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Genetic Studies in Natural Populations of Forest Trees

III. Genetic Differentiation within a Forest of *Cryptomeria japonica*[1]

KAN-ICHI SAKAI and YOUNG-GOO PARK[2]

National Institute of Genetics, Misima (Japan)

Summary. The present study deals with the genetic differentiation within a natural forest of *Cryptomeria japonica*. Needle leaves were collected from approximately 50 trees in each of three sites, called subpopulations I, II and III, located at the apices of a triangle. The distances from each other among these three subpopulations were 760 m or 1,300 m. It was found, by an electrophoretic study of peroxidase, that the mean number of isozyme bands exhibited per individual tree was 11.68 in I, 13.73 in II and 12.53 in III. The t-test revealed that the differences between I and II and between II and III were statistically significant. The frequency of occurrence of each band was compared among the subpopulations. It was found that the variation among the three subpopulations was statistically significant. Not a few papers dealing with the study of pollen dispersal in coniferous trees have been published so far. Some of the authors consider that the distance of pollen dispersal is short and gene flow among subpopulations within a forest seldom occurs, while others are of the opinion that pollen travels long distances and no effective isolation is possible within a forest. Findings from the present study support the former view. They show that subpopulations within a forest of *Cryptomeria japonica* are genetically differentiated from each other, i. e. Sewall Wright's neighbourhood size is small in *Cryptomeria japonica*.

Introduction

There are two opposing views on migration and isolation in forests. Wright (1962) reviewed several papers dealing with pollen dispersion (including one by himself and those by Strand, Silen, Langner and others) and concluded that trees probably exchange pollen only within the close neighbourhood. Andersson (1963), Lanner (1966) and Sarvas (1967), on the contrary, believe that trees receive pollen not only from their neighbours but also from trees growing far away. According to the first view, a natural forest should consist of numerous subpopulations not isolated geographically but more or less differentiated genetically. The latter authors take the view that there are many possibilities for effective migration, and racial differentiation, if present, might have been caused by regional or local environmental selection pressure. All these arguments have been put forward on the sole basis of pollen dispersal without considering a direct analysis of the genetic situation in a forest.

Considerably less is known about genetic differences between adjacent populations than about differences between distant populations of forest trees, as Callaham (1967) has pointed out.

Sakai, Miyazaki and Matsuura (1971) have recently reported that two natural forests of *Thujopsis dolabrata*, which had long been isolated by 10 kilometer wide straits, were genetically different from each other. This conclusion was drawn from a comparative study of peroxidase, and from the measurement of leaf traits. The two forests were considered to be similar ecologically, and it is very likely that the genetic differentiation had been induced not by natural selection, but by random genetic drift followed by geographic isolation.

We have in Japan a number of natural forests of *Cryptomeria japonica*, spontaneously growing in the mountainous regions. This is one of the most important tree species in Japan, and natural forests of this species have long been valuable sources of propagation material for people who make a living from forests.

The present paper describes the results of a study on the genetic differentiation among three subpopulations sampled in a natural forest of *Cryptomeria japonica* located in the Kochi prefecture of Shikoku island.

Materials and Methods

Needle-leaves for the electrophoretic study were collected on an individual tree basis from approximately 50 trees growing in each of three sites located at the apices of an isosceles triangle. A contour map of these sites is shown in Figure 1. The three subpopulations are at approximately the same elevation of 500 to 600 metres above sea-level, and are surrounded by dense forests of *Cryptomeria japonica* mixed with trees of broad-leaved species.

As seen in Figure 1, ravines, each with a stream, form the boundaries of the three sites, though they can hardly be considered to serve as barriers against gene flow. The straight line distances among the three subpopulations were 760 m between I and II, and 1,300 m between I and

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[1] Contributions from National Institute of Genetics, Misima, No. 790.

[2] Researcher in Institute of Forest Genetics, Suwon, Korea, temporarily working in National Institute of Genetics, Misima.

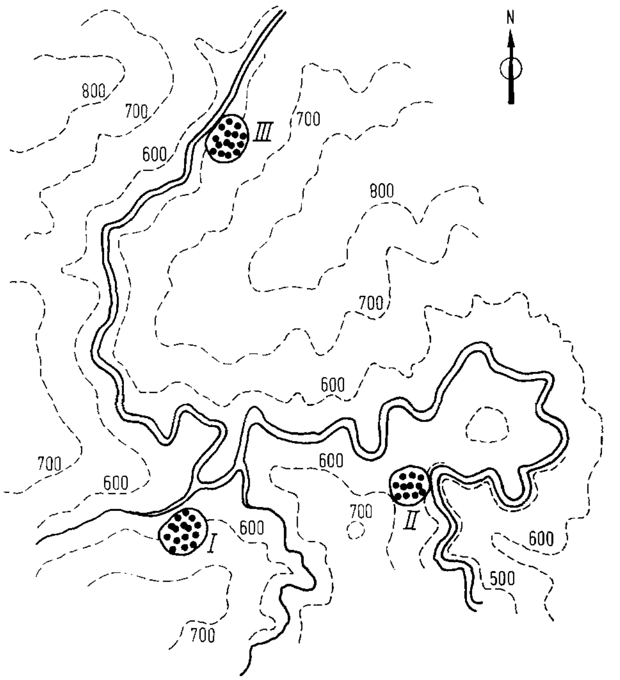

Fig. 1. Countour map of Yanase natural forest of *Cryptomeria japonica*, where three subpopulations were sampled

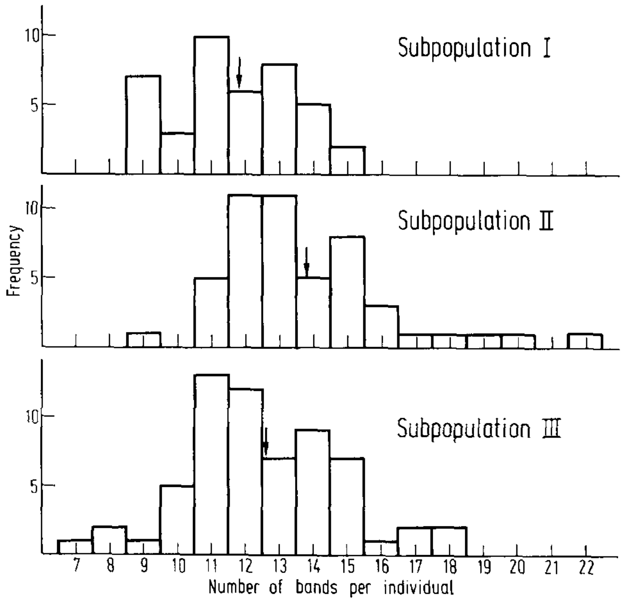

Fig. 2. Frequency distribution of the number of isoperoxidase bands per individual in three subpopulations of *Cryptomeria japonica*. Arrows indicate mean number of bands per individual

III and between II and III. The number of trees sampled was 41, 49 and 62 in subpopulations I, II and III, respectively. A long branch, about 30 cm long with needle leaves, was taken from each tree. The collected leaves were stored in a deep-freezer until needed for electrophoretic study.

The technique adopted for the biochemical study was almost the same as that given in the previous paper (Miyazaki and Sakai, 1969), except for a slight modification in H_2O_2 concentration of the staining reagent, i.e. 0.5% instead of the 1% used previously. Measurement of zymograms was performed after discarding erroneous bands by a statistical examination (Sakai, Miyazaki and Matsuura, 1971).

Results of Study

The maximum number of peroxidase bands established in needle-leaves of *Cryptomeria japonica* was 36, though the number actually counted for each tree was far less than that. Table 1 and Fig. 2 show the frequency distribution of the number of peroxidase bands exhibited per tree in the three subpopulations.

Table 1 and Figure 2 show that subpopulation II included trees with an extraordinarily high number of isozyme bands. Subpopulation I was least variable, while in subpopulation III trees with as few as 7 or 8 bands were found. The mean number of isozyme bands shown by individual trees in subpopulations I, II and III was 11.68, 13.73 and 12.53, respectively. The t-test revealed that the differences between I and II and between II and III were significant statistically, whereas the difference between I and III was not.

Frequency of occurrence of isozyme bands in the three subpopulations was compared. Figure 3 is its graphical representation, with figures above the bars standing for an average value of the respective frequencies in the three subpopulations.

It was found (Fig. 3) that several bands appeared with higher frequencies. For example, band No. 9 appeared with the highest frequency of 87%; three bands, Nos. 3, 12 and 31, had incidences higher than 75%. Bands observed with low frequencies, were Nos. 5, 6, 25 and 26. Occurrence of some bands, however, was highly variable among the subpopulations: band No. 2 occurred as frequently as 69% in subpopulation III but only at 27% in II.

The differences in incidence of isozyme bands between I and II, II and III and I and III were tested statistically by measuring z value. This is $(x_{si}/N_s -$

Table 1. *Variation in number of isoperoxidase bands per tree in three subpopulations of Cryptomeria japonica*

Subpopulation	Number of trees	Number of isozyme bands per tree																Average ($m \pm \sigma_m$)
		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
I	41			7	3	10	6	8	5	2								11.68 ± 0.28
II	49			1	0	5	11	11	5	8	3	1	1	1	1	0	1	13.73 ± 0.35
III	62	1	2	1	5	13	12	7	9	7	1	2	2					12.53 ± 0.29

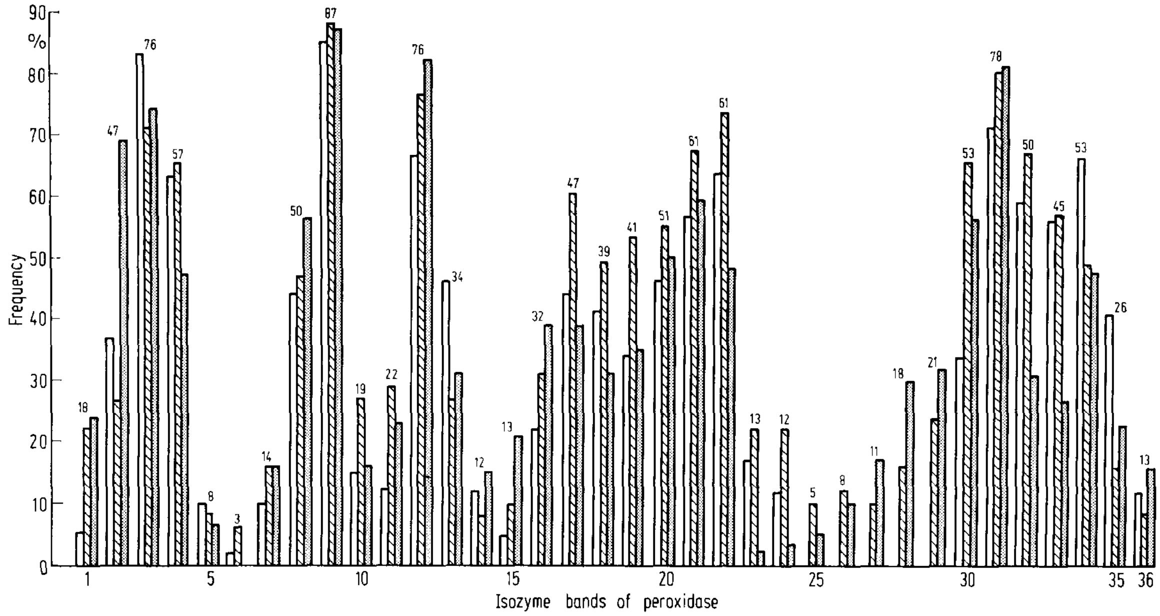

Fig. 3. Frequency in percent of 36 isozyme bands of peroxidase in three subpopulations of *Cryptomeria japonica* in Yanase natural forest. Open bar stands for subpopulation I, hatched bar, subpopulation II and mottled bar, subpopulation III. Figures above bars stand for average incidences in per cent of the numbered isozymes

$x_{ti}/N_t)/\sigma$, where x_{si} is the number of trees with *i-th* band in *s-th* population, while N_s the total number of trees investigated in the same subpopulation. The σ is $\sqrt{C(1-C)(1/N_s + 1/N_t)}$ where $C = (x_{si} + x_{ti})$ $(N_s + N_t)$. When x_i is less than 5, the χ^2 test by the use of 2×2 contingency table was adopted with a correction by Yates. It was found from these comparisons that certain bands showed significant differences between subpopulations. The number of statistically significant differences between I and II was 10 out of 36, which was the total number of isozyme bands, 12 between II and III, and 15 between I and III. The bands detected to be significantly different are shown in Table 2.

If these subpopulations were samples from a homogeneous population, then the number of pairs of isozyme bands which could show significant difference by chance would be less than 5% or $36 \times 0.05 = 2$. The actual number of statistically significant differences was 10 to 15, as shown above, thus it may be concluded that the three subpopulations were different from one another.

The magnitude of the differences among the three subpopulations was estimated by the measure of divergence or D^2 according to Berry (1963), which is calculated by $D^2_{s,t} = \sum_i \{(\Theta_{si} - \Theta_{ti})^2 - (1/N_s + 1/N_t)\}$, where $\Theta_{si} = \sin^{-1}(1 - 2p_{si})$ in which p is the frequency of occurrence of *i-th* band in *s-th* subpopulation. The D^2 calculated was 5.770 between I and II subpopulations and 5.434 between II and III, while it was 8.850 between I and III. It is thus concluded that the I and III subpopulations were very different in view of the distribution of isozyme bands of peroxidase.

Table 2. *Statistically significant differences in the frequency of 36 peroxidase bands among three subpopulations. Asterisk stands for a significant difference*

Band number	I – II	II – III	I – III	Band number	I – II	II – III	I – III
No. 1	*	—	*	Nos. 20, 21	—	—	—
No. 2	—	*	*	No. 22	—	*	—
No. 3	—	—	—	No. 23	—	*	*
No. 4	—	*	*	No. 24	—	*	—
No. 5	—	—	—	No. 25	—	—	—
No. 6	*	—	—	No. 26	*	—	—
Nos. 7—10	—	—	—	No. 27	—	—	*
No. 11	*	*	—	No. 28	*	*	*
No. 12	—	—	*	No. 29	*	—	*
No. 13	*	—	—	No. 30	*	—	*
No. 14	—	—	—	No. 31	—	—	—
No. 15	—	—	*	No. 32	—	*	*
No. 16	—	—	*	No. 33	—	*	*
No. 17	—	*	—	No. 34	—	—	*
No. 18	—	*	—	No. 35	*	—	*
No. 19	*	*	—	No. 36	—	—	—

Discussion

We have at present insufficient knowledge of the genetic basis for isozyme differentiation of peroxidase in *Cryptomeria japonica*. The comparative study among subpopulations within a natural stand of the species described in this paper is accordingly based on isozyme bands, tentatively marked with numbers (1–36).

The mountainous region of the Yanase district in Shikoku island, where the materials for this study were collected, is covered with continuous natural stands of *Cryptomeria japonica* often mixed with some broad-leaved species. The whole area spreads over 20,000 hectares. The forest is nearly continuous except for valleys, roads and open spaces where living quarters for forestrymen are located.

We do not know to what extent a natural forest of *Cryptomeria japonica* is genetically homogeneous. As described in the introduction to this paper, there are two opposing views of migration and isolation in a natural forest. J. W. Wright (1962) reviewed papers dealing with pollen migration and isolation of forest trees; among the authors cited were J. W. Wright, L. Strand, R. Silen, and W. Langner, who reported their investigations during the decade before 1962. The general view obtained by Wright from these authors was that the pollen of forest trees, mostly conifers, travels in quantity for relatively short distances. Ehrlich and Raven (1969) have recently reviewed a number of reports dealing with pollen dispersal. They consider that a plant will normally be pollinated with pollen from nearby sources; this would form the basis for nearly complete genetic discontinuity even in a wind-pollinated plant. All the authors mentioned above appear to consider that a subpopulation within a population is likely to be effectively isolated from others.

On the other hand, there are some botanists who insist that pollen can travel a long distance. For example, Andersson (1963) described the results of a few experiments which showed that pollen of Norway spruce and *Pinus silvestris* can, under certain circumstances, move over considerable distances. Sarvas (1967) believes that, in *Pinus silvestris*, pollination by trees growing in a neighbourhood within a radius of about 22 metres may attain 50%, while the remaining 50% would come from sources outside this area. It would be expected that this fairly high rate of gene flow in or between forests might prohibit racial differentiation, unless some other regional or local environmental selection pressure is operating.

Lanner (1966) emphasized the long distance dispersal of pollen in forest trees. He suggested, from a combination of fragments of evidence, that the mass movement of pollen is probably caused by a thermal or a vortex shell which traps masses of pollen. The shell is carried by winds for at least 24 to 32 kilometres. Such masses of pollen can be deposited on the earth by raindrops.

To summarize, as Libby, Stettler and Seitz (1969) have stated, two conflicting pictures of pollen migration exist: one considers that the distances of pollen dispersal are small, while the other insists that some long-distance migration is possible.

In the second report of this series, Sakai and Miyazaki (1971) described that, in *Thujopsis dolabrata*, genetically related trees were found growing in an area with a radius of 20 or 25 metres. This finding was based on a comparative study of the isozyme variation of peroxidase among individual trees. At any rate, the conclusion that trees in close proximity are likely to resemble each other may indicate that the distance of pollen dispersal is very small in a natural forest of *Thujopsis dolabrata*.

In our present work, it has been found that three subpopulations sampled within a natural forest of *Cryptomeria japonica* were genetically different from one another. The distances between these subpopulations ranged from 700 to 1,300 metres. Although ravines formed boundaries between these three subpopulations, and broad-leaved trees were admixed, they may not have been effective barriers against pollen dispersal. We may conclude that pollen migration in *Cryptomeria japonica* takes place only within short distances, resulting in the fairly effective isolation of subpopulation from subpopulation in a forest. Thus, one natural forest of *Cryptomeria japonica* could not be genetically uniform. In these circumstances, we may consider the practical aspect of forest reserves. It is the practice of foresters to appoint one single area within a stretch of natural forest to be the reserve for its genetic variability. It should be recommended, from the results of the present study, that several relatively small spaces should be designated as reserves instead of only one large area of the forest.

So far, a large number of investigations have been conducted on the geographic variability of forest trees. Evidence for local genetic differentiation within a forest, however, is quite scanty so far as the present writers are aware. Recently, Sakai, Miyazaki and Matsuura (1971) reported that two natural forests of *Thujopsis dolabrata*, which are separated by 10 kilometre wide straits, are different in the isozyme patterns of peroxidase and also in a few measurable leaf traits.

They suggested that the differentiation observed between the two populations of *Thujopsis dolabrata* might have been brought about by random genetic drift rather than by natural selection due to edaphic, climatic or geographic conditions.

The case reported in the present paper again may indicate the effect of random genetic drift and isolation by distance in a natural forest.

Conclusion

It is concluded from the present study that a natural forest of *Cryptomeria japonica* is not genetically homogeneous but may involve numerous subpopulations which are more or less genetically different from one another. A comparative study was made of three subpopulations with regard to the number of isozyme bands of peroxidase per tree, and the frequency of occurrence of 36 isozyme bands. The distances between these subpopulations were 700 or 1,300 metres. There have been two conflicting views on pollen migration and isolation in forests. From one point of view, the distance of pollen transportation is generally so small that there would be little gene flow within a forest, while from the other, there is no isolation among subpopulations due to the long distances over which pollen is dispersed. It should be noticed that all these opinions are based on the results of pollen dispersion studies, not on a genetic analysis of forest trees.

The results of our comparative study of electrophoretic variability among subpopulations support the former view, since they show that the subpopulations sampled in a forest are apparently differentiated. It is suggested that random genetic drift followed by effective isolation may have taken place in a natural forest of *Cryptomeria japonica*.

One area is often allocated in a natural forest to be left free of any artificial treatment in order to reserve its genetic variability. The present study indicates, however, that not one but several areas distributed widely should be allocated for this purpose, because one forest is as a whole not uniform genetically.

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Zusammenfassung

Die vorliegende Arbeit befaßt sich mit der genetischen Differenzierung innerhalb eines natürlichen Waldbestandes von *Cryptomeria japonica*. Von drei als Subpopulation bezeichneten, in Dreiecksform angeordneten Standorten wurden Nadeln von jeweils etwa 50 Bäumen geerntet. Der Abstand der Subpopulationen voneinander betrug 760 bzw. 1300 m. Mit Hilfe elektrophoretischer Untersuchung der Peroxydasen wurde gefunden, daß die durchschnittliche Zahl der Isozymbanden je Individuum 11.68 in I, 13.73 in II und 12.53 in III betrug. Der t-Test ergab eine signifikante Differenz zwischen I und II und zwischen II und III.

Die Häufigkeit des Auftretens jedes Bandes wurde zwischen den Subpopulationen verglichen. Es wurde beobachtet, daß die Variation zwischen diesen drei Subpopulationen statistisch signifikant war.

Es gibt nicht wenige Arbeiten, in denen über Untersuchungen zur Pollenverbreitung bei Koniferen berichtet wird. Einige der Autoren nehmen an, daß die Entfernung der Pollenverbreitung gering ist und daß der Genfluß zwischen Subpopulationen innerhalb eines Waldbestandes selten auftritt, während andere der Ansicht sind, daß Pollen über große Entfernungen verbreitet wird und eine wirksame Isolation innerhalb eines Forsts nicht möglich ist.

Die Beobachtungen der vorliegenden Untersuchungen unterstützen die erste Ansicht. Sie zeigen, daß Subpopulationen innerhalb eines Waldbestandes von *Cryptomeria japonica* genetisch voneinander verschieden sind, d. h., daß Sewall Wright's „Nachbarschaftsgröße" bei *Cryptomeria japonica* klein ist.

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Dr. K.-I. Sakai
Young-Goo Park
National Institute of Genetics
Yata 1111
Misima, Sizuoka-ken 411 (Japan)