Genetic Studies in Natural Populations of Forest Trees

III. Genetic Differentiation within a Forest of Cryptomeria japonica

KAN-ICHI SAKAI and YOUNG-GOO PARK

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 Thujaopsis 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
Kan-Ichi Sakai and Young-Goo Park: Theoret. Appl. Genetics

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

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 \( z = \frac{x_i - N \mu}{\sigma_m} \) where

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>Number of trees</th>
<th>Number of isozyme bands per tree</th>
<th>Average ( (m \pm \sigma_m) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>41</td>
<td>7 3 10 6 8 5 2</td>
<td>11.68 ± 0.28</td>
</tr>
<tr>
<td>II</td>
<td>49</td>
<td>1 0 5 11 11 5 8 3 1 1 1 0 1</td>
<td>13.73 ± 0.35</td>
</tr>
<tr>
<td>III</td>
<td>62</td>
<td>1 2 1 5 13 12 7 9 7 1 2 2 1</td>
<td>12.53 ± 0.29</td>
</tr>
</tbody>
</table>