

Genetic Studies in Natural Populations of Pinus

II. Geographical Variation in Relation to Natural Selection

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Introduction

In the preceding paper (Sakai, Hayashi and Iyama⁸⁾), genetic variation of isoperoxidases among local populations of Japanese black pine, *Pinus thunbergii*, was reported. Collection of needle leaves in that study was made from 20 populations in six prefectures of the Japanese islands. Further studies continued thenceforth, allow at present the writers to survey the electrophoretic variability in 40 populations in toto, along with inter-population variability in several exterior and interior structures of needle-leaves in 30 populations. This paper describes results of those investigations aiming at elucidating relationships between character variation and geographic parameters.

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Materials and Methods

Twigs bearing two year-old needle-leaves were collected on an individual tree basis in 40 populations of *Pinus thunbergii*. Locations of the 40 populations providing those twigs are sketched in Figure 1, and the relevant geographical data are given in Table 1.

The collected twigs were preserved in a refrigerator kept at -20°C for some time until they were used for electrophoretic as well as morphological investigations. The technique of the electrophoresis and the schema of the isoperoxidase pattern in *Pinus thunbergii* were given in the previous paper (Sakai, Hayashi and Iyama⁸⁾), hence no repetition of them here.

Observation and measurement were performed with 10 two year-old needle-leaves taken at random from each tree. After being measured for length, the needle-leaves were sectioned transversely in the middle part by razor blade and were measured under a low-powered microscope for their width and thickness. The number of stomatal rows distributed on dorsal and ventral sides and that of resin-canals were counted.

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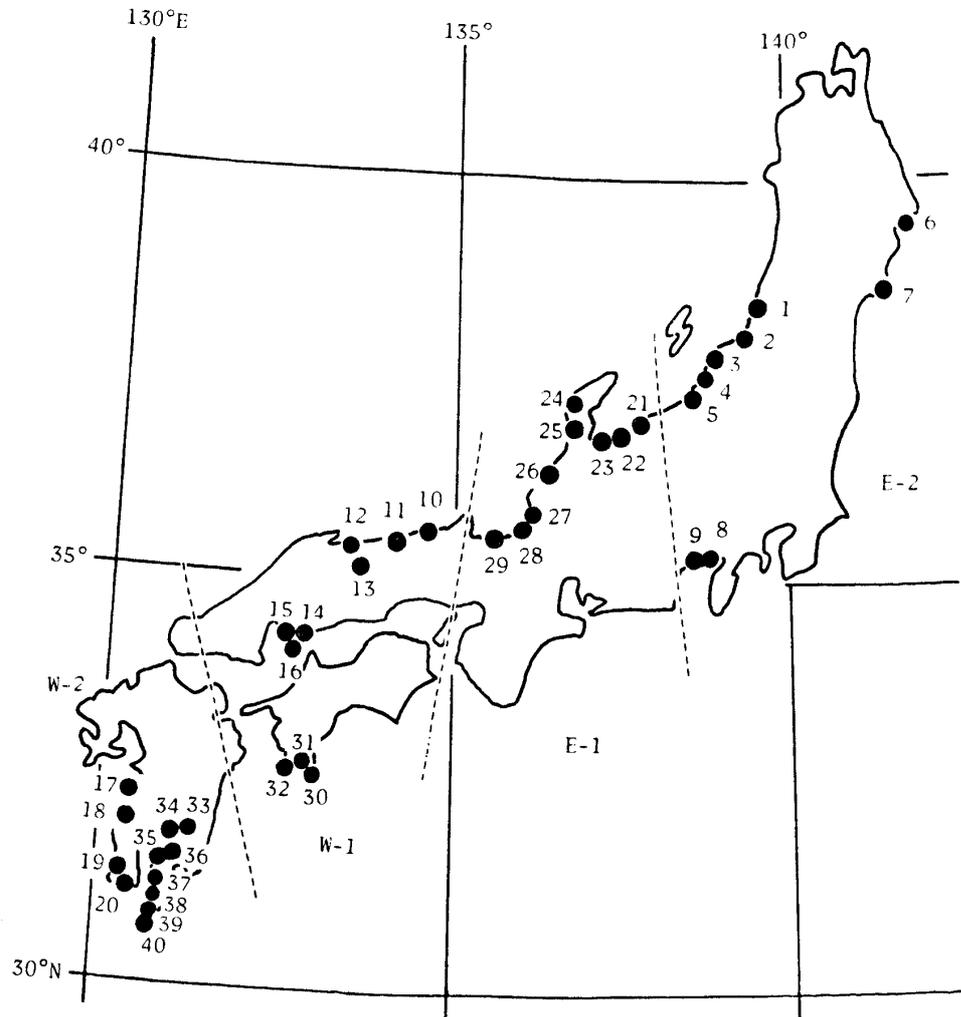


Fig. 1. A sketch map of the Japanese islands showing sites of collection of needle-leaves of *Pinus thunbergii*. For geographical details of those 40 sites, see Table 1.

Results

Isoperoxidase study

It has been shown in the previous study that there was a variation not only among trees within population, but also among populations in the pattern of 14 isoperoxidase bands. Of special interest was the variation in its number of isoperoxidase bands manifested per tree. Data from 40 populations including 20 old and 20 new ones, are given in Table 2.

It is found from Table 2 that in the E-2 district or in the most north-eastern group of populations, the trees tended to have lower number of isoperoxidase bands ranging from 4.45 to 5.54 per tree, while in the central district, i.e. E-1 and W-1, the number was 5.72 to 7.71 and in the most south-western district, W-2, it was from 7.60 to 10.03 (See also Figure 3). Analysis of variance shows that the between-districts-, between-provinces- and between-populations-variations were all highly significant (Table 5).

Table 1. Description of natural populations of *Pinus thunbergii* investigated in the present study.

Population number	District	Province	Locality	Latitude	Longitude	Number of investigated trees
1	E-2	I. Niigata	Senami	38°14'	139°28'	45
2			Fujizuka	38°03'	139°18'	42
3			Yotsugoya	37°51'	138°53'	43
4			Mase	37°44'	138°48'	39
5			Osaki	37°30'	138°38'	37
6		II. Miyagi	Okama	38°52'	141°40'	73
7			Kurosaki	38°16'	141°32'	78
8		III. Shizuoka	Numazu	35°07'	138°50'	53
9			Ipponmatsu	35°08'	138°47'	33
21		IV. Toyama	Iino	36°56'	137°27'	66
22	Kyoda		36°51'	137°25'	57	
23	E-1	V. Noto	Amebarashi	36°49'	137°03'	64
24			Akazumi	37°03'	136°44'	28
25			Shibagaki	36°56'	136°46'	51
26	VI. Fukui		Kitagata	36°16'	136°13'	53
27			Shiraki	35°44'	135°59'	25
28			Niu	35°43'	135°59'	57
29			Tomari	35°33'	135°43'	35
10		VIII. Tottori	Haneo	35°35'	134°21'	62
11			Natsudomari	35°32'	134°01'	52
12			Nawa	35°31'	133°30'	52
13			Daisen	35°23'	133°32'	54
14	W-1	VIII. Hiroshima	Kure	34°12'	132°32'	30
15			Kurahashi	34°07'	132°29'	25
16			Ondo-Hata	34°09'	132°32'	27
30		IX. Kochi	Ashizuri	32°43'	133°01'	30
31			Tatsukushi	32°47'	132°52'	42
32			Kanozaki	32°45'	132°48'	69
17		X. N-Satsu	Sendai	31°52'	130°12'	38
18			Kushikino	31°42'	130°16'	39
19		XI. S-Satsu	Ooura	31°25'	130°14'	40
20			Bonotsu	31°18'	130°14'	41
33	W-2	XII. Kirishima	Kirishima	31°52'	130°53'	35
34			Makizono	31°52'	130°48'	25
35			Sakurajima	31°34'	130°40'	39
36			Ushine	31°34'	130°45'	67
37		XIII. Oosumi	Oosumi-1	31°24'	130°47'	19
38			Oosumi-2	31°22'	130°48'	38
39			Oosumi-3	31°20'	130°49'	37
40			Oosumi-4	31°17'	130°47'	21

Table 2. The number, per tree, of isoperoxidase bands in 40 natural populations of *Pinus thunbergii*.

Population number	Geographical group	Number of isoperoxidase bands per tree														Number of trees	Mean	Standard deviation	
		2	3	4	5	6	7	8	9	10	11	12	13	14					
1	E-2-1			6	27	9	2	1									45	5.22	0.823
2	E-2-1		6	15	18	2	1										42	4.45	0.889
3	E-2-1	1	2	9	26	5											43	4.74	0.790
4	E-2-1	1	4	11	18	4	1										39	4.59	0.993
5	E-2-1		1	5	20	8	3										37	5.19	0.877
6	E-2-II	1	0	13	20	28	9	2									73	5.49	1.095
7	E-2-II		2	8	23	36	9										78	5.54	0.922
8	E-2-III		2	16	22	12	1										53	4.89	1.054
9	E-2-III		1	13	16	3											33	4.64	0.688
21	E-1-IV			10	10	22	19	14	1								66	6.61	1.028
22	E-1-IV		2	6	18	14	10	6	1								57	5.81	1.330
23	E-1-V				2	14	25	16	6								64	7.16	0.972
24	E-1-V				3	7	6	4	2								28	7.25	1.430
25	E-1-V				0	16	10	17	6	1							51	7.25	1.186
26	E-1-VI		1		5	14	15	11	6	1							53	7.11	1.327
27	E-1-VI				6	6	6	7	5								25	6.60	1.224
28	E-1-VI				5	24	16	7	1								57	6.70	1.075
29	E-1-VI				2	8	17	7	1								35	6.91	0.886
10	W-1-VII			8	14	26	10	4									62	5.81	1.069
11	W-1-VII			1	13	25	11	1									52	5.96	0.791
12	W-1-VII		1	3	14	15	16	2	1								52	6.00	1.155
13	W-1-VII			4	19	23	6	1	0	1							54	5.72	1.036
14	W-1-VIII				2	4	14	6	2	1							30	7.30	1.291
15	W-1-VIII				1	2	12	7	3								25	7.63	0.952
16	W-1-VIII				6	9	10	2									27	6.30	0.912
30	W-1-IX			3	13	8	4	1									29	6.55	0.968
31	W-1-IX			1	1	8	23	10	1								43	7.05	0.776
32	W-1-IX			2	5	21	24	10	5								70	7.71	1.110
17	W-2-X				1	4	9	10	12	1							38	8.92	1.260
18	W-2-X				3	8	15	9	2	1							39	7.97	1.203
19	W-2-XI		1		1	6	8	15	7	2							40	7.60	1.293
2)	W-2-XI				3	11	23	4	4								41	7.68	0.756
33	W-2-XII				4	11	9	8	3								35	7.86	1.167
34	W-2-XII				3	4	14	3	1								25	7.80	0.957
35	W-2-XII				2	6	14	6	8	1							39	8.56	1.392
36	W-2-XII			1	10	14	14	9	1	2							43	7.81	1.304
37	W-2-XIII				3	12	9	6	4	2							19	9.21	1.272
38	W-2-XIII				1	1	5	6	9	5							38	9.03	1.197
39	W-2-XIII				2	11	14	4	2	4							37	10.03	1.500
40	W-2-XIII				3	6	7	2	3	2							21	8.86	1.352

Table 3. Frequency of occurrence of main bands of isoperoxidase in natural populations of *Pinus thunbergii*.

Population number	Geographical group	Number of trees	Frequency of occurrence in percent											
			A	B	E	G	H	K	M	N	Q	S	V	Y
1	E-2-I	45	2	2	0	100	18	96	100	89	16	0	0	98
2	E-2-I	42	0	0	0	100	2	95	100	64	5	2	0	76
3	E-2-I	43	2	0	0	100	9	84	100	81	7	0	0	88
4	E-2-I	39	5	0	0	100	10	95	97	74	10	0	0	54
5	E-2-I	37	3	3	0	100	14	95	100	97	27	0	0	81
6	E-2-II	73	3	4	0	100	67	67	100	96	23	1	0	85
7	E-2-II	78	0	1	0	100	63	87	99	95	17	0	0	92
8	E-2-III	53	4	0	0	100	11	94	98	92	19	0	0	64
9	E-2-III	33	12	9	6	100	12	97	97	45	6	0	0	76
21	E-1-IV	66	44	8	0	100	33	98	89	94	70	9	0	100
22	E-1-IV	57	26	4	0	100	28	100	100	63	54	2	2	88
23	E-1-V	64	58	3	0	100	36	100	100	100	81	2	36	100
24	E-1-V	28	21	0	36	100	71	100	100	100	54	0	0	100
25	E-1-V	51	45	0	2	100	51	100	80	94	86	25	0	100
26	E-1-VI	53	53	13	2	100	34	100	70	100	79	36	9	100
27	E-1-VI	25	60	4	0	100	12	100	100	76	84	24	0	100
28	E-1-VI	57	9	0	0	100	30	100	14	98	91	81	12	100
29	E-1-VI	35	46	80	6	100	3	97	100	80	63	20	14	77
10	W-1-VII	62	6	0	0	100	50	90	100	95	52	3	0	76
11	W-1-VII	52	12	0	2	100	54	94	100	94	44	2	0	92
12	W-1-VII	52	13	6	2	100	60	81	98	92	44	0	0	96
13	W-1-VII	54	13	2	0	100	13	85	100	98	65	6	0	93
14	W-1-VIII	30	10	0	3	100	80	97	100	100	93	33	7	97
15	W-1-VIII	25	20	12	0	100	96	88	96	100	96	20	0	96
16	W-1-VIII	27	4	0	0	100	59	100	100	93	74	4	0	96
30	W-1-IX	30	57	7	0	100	43	100	93	73	47	3	0	100
31	W-1-IX	42	71	5	2	100	86	95	90	88	29	2	0	100
32	W-1-IX	69	69	7	3	81	77	96	93	93	74	10	4	100
17	W-2-X	38	97	3	3	100	74	97	82	100	100	58	0	97
18	W-2-X	39	95	0	0	100	85	100	64	100	95	31	0	100
19	W-2-XI	40	84	0	0	100	63	100	100	100	88	13	3	90
20	W-2-XI	41	100	0	0	100	66	98	100	98	90	12	0	98
33	W-2-XII	35	86	0	14	100	28	89	100	100	94	37	0	100
34	W-2-XII	25	88	0	4	100	40	100	92	100	100	56	0	100
35	W-2-XII	39	82	8	5	100	36	97	82	100	97	38	0	97
36	W-2-XII	67	0	2	2	100	40	95	88	100	91	5	0	100
37	W-2-XIII	19	100	5	0	100	100	100	100	100	100	37	0	95
38	W-2-XIII	38	92	3	5	100	95	97	92	97	100	47	0	100
39	W-2-XIII	37	100	3	14	100	84	100	89	100	100	49	0	97
40	W-2-XIII	21	95	10	14	100	100	97	67	100	100	43	0	95

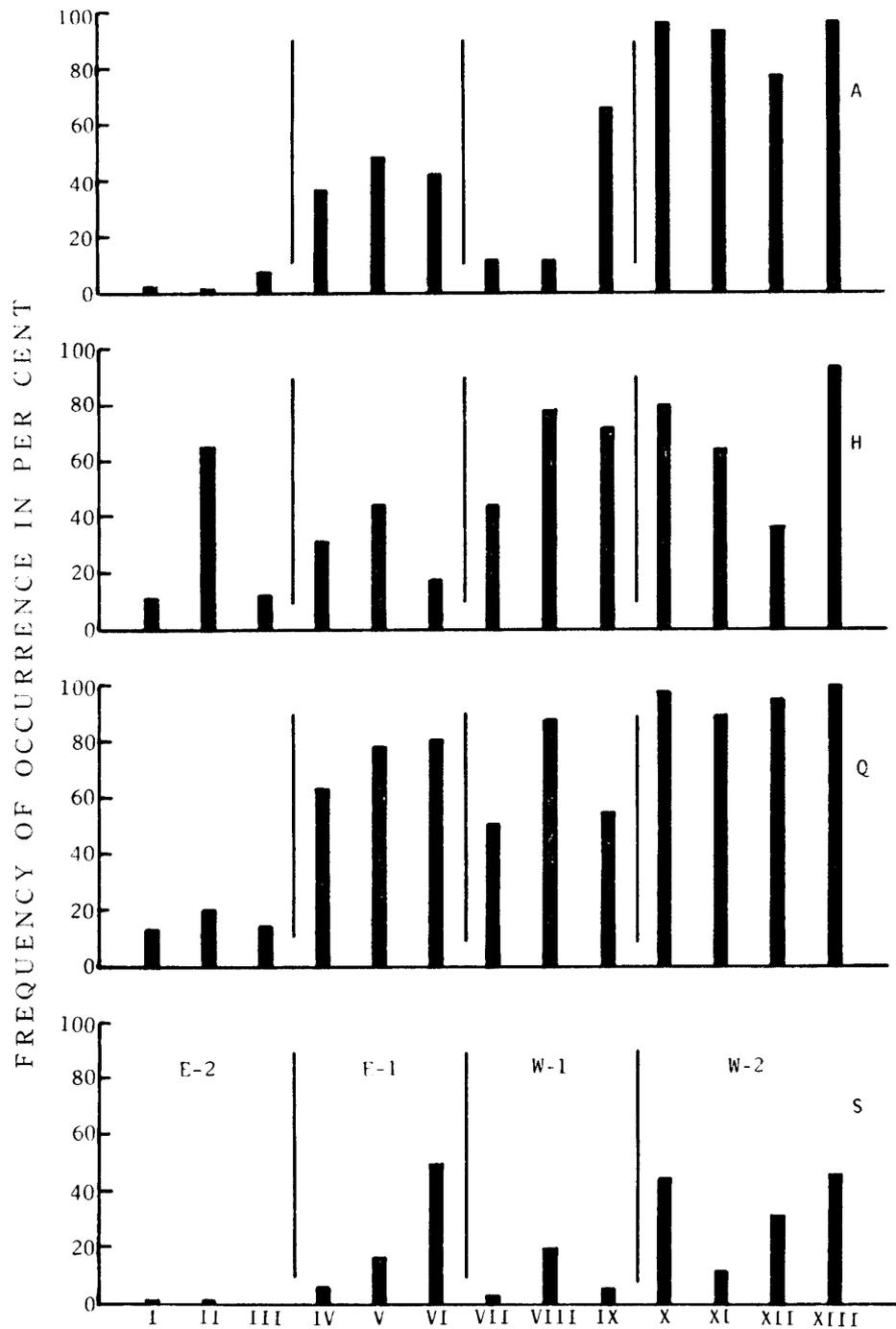


Fig. 2. Frequency of occurrence of four isoperoxidase bands. A, H, Q and S, in 40 populations of *Pinus thunbergii*, averaged for 13 provinces in four districts.

Frequency of occurrence was counted for twelve bands designated A, B, E, G, H, K, M, N, Q, S, V and Y, although there were still other bands which were weak in stainability and rather infrequently occurring (Table 3). Of those bands, G, K, M, N and Y were generally of high occurrence in most populations, while B, E and V were rather rare. Very variable and accordingly interesting for comparative study were the frequencies of A, H, Q and S bands (Figure 2).

It is found from Fig. 2 that in the E-2 district, the frequency of occurrence of

Table 4. Length, width and thickness of needle-leaves, the number of stomatal rows and that of resin-canals per leaf in 31 natural populations of *Pinus thunbergii*.

Population number	Geographical group	Number of trees	Needle length (cm)	Needle width*	Needle thickness*	Number of stomatal rows	Number of resin canals
6	E-2 II**	75	8.33	26.27	18.43	17.87	5.02
7	E-2 II	76	8.23	26.71	18.43	18.17	4.07
9	E-2-III	32	9.41	26.22	17.78	17.34	4.34
21	E-1-IV	65	11.41	27.38	19.56	17.09	5.05
22	E-1-IV	61	12.29	26.68	19.11	18.01	5.16
23	E-1-V	63	12.06	28.60	20.12	19.60	6.26
24	E-1-V	65	12.61	28.73	20.04	19.16	6.50
25	E-1-V	63	12.10	28.36	19.53	18.28	5.87
26	E-1-VI	56	11.41	26.54	18.36	16.57	4.91
27	E-1-VI	64	11.16	27.11	18.95	17.78	5.59
28	E-1-VI	58	12.09	26.92	18.69	18.10	5.78
29	E-1-VI	35	11.24	26.21	18.36	17.70	6.04
10	W-1-VII	62	9.13	25.89	17.02	15.27	3.98
11	W-1-VII	52	11.67	28.52	18.87	17.94	6.29
12	W-1-VII	51	10.50	26.32	17.50	16.98	4.28
13	W-1-VII	54	9.81	28.00	18.52	19.06	5.69
14	W-1-VIII	30	12.23	26.93	18.47	19.20	6.67
15	W-1-VIII	24	12.38	26.15	17.38	17.79	6.13
16	W-1-VIII	27	12.58	26.43	17.94	18.65	5.98
17	W-2-X	36	10.53	26.42	18.03	17.89	6.75
18	W-2-X	39	11.83	26.35	18.14	16.99	6.42
19	W-2-XI	33	11.04	23.77	16.11	14.68	5.14
20	W-2-XI	29	11.36	23.84	15.78	14.36	4.88
33	W-2-XII	30	10.80	24.07	16.53	15.40	6.20
34	W-2-XII	24	10.58	25.13	17.08	17.38	6.38
35	W-2-XII	38	11.42	25.47	17.26	17.01	6.24
36	W-2-XII	44	11.77	23.82	16.20	14.77	4.93
37	W-2-XIII	19	11.34	23.08	15.87	13.87	4.61
38	W-2-XIII	39	10.35	24.96	17.19	16.22	6.78
39	W-2-XIII	37	10.50	26.80	18.64	17.17	7.74
40	W-2-XIII	21	10.40	25.17	17.31	15.93	6.26

* On an arbitrary scale.

** E-2 stands for the district, while II for the province.

those 4 bands is very low, excepting for H in province II of E-2 district, while in the W-2, it is generally higher than in other districts for A, H, Q and S. The trend here observed is in accord with the trend manifested by the number of isoperoxidase bands shown in Table 2.

Needle-leaf characters

Measurement of length, width and thickness of needle-leaves and number of stomatal rows and that of resin-canals were recorded for 31 populations of *Pinus thumbergii*. Average values from each population are given in Table 4.

Analysis of variance of the investigated characters is presented in Table 5. It indicates that the three external characters of needle-leaves show highly significant variations between-provinces as well as between-populations within provinces. In respect of the number of stomatal rows and that of resin-canals, the between-districts-and-between-provinces-variations did not reach the significance level, though the between-population-variation was highly significant.

Table 5. Analysis of variance of needle characters in natural populations of *Pinus thumbergii*.

Source of variation	Mean squares							
	D. F.	Isozyme number	D. F.	Needle length	Needle width	Needle thickness	Stomatal rows	Resin canals
Between districts	3	799.53**	3	516.51**	476.61*	364.59*	330.32	110.11
Between provinces within districts	9	52.59**	7	57.59*	92.32*	52.01*	96.17	48.89
Between populations within provinces	27	9.83**	20	17.12**	28.96**	15.33**	47.47**	23.75**
Between trees within populations	1698	1.16	1371	2.63	5.42	2.28	6.17	3.44

*, ** stand for 5% and 1% levels of probability, respectively.

The average values of needle-length, the number of stomatal rows, that of resin-canals and that of isoperoxidase bands per tree are graphically represented in Figure 3. Looking at Figure 3, we are impressed by the fact that the number of stomatal rows and that of isoperoxidase bands are likely to show some geographical clines, one is gradually decreasing, while another increasing from E-2 to W-2 districts. It may be, then, of great interest to find the possible relationship between investigated characters and the geographical parameters. Table 6 presents the correlation coefficients between characters themselves and between characters and latitude or longitude.

First of all, correlation among characters in Table 6 may be mentioned. Extremely high correlations were found among needle-width, needle-thickness and the number of stomatal rows, while none of them was significantly correlated with needle-length. It was also found that the number of isoperoxidase bands was positively correlated with that of resin-canals, but negatively with needle-width and the number of stomatal rows, although the correlation was not so high as those described above.

Regarding correlations between characters and latitude or longitude, it was found that all characters, excepting for needle-length, were highly correlated with the geo-

graphic parameters. It means that characters dealt with in this study, show geographical clines with an exception of needle-length. In the meantime, it should be pointed out that latitudes and longitudes in Japan are characteristically highly correlated, the coefficient being so high as 0.9369 (See Table 6). Then, it is quite probable that a character which is substantially not at all correlated with latitude, may be so, provided it is correlated with the longitude, and *vice versa*.

But since whether a given character is primarily correlated with latitude or with longitude is important in regarding the character as ecologically neutral or not, further information which is to be obtained by examining partial correlations between the character and the geographical parameters, is requested. Partial correlation coefficients calculated are presented in Table 7.

In Table 7, we notice that such characters as the number of stomatal rows or needle-thickness are significantly correlated with latitude but not with longitude; while others such as the number of isoenzymes is correlated with longitude, but not with latitude. The number of resin-canals, on the contrary, is correlated with neither latitude nor longitude. Needle-width is a character significantly correlated with both latitude and longitude.

Table 7. Partial correlation coefficients between various needle-characters and geographical parameters.

	Constant variable	Partial correlation coefficient †)				
		Number of isozymes	Number of stomatal rows	Number of resin canals	Needle width	Needle thickness
Latitude	Longitude	-0.1076	0.4656**	0.1048	0.6207**	0.4755**
Longitude	Latitude	0.3576*	-0.2553	-0.2578	-0.4022*	-0.1750

†) Number of degrees of freedom for number of isozymes is 40, while for the remaining 4 characters 35.

Table 8. Total, genetic and environmental correlation coefficients among several needle-characters in *Pinus thumbergii* collected in the arboretum of Kagoshima University.

		Needle width	Number of stomatal rows	Density of stomata
Phenotypic correlation	Needle length	0.4346**	0.1451*	-0.1801*
	Needle width		0.7189**	0.1055
	No. stomatal rows			0.0585
Genetic correlation	Needle length	0.4870	0.1728	-0.2675
	Needle width		0.8593	0.1787
	No. stomatal rows			0.1287
Environmental correlation	Needle length	0.1055	0.0242	-0.0672
	Needle width		0.2406	-0.0007
	No. stomatal rows			-0.0350

Phenotypic, genetic and environmental correlations

In order to investigate phenotypic, genetic and environmental interrelationships among leaf-characters, needle-leaves were collected from a number of trees of *Pinus thunbergii* growing in the campus of Kagoshima University. The collected needle-leaves were investigated for length and width of needles, the number of stomatal rows and the density of stomata or the number of stomata per unit length of a stomatal row (Table 8).

In Table 8, we find that so far as phenotypic correlation is concerned, needle-width is highly correlated with either the number of stomatal rows or needle-length. It is interesting to find that significantly high phenotypic correlation between needle-width and the number of stomatal rows, i. e. $r = 0.7189$ is partitioned into so high a genetic correlation as 0.8593 and an environmental correlation of 0.2406. The same is also true for correlation between needle-width and needle-length, i. e. the phenotypic correlation 0.4346 is partitioned into genetic correlation of 0.4870 and environmental correlation of 0.1055.

Discussion

In connection with geographic variation in forest trees, there have been a number of papers published in recent years. Some of them were reviewed in the previous paper (Sakai, Hayashi and Iyama⁸⁾), and we may not have to repeat the same here, excepting for a few which have not taken up there.

Simak⁹⁾ found from his study on more than 100 populations of *Larix decidua*, that seed-weight, which is little affected by environments and thus highly heritable, was very variable among provenances and there was a close relationship between seed-weight and the altitude of the origin. Eriksson³⁾ collected seeds of *Pinus silvestris* from 20 provenances in Europe and Siberia and grew them in a suburb of Stockholm. He found that seedlings from those seeds were very variable with respect to survival and seedling height. Local differentiation in growth-characters of seedlings was also corroborated with Rocky Mountain Douglas-fir by Rehfeldt⁶⁾. The differentiation in this species was found to be not at all related to such topographical conditions as elevations or the north vs. south aspects.

Bergmann¹⁾ investigated geographic variation in the gene frequencies of isoenzymes of esterase and leucine-aminopeptidase in eight Swedish *Picea abies* populations. He found that some of the gene frequencies showed clinal variation from north to south. He considers that this clinal variation is an evidence of natural selection, caused by the gradually changing environments.

Muhs⁵⁾ made a study on inter-provenance variation in 14 populations of Douglas-fir by the aid of isoperoxidases. He found that the investigated populations were highly heterogeneous on the basis of frequency of five isoperoxidases. He further found that the isoenzyme frequencies were neither correlated with latitude nor with distance between populations.

Of special interest from the writers' present viewpoint are a few papers dealing with electrophoretic variation and/or geographical variation in stomatal frequency or the number of resin-canals.

One of them is the report by Robinson and Thor⁷⁾ which deals with the genetic relationship among three taxonomic groups of *Abies* which are geographically isolated

from each other. They are *Abies balsamea* growing between 40° and 42°N latitude, *A. balsamea* var. *phanerolepis* between 38° and 39°N, and *A. fraseri* growing at the latitudes of 35° to 36°N. The authors examined seven foliage- and six cone-characteristics, and found that most characteristics changed gradually from *A. balsamea* to var. *phanerolepis* with no apparent discontinuity between them, whereas *A. fraseri* was quite distinct from the other two. They calculated correlations between various characteristics and southward map-distance from the northernmost populations. They found that the correlation was high for many characteristics. Of special interest in the report was to find that the number of stomatal rows in the lower and upper sides of needles was highly correlated with the map-distance with $r = +0.88$ and $r = -0.65$, respectively. The stomatal frequency observed in a stomatal row on the under-side of the leaf showed no variation among taxonomic groups, although variation among stands as well as among trees within stand, was very obvious.

Another paper by Feret⁴⁾ describes an investigation on genetic differences among three stands of *Pinus pungens* on the basis of isoenzymes and some morphological characters including the number of rows of stomata. He found that the between-stands variation was significant in either isoenzyme frequencies, the number of rows of stomata or needle-length.

Yim¹⁰⁾ reported geographic variability of the number of resin-canals in needle-leaves of *Pinus thunbergii*. He found that the number was very variable among provenances, ranging from 4.23 to 7.15. He concluded that the variation was random, bearing no relation to geographical as well as edaphic or climatic factors.

From papers described above and also from those cited in our previous paper, we can draw a few conclusions as follows:

- (1) There is an inter-population variation among morphological, physiological or biochemical characters in forest tree species.
- (2) Biochemical variation is understood by some to be correlated with natural selection, while by others to be selectively neutral.
- (3) The number of stomatal rows and the number of resin-canals are also characters which are variable from population to population.

In the present study, it is found that some characters in *Pinus thunbergii* are variable from population to population, from province to province as well as from district to district in the Japanese islands. Characters showing such geographic variation were the number of isoperoxidase bands per tree, needle-length, needle-width and needle-thickness. The number of stomatal rows and that of resin-canals were obviously variable among populations, but variation among districts or among provinces failed to reach the significance level.

In our previous paper we have classified genetic variability in natural population of forest trees into six categories (Sakai, Hayashi and Iyama⁸⁾): (1) Phylogenetic variation; (2) Latitudinal variation (this was designated geographic variation in the previous paper); (3) Ecological variation; (4) Distributional variation; (5) Family variation and (6) Individual variation. Of the six variations just mentioned, family and individual variations are excluded from the present discussion, because the individual variation is variation brought about by genetic segregation and mutation and therefore it is ubiquitous in Mendelian populations, while the family variation is variation within populations caused by gregarious distribution of genetically related individuals due to dispersive

power of pollen and seeds.

The remaining four variations can be divided into two groups according to whether they are related to environmental stresses or not. The latitudinal as well as ecological variation is the outcome of climatic or ecological stresses of the habitat, respectively. They are included in the ecological variation in a broad and theoretical sense, but in forest genetics, it seems to be more convenient to separate the variation related to latitudinal conditions from a crowd of variations responding to various sporadic ecological factors. The phylogenetic variation is a variation among populations caused by genealogical differences within a species such as that found by Chylarecki and Giertych²⁾ in *Picea abies* of Poland. The distributional variation is a variation caused by the genetic random drift along migration routes.

It should be pointed out here that all these four variations may be capable of showing a more or less geographic cline. Thus it is quite apparent that the presence of a geographic cline alone, can not be an evidence for the claim that the variation is selection-dependent or it is selectively neutral.

The characters we have measured in *Pinus thunbergii* in the present study are six, that is, the number of isoperoxidase bands per tree, needle-length, needle-width, needle-thickness, the number of stomatal rows and that of resin-canals. It is then of interest to find what categories of genetic variations above mentioned, they belong to.

A character may be correlated predominately with latitude or longitude, or with neither. Thus, we may provisionally assume that :

(1) If mean value of a character is correlated with latitude, the variation may be regarded as latitudinal, excepting for a possible case that the correlation is made merely by chance.

(2) If the mean value of a character is correlated with longitude or forming a cline along with a possible migration route, it may be regarded as a distributional variation, or, in certain circumstances, as a phylogenetic variation.

(3) If a character to which differentiation among populations is evident, is correlated with neither latitude nor longitude, it may be regarded as ecological. In other words, it is assumed that differentiation among populations without any noticeable cline, may tell that the differentiation has been determined by the environmental heterogeneity.

With provisos as mentioned above in mind, correlation was measured among six characters, on the one hand, and between characters and the latitude or longitude, on the other. Then we found, as shown in Table 6, that needle-width, needle-thickness and the number of stomatal rows were highly correlated with each other, r being so high as $+0.8 \sim +0.9$. It is possible that these three characters are governed by common genes pleiotropically operative. Another correlation which was high enough was that between the number of isoperoxidase bands and that of resin-canals.

In respect of the correlation between plant characters and geographic parameter, it was found that the number of isoperoxidase bands and various needle characters, with an exception of needle-length, were correlated with either latitude or longitude. Since the latitude and the longitude were highly correlated with $r = +0.9369$ in Japan, a character which is essentially correlated with one of them, should naturally be correlated with another. So, in order to find the true state of things, the partial correlation, leaving one of the two constant, was investigated. From this investigation, three

groups of characters classified in respect of the relation to geographic parameters, were identified as seen in Table 7.

The first group consists of those characters highly correlated with latitude but little with longitude. Characters belonging to this group are the number of stomatal rows, needle-width and needle-thickness. The character in the second group which exhibits correlation with longitude but not with latitude is the number of isoperoxidase bands per tree. The third group includes the number of resin-canals which neither latitude nor longitude correlates.

In accord with the assumptions given before, we may classify characters of *Pinus thunbergii* in the following way:

(1) Latitudinal variation: the number of stomatal rows, needle-width and needle-thickness.

(2) Distributional or phylogenetic variation: the number of isoperoxidase bands per tree.

(3) Ecological variation: the number of resin-canals.

With regard to needle-length, no correlation was detected with either latitude or longitude even by the simple-correlation analysis. It was, however, variable from population to population, from province to province and from district to district, and we cannot say at present to what group the needle-length may belong.

Another fact worthy of notice is that the number of isoperoxidase bands is significantly correlated with the number of resin-canals (r being 0.6082** in Table 6). Since partial correlation coefficients between the number of isoperoxidase bands or that of resin-canals with longitude is -0.3576^* and -0.2578 , respectively, further enquiry into the relation between the number of isoperoxidase bands and that of resin-canals was investigated, leaving the longitude constant. The partial correlation coefficient thus obtained between isoperoxidases and resin-canals was 0.4907, which was statistically highly significant. The reason why the number of isoperoxidase bands and that of resin-canals are associated is not known.

The statistical features and supposed nature of geographical variation of six characters investigated in *Pinus thunbergii* are summarized in the following table:

Character	Statistical significance					Nature of geographical variation
	Variation between			Correlation with		
	Populations	Provinces	Districts	Latitude	Longitude	
No. Peroxidases	**	**	**	—	*	Distributional (or phylogenetic)
Needle-length	**	*	**	—	—	Unknown
Needle-width	**	*	*	**	*	} Latitudinal
Needle-thickness	**	*	*	**	—	
No. stomatal rows	**	*	*	*	—	
No. resin-canals	**	—	—	—	—	Ecological

*, ** stand for the 5% and 1% levels of significance, respectively.

In the above table, needle-width, needle-thickness and stomatal rows behave in a similar way in the analysis of variance as well as in the correlation analysis. Remind-

ing the fact that these three characters are mutually highly correlated with $r = +0.8 \sim +0.9$, we presume that these three characters are governed by common pleiotropic genes. As a matter of fact, it has been found in a study of genetic and environmental correlations among several needle-characters that the genetic correlation between the number of stomatal rows and needle-width was so high as $+0.8593$ (See Table 8).

Of these three characters which should biologically respond to latitude? Although no evidence is available at present, we tend to assume that warmer climate throughout the year in southern provinces may favor less transpiration by smaller number of stomata per leaf. In this connection, we have to mention that the phenotypic or genetic correlation between the number of stomatal rows and the density of stomata or the number of stomata per unit length of a stomatal row was approximately zero (See Table 8). It means that the number of stomatal rows may be proportional to the total number of stomata per leaf.

Conclusion

Investigation of geographic variability in 40 populations of *Pinus thumbergii* growing in the Japanese islands has demonstrated that geographic differentiation was evident in the number of isoperoxidase bands per tree, needle-length, needle-width, needle-thickness, the number of stomatal rows and that of resin-canals per leaf. Of these six characters, the number per tree of isoperoxidase bands showed distributional (or phylogenetic) variation, whereas that of stomatal rows in association with width and thickness of needle-leaves, latitudinal variation, and the number of resin-canals showed ecological variation. It is found that the number of stomatal rows in southern provinces was apparently smaller than that in northern, and it has been concluded that the warmer climate throughout the year in southern provinces, may have favored limited transpiration in *Pinus thumbergii*.

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