* and *Got2*.

2. *Genetic Control of Electrophoretic Patterns*

In order to ascertain the genetic control of electrophoretic patterns, genetic analysis was carried out by crossing individuals with given banding patterns for EST, GOT2, 6PGDH, PGI and SKDH. Genetic analysis of GOT1 and IDH was not possible because no parental couple with different electrophoretic patterns was available for crossing-breeding experiments. Phenotypes of F_1 progeny were observed using enzyme extracts of seedlings. To obtain seedlings, the dormancy of F_1 seeds was broken by chilling, with a one-month incubation at 5°C followed by 20 days incubation at 20°C (Okagami and Kawai, 1982). This treatment allowed only about half of the seeds to germinate. The segregation of phenotypes of progeny that resulted from the crosses of parents of given phenotypes are shown in Table 1 and Table 2. Segregation ratios were compared to the genetic models of either 1 : 1, 1 : 2 : 1 or 1 : 1 : 1 : 1 ratios when two, three and four phenotypes were observed in the progeny. The overall results clearly allow us to assign alleles to electrophoretic bands, and the genotype of an individual could be directly inferred by observing electrophoretic patterns. Nevertheless, some statistically significant deviation of the observed segregation from the expected ratios were found in crosses of EST-no. 3 (Table 1), PGI-no. 4 and PGI-no. 6 (Table 2). For *Pgi*, the deviation was always encountered in the cross using parents with allele *Pgi-c*. This observation will be discussed further below. As a result, it was revealed that two alleles were contained in four loci, *Got1*, *Got2*, *Idh* and *Skdh*, three alleles in two loci, *Est* and *6pgdh*, and four alleles in one locus, *Pgi*.

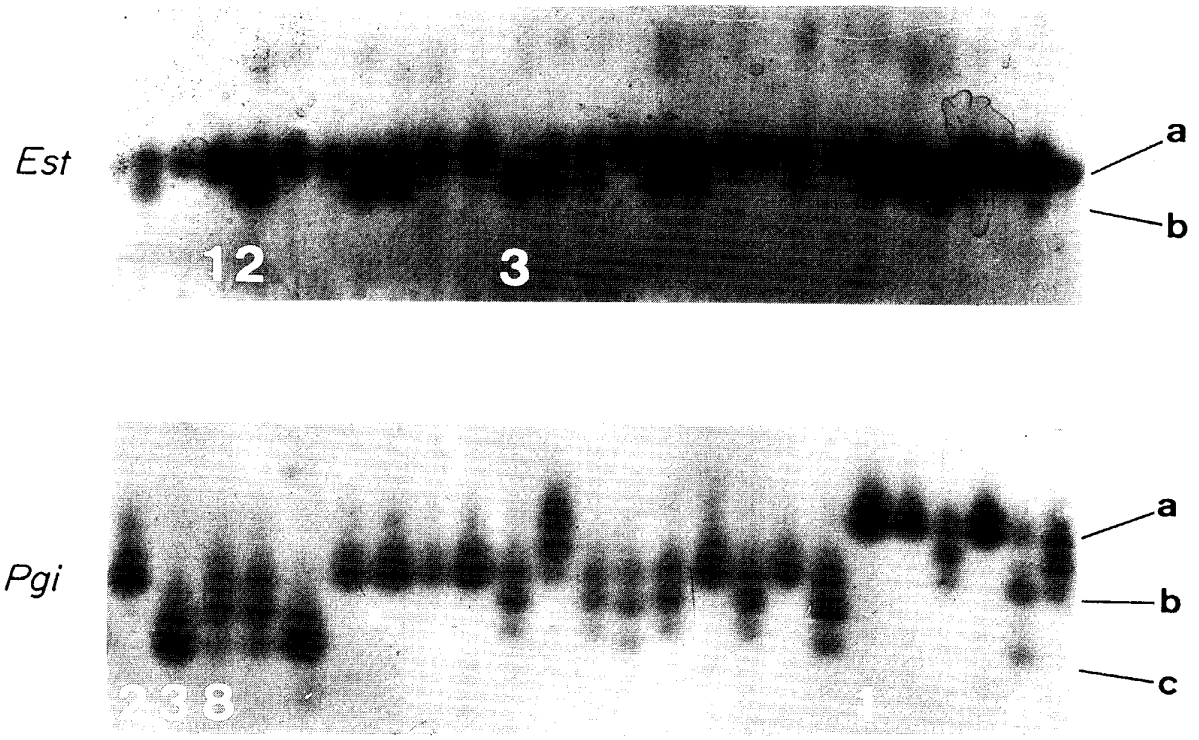

Fig. 2. Electrophoretic patterns of EST (upper) and PGI (lower) allozymes of *D. tokoro*. Alphabets and numbers indicate alleles and phenotypes, respectively.

Table 1. Segregation of progeny phenotypes resulting from the crosses between the parents of given phenotypes of EST, 6PGDH, GOT2 and SKDH.

Enzyme	Cross no.	Phenotypes of parents ♀	×	♂	Phenotypes of F_1 progeny[1] 1	2	3	Total	χ^2
EST	1	1		2	53	45		98	0.65(1 : 1)
	2	2		1	36	37		73	0.01(1 : 1)
	3	2		2	19	16	13	48	6.83(1 : 2 : 1)*
6PGDH	1	1		2	13	15		28	0.14(1 : 1)
	2	1		3		72		72	—
GOT2	1	1		2	12	15		27	0.33(1 : 1)
	2	2		3		11	7	18	0.89(1 : 1)
	3	2		1	18	20		38	0.11(1 : 1)
	4	2		2	11	23	14	48	0.46(1 : 2 : 1)
SKDH	1	1		2	7	8		15	0.07(1 : 1)

[1] Phenotypes 1=band **a**, 2=band **a**+band **b**, 3=band **b** for all loci; * Significant deviation of the observed segregation from the expected at $P<0.05$.

3. *Allele Frequencies*

Allele frequencies of seven loci examined on 26 populations are shown in Table 3. Some data are lacking because the detection of electrophoretic bandings failed as a result of the lack of enzyme activity. At the 99% criterion, all loci except *Idh* were polymorphic. Among six polymorphic loci, four loci, *Est*, *Got2*, *6pgdh* and *Pgi*, were highly polymorphic, with neither of the alleles exceeding 90%. The percentage of polymorphic loci over all populations was 85.7%. As to *Est*, in all but three populations, the frequency of allele *Est-a* was higher than that of *Est-b*. The locus *Got1* was fixed to allele *Got1-a* in all populations other than KT3, KT5 and NG1. For *Got2* locus, the allele *Got2-a* was more frequent than *Got2-b*, at 66% vs. 34%. *Idh* was a monomorphic locus, letting only two individuals possess allele *Idh-a* in one population, FK1. Of the three alleles found at the *6pgdh* locus, one, *6pgdh-a*, was predominant, and *6pgdh-c* was detected in only

Table 2. Segregation of progeny phenotypes resulting from the crosses between the parents of given phenotypes of PGI.

Cross no.	Phenotypes of parents ♀	×	♂	Phenotypes of F_1 progeny[1] 1	2	3	4	5	6	7	8	9	10	Total	χ^2
1	5		2		4			11						15	3.3(1 : 1)
2	5		5	1	5			4						10	3.6(1 : 2 : 1)
3	6		2					9			11			20	0.2(1 : 1)
4	6		3			9			32					41	12.9(1 : 1)**
5	6		5	4				7	6		3			20	2.0(1 : 1 : 1 : 1)
6	6		8			9		7	10		22			48	11.5(1 : 1 : 1 : 1)*
7	8		2		15						11			26	0.6(1 : 1)
8	8		8		12	4					12			28	5.2(1 : 1 : 2)
9	8		10			7					11	15	17	50	4.7(1 : 1 : 1 : 1)

[1] Phenotypes of 1=band **a**, 2=band **b**, 3=band **c**, 4=band **d**, 5=band **a**+band **b**, 6=band **a**+band **c**, 7=band **a**+band **d**, 8=band **b**+band **c**, 9=band **b**+band **d**, 10=band **c**+band **d**; * Significant deviation of the observed segregation from the expected at $P<0.05$; ** Significant deviation of the observed segregation from the expected at $P<0.01$.

Table 3. Allele frequencies at seven loci in *Dioscorea tokoro*.

Population	N	*Est*			*Got-1*		*Got-2*		*Idh*		*6-pgdh*			*Pgi*				*Skdh*	
		a	*b*	*c*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*c*	*a*	*b*	*c*	*d*	*a*	*b*
WA1	32	0.83	0.17	0.00	1.00	0.00	0.45	0.55	0.00	1.00	0.56	0.44	0.00	0.06	0.03	0.91	0.00	1.00	0.00
WA2	37	0.81	0.19	0.00	1.00	0.00	0.59	0.41	0.00	1.00	0.76	0.24	0.00	0.18	0.23	0.59	0.00	0.97	0.03
WA3	41	0.98	0.02	0.00	1.00	0.00	0.56	0.44	0.00	1.00	0.78	0.21	0.01	0.16	0.04	0.80	0.00	0.98	0.02
WA4	31	0.89	0.11	0.00	1.00	0.00	0.61	0.39	0.00	1.00	0.63	0.37	0.00	0.18	0.02	0.81	0.00	1.00	0.00
WA5	18	0.31	0.69	0.00	1.00	0.00	0.50	0.50	0.00	1.00	0.92	0.08	0.00	0.33	0.47	0.20	0.00	1.00	0.00
WA6	12	0.46	0.54	0.00	1.00	0.00	0.58	0.42	0.00	1.00	1.00	0.00	0.00	0.07	0.39	0.43	0.11	1.00	0.00
WA7	10	0.40	0.60	0.00	1.00	0.00	0.40	0.60	0.00	1.00	0.95	0.05	0.00	0.35	0.35	0.10	0.20	1.00	0.00
WA8	29	0.65	0.35	0.00	1.00	0.00	0.69	0.31	0.00	1.00	0.88	0.12	0.00	0.38	0.21	0.41	0.00	1.00	0.00
NA1	19	0.74	0.26	0.00	1.00	0.00	0.71	0.29	0.00	1.00	0.97	0.03	0.00	0.11	0.63	0.26	0.00	0.97	0.03
NA2	15	—	—	—	1.00	0.00	—	—	0.00	1.00	1.00	0.00	0.00	0.30	0.47	0.23	0.00	1.00	0.00
OS1	209	0.80	0.20	0.00	1.00	0.00	0.67	0.33	0.00	1.00	0.93	0.07	0.00	0.27	0.34	0.39	0.00	0.99	0.01
OS2	26	0.69	0.31	0.00	1.00	0.00	0.70	0.30	0.00	1.00	0.94	0.06	0.00	0.31	0.23	0.46	0.00	1.00	0.00
KT1	44	0.72	0.28	0.00	1.00	0.00	0.75	0.25	0.00	1.00	0.95	0.05	0.00	0.38	0.44	0.19	0.00	1.00	0.00
KT2	32	—	—	—	1.00	0.00	—	—	0.00	1.00	0.91	0.09	0.00	0.30	0.53	0.17	0.00	0.98	0.02
KT3	53	0.76	0.24	0.00	0.99	0.01	0.87	0.13	0.00	1.00	0.91	0.09	0.00	0.46	0.19	0.35	0.00	0.96	0.14
KT4	125	0.83	0.17	0.00	1.00	0.00	0.86	0.14	0.00	1.00	0.80	0.20	0.00	0.31	0.45	0.24	0.00	0.97	0.03
KT5	63	0.85	0.15	0.00	0.98	0.02	0.63	0.37	0.00	1.00	0.91	0.09	0.00	0.41	0.29	0.31	0.00	1.00	0.00
KT6	82	—	—	—	—	—	0.71	0.29	0.00	1.00	0.91	0.09	0.00	0.55	0.29	0.16	0.00	—	—
KT7	23	1.00	0.00	0.00	1.00	0.00	—	—	0.00	1.00	0.89	0.11	0.00	0.24	0.61	0.15	0.00	0.98	0.02
KT8	17	0.77	0.23	0.00	1.00	0.00	—	—	0.00	1.00	0.97	0.03	0.00	0.18	0.41	0.41	0.00	1.00	0.00
KT9	57	0.97	0.03	0.00	1.00	0.00	0.54	0.46	0.00	1.00	0.94	0.06	0.00	0.44	0.46	0.11	0.00	0.95	0.05
SG1	29	0.83	0.17	0.00	1.00	0.00	—	—	0.00	1.00	0.97	0.03	0.00	0.24	0.36	0.40	0.00	1.00	0.00
SG2	24	0.71	0.29	0.00	1.00	0.00	—	—	0.00	1.00	1.00	0.00	0.00	0.58	0.29	0.13	0.00	1.00	0.00
FK1	42	0.76	0.24	0.00	1.00	0.00	—	—	0.03	0.97	0.85	0.15	0.00	0.53	0.30	0.18	0.00	1.00	0.00
FO1	20	0.55	0.12	0.33	1.00	0.00	—	—	0.00	1.00	1.00	0.00	0.00	0.08	0.92	0.00	0.00	1.00	0.00
NG1	38	—	—	—	0.93	0.07	—	—	0.00	1.00	0.96	0.04	0.00	0.07	0.43	0.50	0.00	0.95	0.05
Total	1128	0.79	0.20	0.01	0.99	0.01	0.66	0.34	0.00	1.00	0.89	0.11	0.00	0.31	0.34	0.34	0.00	0.98	0.02

one population, WA3. In four populations located in the southern extremity of the Kii Peninsula, namely, WA1, WA2, WA3 and WA4, the frequency of *6pgdh-b* exceeded 20%. *Pgi* was a peculiar locus, accommodating four alleles. Three of these four alleles, *Pgi-a*, *Pgi-b* and *Pgi-c*, showed the same degree of abundance in most populations. The fourth allele, *Pgi-d*, was detected only in two populations located in the center of the Kii Peninsula. The locus *Skdh* showed low polymorphism, and populations with allele *Skdh-b* were dispersed all over the Kii Peninsula.

For the sake of quantifying the genetic differentiation of *D. tokoro* populations, genetic identity and genetic distance were calculated for every pair of populations after the method of Nei (1972). Because of the absence of data, one locus, *Got2*, and four populations, NA2, KT2, KT6 and NG1, were excluded from calculation of these measures. The mean genetic identity over all pairs of populations was $I=0.955$, which is very similar to that reported for other outcrossing plant species: $I=0.956$ (Gottlieb, 1981). Cluster analysis of the UPG method (Sneath and Sokal, 1973) was employed for grouping *D. tokoro* populations based on genetic similarity (Fig. 3). Four clusters were formed at the level of $D=0.04$, which was proposed by Nei (1976) as a criterion of the genetic differentiation at the subspecies level. Four clusters are represented by (1) southernmost populations consisting of WA1, WA2, WA3 and WA4, (2) populations of the central part of the Kii Peninsula, WA5, WA6 and WA7, (3) northern Kinki populations, and (4) one population from Kyushu, FO1. Populations of southernmost Kii Peninsula are located genetically far apart from other populations. The peculiarity of this cluster

Table 4. Interpopulational heterozygosities estimated for 26 populations of *Dioscorea tokoro*.

Population	N	A^*	P^{**}	H_{obs}	H_{exp}	F
WA1	32	1.7	57.1	0.344	0.368	0.063
WA2	37	1.9	71.4	0.329	0.360	0.076
WA3	41	2.0	71.4	0,185	0.219	0.271
WA4	31	1.7	57.1	0.363	0.368	0.023
WA5	18	1.7	57.1	0.324	0.434	0.214
WA6	12	1.7	42.9	0.421	0.560	0.293
WA7	10	1.9	57.1	0.400	0.463	0.124
WA8	29	1.7	57.1	0.403	0.442	0.078
NA1	19	1.9	71.4	0.211	0.290	0.180
NA2	15	1.4	20.0	0.800	0.660	−0.221
OS1	209	1.9	71.4	0.288	0.319	0.073
OS2	26	1.7	57.1	0.369	0.406	0.074
KT1	44	1.7	62.5	0.392	0.394	0.009
KT2	32	1.8	60.0	0.369	0.319	−0.094
KT3	53	2.0	85.7	0.223	0.255	0.018
KT4	125	1.9	71.4	0.290	0.343	0.137
KT5	63	1.9	71.4	0.320	0.339	0.030
KT6	82	2.0	75.0	0.402	0.387	−0.046
KT7	23	1.7	50.0	0.268	0.255	0.092
KT8	17	1.7	50.0	0.373	0.359	−0.021
KT9	57	1.9	71.4	0.277	0.249	−0.069
SG1	29	1.7	50.0	0.287	0.340	0.127
SG2	24	1.5	33.3	0.438	0.497	0.158
FK1	42	1.8	66.7	0.281	0.322	0.053
FO1	20	1.5	33.3	0.179	0.216	0.074
NG1	38	2.0	80.0	0.224	0.216	−0.041
Mean	43.3	1.8	59.7	0.230	0.258	0.109

* Mean number of alleles per average polymorphic locus; ** Percentage of loci polymorphic.

reflects its high frequencies of *Est-a*, *6pgdh-b* and *Pgi-c* alleles. Although allozyme data revealed a large genetic differentiation among these four clusters, there was actually no indication of morphological differentiation among them.

4. *Amount of Heterozygosity*

Averaging the values obtained for six polymorphic loci, estimates of intrapopulational heterozygosities are given in Table 4. In this table, mean number of alleles per average polymorphic locus (A) and the percentage of polymorphic loci (P) were also presented. The value of A ranged from 1.4 to 2.0 with the mean of 1.8, and P ranged from 20.0% to 85.7% with the mean of 59.7%. The average values of A and P known for other outcrossing plant species are 2.90 and 51%, respectively (Gottlieb, 1981). A *D. tokoro* population has a smaller value for A but a larger value for P compared with these averages. Observed heterozygosity (H_{obs}) ranged from a maximum of 0.800 at population NA2 to a minimum of 0.179 at population FO1, with the weighted mean $H_{obs}=0.230$. The maximum and minimum values of expected heterozygosity (H_{exp}) adjusted for small sample sizes (Nei, 1977) were 0.660 for population NA2 and 0.216 for populations FO1 and NG1, with the weighted mean $H_{exp}=0.258$. The fixation index (F) ranged from −0.221 (NA2) to 0.293 (WA6). No correlation was detected between the value of F and population conditions such as the population size and density, which might be presumed to affect the genetic structure of the population.

5. *Apportionment of Genetic Diversity*

Nei (1973) used the amount of heterozygosity for describing genetic diversity within and among populations. The term H_T is used in place of H_{exp} for describing genetic diversity in the total population. Total genetic diversity (H_T) is subdivided into genetic diversity within populations (H_S) and among (D_{ST}), thus, $H_T=H_S+D_{ST}$. The degree of differentiation among populations is described by $G_{ST}=D_{ST}/H_T$, where G_{ST} ranges from 0 (no genetic heterogeneity among populations) to 1.0 (populations are fixed to different alleles). The summary of genetic diversity of *D. tokoro* is given in Table 5. The *D. tokoro* population had large genetic diversity, with the mean H_T being 0.282. This value is slightly larger than the 0.251, reported as the mean H_T for 76 predominantly outcrossing species, and almost twice as large as 0.155, the H_T value previously reported for three dioecious species (Loveless and Hamrick, 1984). The mean genetic diversity within populations (H_S) was 0.258, which is far larger than the values reported for outcrossing species ($H_S=0.214$) and dioecious species ($H_S=0.121$) (Loveless and Hamrick, 1984). These data indicate that *D. tokoro* populations have much more intrapopulational diversity compared with other dioecious species. This finding is thought to reflect the fact that high frequencies are evenly shared by two or more alleles at each locus. G_{ST} of *D. tokoro* was 0.096, which indicates that about 90% of total genetic diversity found in a *D. tokoro* population is attributable to the genetic diversity among individuals within every population, and only 10% to the genetic diversity

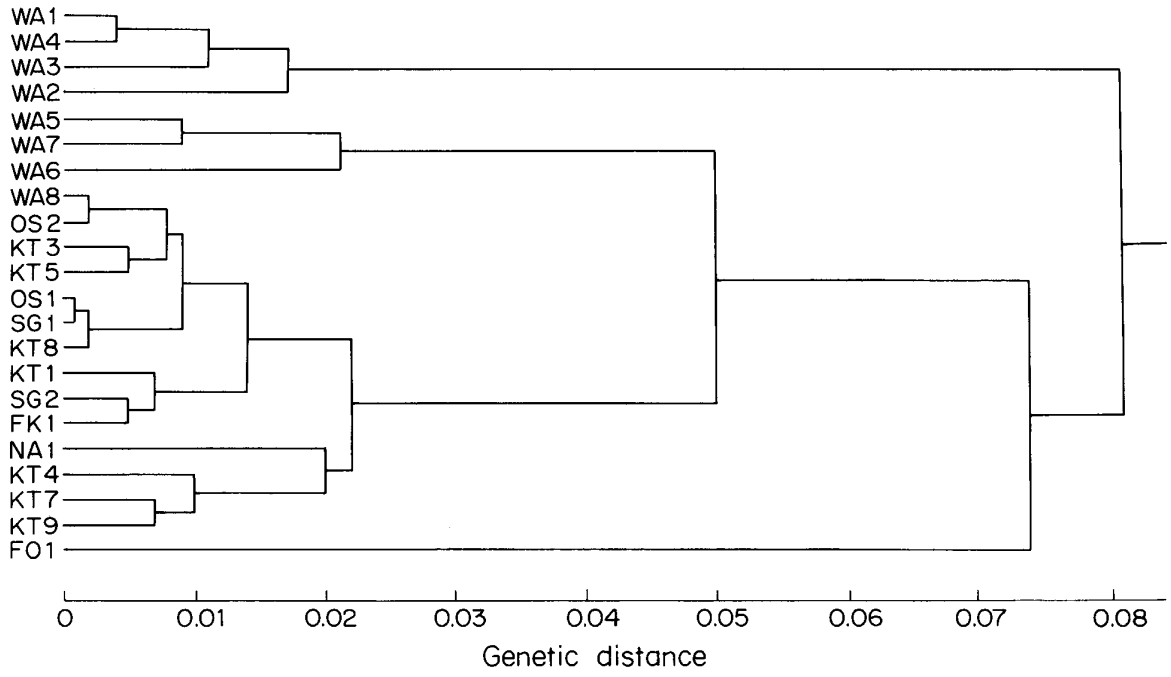

Fig. 3. Clustering of *D. tokoro* populations (UPG method) based on Nei's (1972) genetic distance between every pair of populations.

among populations. This value is smaller than 0.118, the mean of outcrossing species, and 0.109 for three dioecious species (Loveless and Hamrick, 1984).

6. *The Organization of Variation Expressed by F-statistics*

The genetic structure of a subdivided population can be analyzed by means of F-statistics developed by Wright (1951). Total expected heterozygosity is given by $H_T=1-\Sigma p_i^2$, where pi is the weighted mean of allele frequency of the i-th allele over the total population. Deviation of the amount of observed heterozygosity (H_I) from that expected (H_T) is described as $F_{IT}=(H_T-H_I)/H_T$. Division of total population into subpopulations brings about a decrease in heterozygosity even if panmixis is maintained within each subpopulation (Wahlund, 1928). The extent of decrease in heterozygosity by this effect is given by $F_{ST}=(H_T-H_S)/H_T$, where H_S is the weighted mean of H_{exp} over all subpopulations. The observed amount of heterozygosity H_I is found to be less than H_S and this is because of inbreeding within subpopulations. The extent of decrease in heterozygosity by this effect is described as $F_{IS}=(H_S-H_I)/H_S$. In Table 6, the summary of F-statistics for *D. tokoro* is presented. If it is assumed that all loci examined are selectively neutral and that the

Table 5. Nei's genetic diversity calculated for the natural population of *Dioscorea tokoro*.

Locus	H_T	H_S	D_{ST}	G_{ST}
Est	0.325	0.294	0.031	0.095
Got1	0.010	0.008	0.002	0.200
Got2	0.446	0.429	0.017	0.038
6pgdh	0.202	0.186	0.016	0.079
Pgi	0.666	0.590	0.076	0.114
Skdh	0.041	0.039	0.002	0.049
Mean	0.282	0.258	0.024	0.096

Table 6. Summary of F-statistics calculated for the natural population of *Dioscorea tokoro*.

Locus	F_{IS}	F_{IT}	F_{ST}
Est	0.138	0.258	0.095
Got1	−0.007	−0.100	0.200
Got2	0.214	0.260	0.038
6pgdh	0.004	0.099	0.079
Pgi	0.013	0.134	0.114
Skdh	0.037	0.098	0.049
Mean	0.067	0.125	0.096

differentiation of populations is random, the magnitude of F_{IS} and F_{ST} relative to F_{IT} must be similar throughout all loci. But this is not the case with *D. tokoro*. The mean F_{IT} over all loci was 0.125, which was little smaller than the 0.19 reported as the mean F_{IT} for three other outcrossing perennial herbs (Brown, 1979). For this observed F_{IT}, the subdivision of population (F_{ST}) contributes to the same extent as the effect of inbreeding (F_{IS}), as indicated by the values $F_{ST}=0.096$ and $F_{IS}=0.067$.

Discussion

1. *Geographical Cline of* Pgi *Allele Frequencies*

Among four highly polymorphic loci (*Est*, *Got2*, *6pgdh* and *Pgi*), *Pgi* showed the highest G_{ST} value (Table 5), which indicates that the geographical differentiation is most evident in *Pgi*. This assumption is confirmed by glancing at the pie graph of *Pgi* allele frequencies (Fig. 4). The frequency of allele *Pgi-a* increased from south to north. In contrast, the frequency of *Pgi-c* was extremely high in the southernmost population but decreased northward. In Fig. 5, the correlations between allele frequencies of *Pgi* and latitude are depicted. The correlation coefficients between latitude and allele frequencies are significant at the probability of 0.01 for *Pgi-a* and *Pgi-c* with $r=0.612$ and $r=-0.675$, respectively. Geographical clines of allele frequencies are thought to be the results of either of two mechanisms: (1) by a migration between two well-differentiated populations with differently fixed alleles, and (2) by the combination of a differential selection on that locus in various parts of the population and a restricted gene flow among subpopulations. The possibility of "genetic hitchhiking" (Hedrick, 1982) of the concerned locus with other closely linked loci under the selection might be safely excluded when we consider a large geographical clines as in this case. Mechanism (1) is well exemplified in the *HLA* cline in humans (Menozzi et al., 1978), and mechanism (2) is used to explain the *Adh* cline of *Drosophila* (Sampsell and Simms, 1982), the *Lap* cline in the marine mussel *Mytilus* (Koehn and Hilbish, 1987),

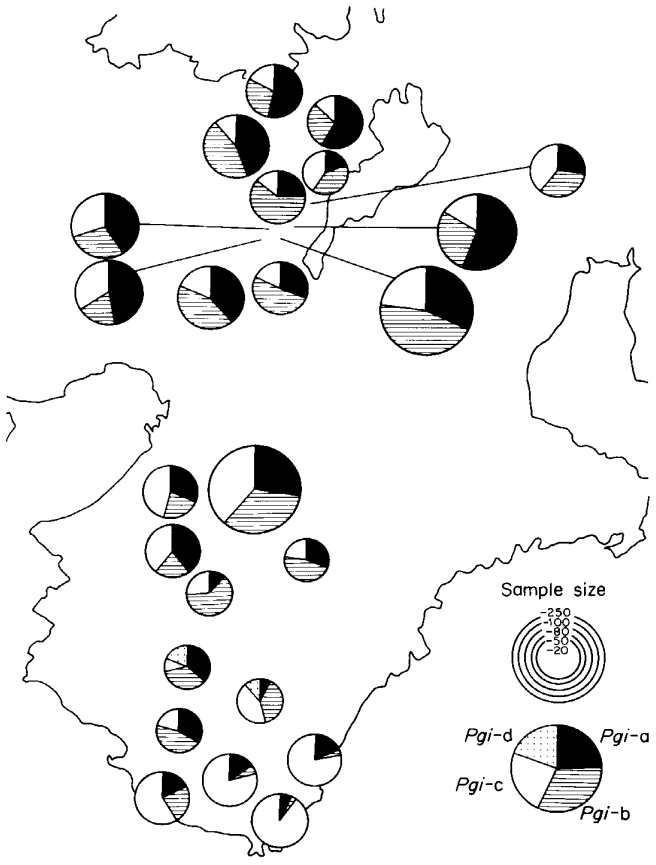

Fig. 4. Geographical variation in allele frequencies at the *Pgi* locus in *D. tokoro*.

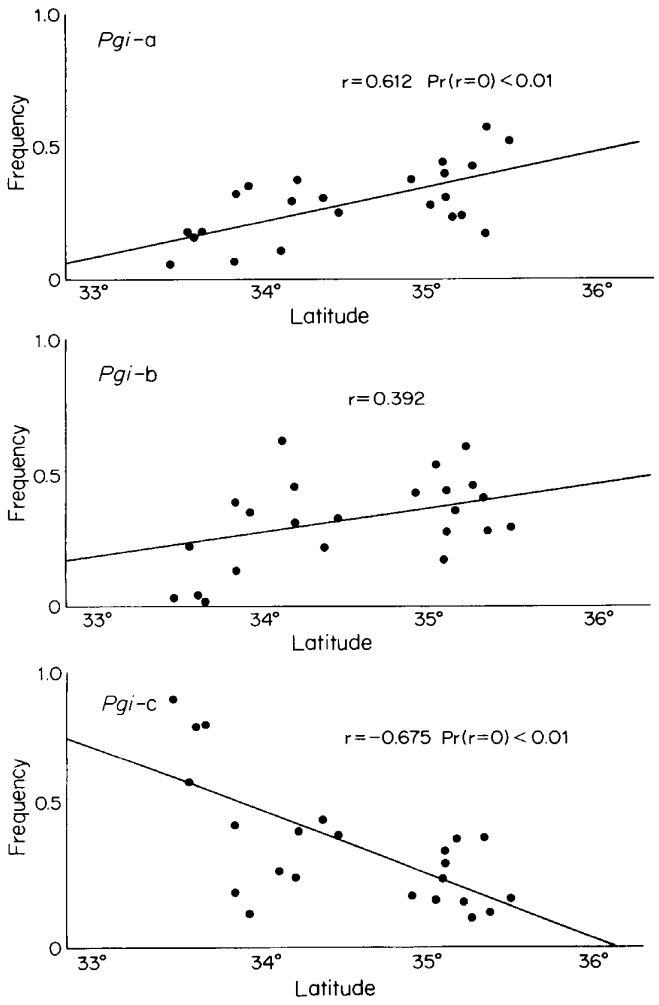

Fig. 5. Correlations between the latitude and allele frequencies at *Pgi* locus in *D. tokoro*.

the *Ldh* cline in the marine fish *Fundulus* (Powers et al., 1983), etc. To explain the *Pgi* cline of *D. tokoro*, it is difficult to invoke mechanism (1), because it is unlikely that two well-differentiated populations had initially existed in the north and south ends of the area in which the present study was carried out, when we consider the facts that no similar cline along the latitude was detected for other loci examined and that no morphological differentiation was observed between populations at the north and south ends. Therefore, mechanism (2) will be more feasible as the reason for the *Pgi* cline of *D. tokoro*. The major environmental gradient formed along the latitude is that of the temperature, when the difference of mean temperature between the locations of populations of north and south ends is assumed to be 3.0°C. Such correlations between the temperature and allele frequencies of *Pgi* are also reported in other organisms such as the butterfly *Colius* (Watt, 1983) and the sea anemone *Metridium* (Hoffman, 1981). In these cases, *Pgi* genotypes are concluded to be subject to direct natural selection. Therefore, it is possible also that for *D. tokoro* the *Pgi* locus is under the selection of the temperature. What supports this assumption are the data on segregation of *Pgi* genotypes among F_1 progeny obtained by the crossing the parents of given *Pgi* genotypes (Table 2). In all crossing experiments expected to give rise to *Pgi-c/Pgi-c* homozygote progeny (cross nos. 4, 6, 8 and 9), this particular genotype was found among progeny with consistently less frequency than expected. Because observed ratios of heterozygotes of *Pgi-c* did not show a deficiency compared with genotypes not containing *Pgi-c*, we cannot attribute this deviation to the selection working on gametes having allele *Pgi-c* (the meiotic drive). What is feasible is that the temperature condition (5°C) of the chilling treatment practiced to break the dormancy of F_1 seeds has not favored the zygote with *Pgi-c/Pgi-c* genotype to germinate, considering the fact that by this treatment about half of the seeds sown have not germinated and their *Pgi* genotypes have not been determined. The temperature of the chilling treatment (5°C) applied for this experiment is close to the mean winter temperature of the northern Kinki district, and about 3°C lower than that of southern Kinki (8°C). Based on these observations, it may be proposed that individuals with *Pgi-c/Pgi-c* genotype are not favored under low temperature conditions (5°C) with respect to their germination ability and/or timing, and this might have resulted in the geographical cline of allele frequencies at *Pgi* locus, as observed in the present study.

2. *Gene Flow in* D. tokoro

High total genetic diversity ($H_T=0.282$) and low interpopulational genetic diversity ($G_{ST}=0.096$) were observed for a dioecious species, *D. tokoro*. This feature was reported to be a common characteristic of outcrossed plant species with a high gene flow level (Gottlieb, 1981). The extent of gene flow required to explain these values is inferred by examining F_{ST} value. In an island model, Wright (1931) showed that at equilibrium $F_{ST}=1/(4N_em+1)$, where N_e is the effective size of the subpopulation and m is the immigration rate to each population from another subpopulation chosen at random. The estimation of N_em for *D. tokoro* was 2.4 when we replaced F_{ST} by 0.096, the mean F_{ST} over all loci (Table 6). It indicates that each subpopulation would receive 2.4 immigrants per generation from the population as a whole. When we consider a more realistic stepping-stone model (Crow and Aoki, 1984; Levin, 1988), N_em is estimated to be 4.8.

3. *Inbreeding in* D. tokoro

In *D. tokoro*, the mean F_{IS} value for six loci was 0.067. The statistical significance of the deviation of F_{IS} from 0 could not be tested. But, it is noteworthy that both this mean F_{IS} and F values of 20 out of 26 populations (Table 4) are positive, indicating habitual inbreeding in *D. tokoro* populations. Positive F_{IS} values observed for outcrossing species and negative F_{IS} values for selfing species are paradoxical, and Brown (1979) termed this finding the "heterozygosity paradox". Among many of the factors giving rise to this paradox, he pointed out the role of the restricted neighborhood size, which implies that mating individuals are closely adjacent in space and dispersals of the gametes and seeds are limited. For *D. tokoro*, a dioecious species, this factor is supposed to be the primary reason for the observed positive F_{IS}. Hedrick and Cockerham (1986) developed a method to quantify the level of partial inbreeding, or the between-relative matings. When we limit the matings of *D. tokoro* to the first-degree matings, that is, matings between full sibs and between parents and offspring, the frequency of such partial inbreeding required to account for the observed F_{IS} at equilibrium is estimated to be 0.223. It indicates that about 20% of individuals within subpopulations should undergo the first-degree matings to maintain the observed F_{IS} value.

Conclusion

Dioecy is characteristic of the genus *Dioscorea*. The consequences this breeding system has for the genetic structure of *Dioscorea* populations are exemplified using *D. tokoro* as a material. The characteristics of *D. tokoro* population structure thus revealed included high total genetic diversity, low inter-populational differentiation, and low inbreeding within populations. These features were concordant with previous reports for other outcrossing species. What was also revealed is that *D. tokoro* has more total and intrapopulational genetic diversity and less interpopulational diversity than previously reported dioecious tree species of tropical rain forests. This discrepancy would be ascribable primarily to the higher gene flow in a *D. tokoro* population compared with tree species. The high gene flow in a *D. tokoro* population might be the consequence of its habitat and its life form as a vine species. After climbing to the height of the canopy, *D. tokoro* florifies and can disperse winged seeds to the distant places along the fringes of forest. The high dispersal ability and high genetic diversity observed for *D. tokoro* is considered to be the prerequisite for survival of vine species which commonly occur in such unpredictable disturbed habitats as the gaps and the fringes of forests.

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