# Clone Analysis and Genetical Study of Quantitative Characters in a Natural Forest of Cryptomeria japonica[1])

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## Introduction

It has repeatedly been pointed out by TIHARA (1918), IWASAKI (1927), OKIMURA, YAMANE and ONO (1961), ARITA, TOMITA, H. HAYASHI and KAMIYA (1964) and also by SATO, KATO and SUZAKI (1965) that Cryptomeria japonica in natural forests propagates either vegetatively or by seed. If so, the present forest of Cryptomeria japonica growing wild must be composed of vegetative propagules and seed progeny, which have arisen during the long term in the past. The purpose of the present study was to develop a method of distinguishing clonal groups in the natural forest, drawing up a map of distribution of clones and to estimate genetic parameters and also genetic variability.

## Materials of the Study

The investigated forest is located in the Shokawa District of Gifu Prefecture, and is called Mumai Natural Forest. The forest as a whole covers an area of some 10 hectares, but the investigated trees were collected from an area of some 750 square meters, 25 meters perpendicular and 30 meters parallel to the slope. The forest involves not only Cryptomeria, but also other conifers such as Chamaecyparis obtusa SIEB. et Zucc. and broad-leaved trees such as Fagus crenata BL., etc. We were informed that the forest was once cleared about 60 years ago. Therefore, most of *Cryptomeria* trees surviving in the forest today are supposed to have naturally reproduced since that time. Investigated were 266 Cryptomeria trees which had larger diameters at breast height than 5 cms.

## Part I. Clone Analysis

(I-1) Method of Analysis: —

Plants belonging to the same clone must have the same genotype except for seldom occurring spontaneous mutations. They should then have the same phenotype if the character is little affected by environment. In general, a character of an organism is understood as a cooperative product of genotype and environment, though the extent of environmental participation may be markedly different from character to character. If characters little affected by environment are chosen for comparison among trees, it may be possible to check whether there is such a close resemblance between two trees compared that they can be considered as propagules originated from the same mother tree or an isogenic clone.

An index for identifying isogenic trees in the forest has been constructed:

$$I_{j(i)} = 1 - \frac{\sum_{m=1}^{n} D^2_{m(j,i)}}{n}$$

where $I_{j(i)}$ is the identity index measuring the extent of similarity between i-th and j-th trees. The D, stands for the difference in the m-th character between the two trees under consideration, and it is defined in the following way: Let measurement of a character be designated by X, then, $D = X_i - X_j$, if $|X_i - X_j| \leqq 1$ or $D = 1$, if $|X_i - X_j| \geqq 1$. The n is the number of characters investigated. The characters should be those which are as little as possible affected by environment. For the rating of the character, three classes are used: +1, 0 and —1, or two classes: 1 and 0, by eye-judgement. For example, the needle-leaf is divided for its colour into three classes: dark (+1), medium (0) and light (—1), or into two classes for thickness: thick (1) and slender (0). It often happened that the classification into a class was difficult because of an intermediate appearance between two classes: In such a case, an intermediate value, ±0.5 was assigned to the character.

In the present study, we have investigated five characters, all of them distinctive traits of needle-leaves. They are: (1) twistedness, (2) angle of insertion, (3) stiffness, (4) density of needles on the twigs, and (5) intensity of colour. Twigs for the study were collected from the largest branch of each tree. Care was taken to take twigs growing at some distance from the distal end of the branch, because it is known that some characters of needle leaves vary according to their position in a tree (MURAI 1950). The computation of the identity index for j-th tree against i-th tree on the basis of those five characters, 1 to 5, is explained by the example given in the following table (*Table* 1).

*Table 1.* — Illustration by example of computation of the identity index between i-th and j-th trees on the basis of five characters, 1 to 5.

| | Character | | | | | $\frac{\Sigma D^2}{n}$ | $I_{j(i)}$ |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | |
| j-th tree (unspecified) | —1.0 | +0.5 | 0 | —1.0 | —1.0 | | |
| i-th tree (specified) | —1.0 | +1.0 | —1.0 | +1.0 | +0.5 | | |
| Difference | 0 | —0.5 | 1.0 | —2.0 | —1.5 | | |
| D (corrected) | 0 | —0.5 | 1.0 | —1.0 | —1.0 | | |
| $D^2$ | 0 | 0.25 | 1.00 | 1.00 | 1.00 | $\frac{3.25}{5}$ | 0.35 |

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If the two trees, i and j, belong to the same clone, all $D^2$ values may be exactly or approximately zero leading the I value toward unity. Accordingly, such a low value as 0.35 obtained in *Table 1* should be unterstood as indicative of i and j trees being not of the same clone. The problem then arises: what would be the lowest value of I which permits to regard the two trees concerned to be sibs from the same clone. Though there is no solid foundation for it, we have chosen 0.80 as the lowest value of I indicating clonal resemblance between the trees i and j. *Table 2* shows various cases of combination of ratings of 5 characters and the corresponding values of identity indexes. Cases (6), (8) and (9) in the table are understood to indicate that the two trees concerned are genetically different.

*Table 2.* — Several cases of combination of five ratings and their I values.

| Case | | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
|---|---|---|---|---|---|---|---|---|---|---|
| D | 0 | 5 | 4 | 3 | 2 | 1 | 0 | 4 | 3 | 2 |
| | 0.5 | 0 | 1 | 2 | 3 | 4 | 5 | 0 | 1 | 2 |
| | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| $\Sigma D^2/5$ | | 0 | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.20 | 0.25 | 0.30 |
| I | | 1.00 | 0.95 | 0.90 | 0.85 | 0.80 | 0.75 | 0.80 | 0.75 | 0.70 |

*Table 3.* — Variability of 5 needle characters.

| Character | -1* | -0,5 | 0 | +0,5 | +1 |
|---|---|---|---|---|---|
| Twistedness | 225 | 2 | 39 | 0 | 0 |
| Angle of insertion | 58 | 21 | 89 | 32 | 66 |
| Stiffness | 44 | 46 | 120 | 28 | 28 |
| Density | 88 | 52 | 92 | 22 | 12 |
| Intensity of colour | 17 | 21 | 98 | 34 | 96 |

*) —1 stands for least twisted, acute angle of insertion, soft needle, lax density or light coloured, respectively.

*Table 4.* — Correlation among five characters (d. f. = 264).

| | Twistedness | Angle of insertion | Stiffness | Density |
|---|---|---|---|---|
| Angle of insertion | —0.22** | | | |
| Stiffness | —0.32** | —0.06 | | |
| Density | —0.23** | —0.61** | 0.04 | |
| Intensity of colour | —0.12 | 0.06 | 0.13 | —0.03 |

(I-2) *Variation pattern of five characters:* —

It is preferable, first of all, to examine the variation pattern of characters investigated. *Table 3* shows the variability of five characters dealt with.

It is found from *Table 3* that, of the five characters, twistedness was comparatively undesirable because of its low variability. Mutual relationships between the five characters are shown in *Table 4*.

*Table 4* shows that angle of insertion of needles is correlated with density with $r = -0.61^{**}$. Twistedness was also found more or less negatively correlated with angle of insertion, stiffness and density, but the correlation coefficient was so low as $-0.2 \approx -0.3$. It is thus concluded that these characters except for twistedness were on the whole suitable for the study.

(I-3) *Analysis of clone distribution by the identity index:* —

Investigation of resemblance by means of the identity index showed that 266 trees in all could be divided into 39 groups including each more than 2 trees, and 13 single trees. The distribution of number of trees per group is found in *Table 5*.

*Table 5.* — Frequency distribution of number of trees per group.

| | Number of trees per group | | | | | | | | | | | | | | | Number of groups | Mean number of trees per group |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 11 | 12 | 13 | 18 | 19 | 28 | | |
| Number of clones | 13 | 9 | 7 | 3 | 3 | 5 | 3 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 52 | 5.12 |

*Figure 1* represents the distribution of groups of trees having the same phenotype. It is concluded that at least

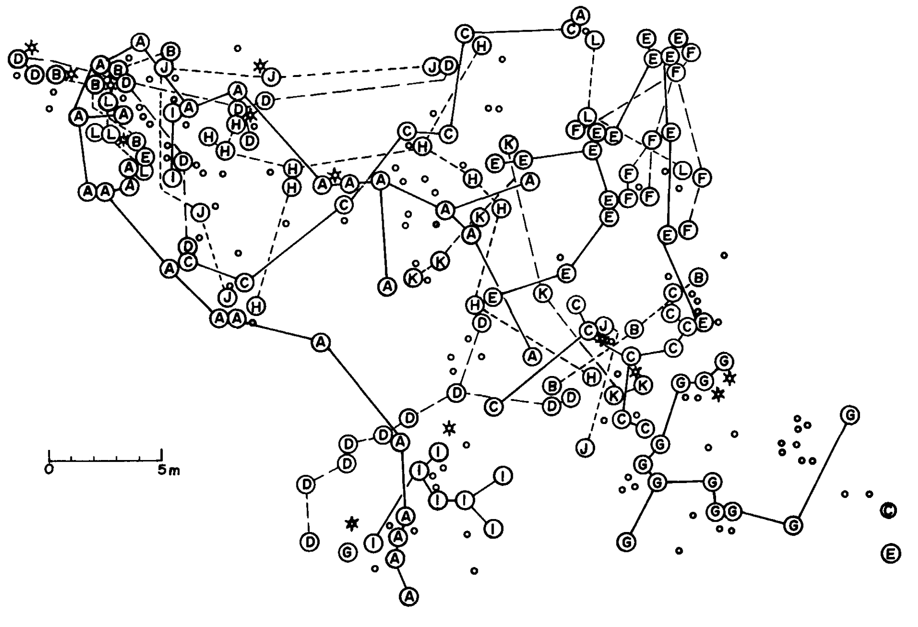

*Figure 1.* — Distribution map of trees of *Cryptomeria japonica* D. Don in the Mumai natural forest. Twelve groups or supposedly clones, A to L, are indicated. Asterisks show solitary trees.

12 groups marked in alphabetical order by A to L, are clones probably propagated by spontaneous rooting of branches of fallen tree or by shooting around surviving stumps.

## Part II. Genetical study of quantitative characters

### (II-1) *Method of study:* —

Since twelve clones have been identified in the forest, it is possible to estimate intraclone variance or environmental variance including some measure of competitional variance. Then, by subtracting the intraclone variance from the total or phenotypic variance of the whole population, we are able to obtain estimates of genotypic variance which yields heritability of a given character on the one hand, and the extent of genetic variability in the forest, on the other.

The characters investigated for the present purpose are those of needles: chord length of needle, height of arched needle, bendedness expressed as height of arched needle divided by chord length, and needle expanse or the width of expanse of needles on a twig (see *Figure 2*). These characters are not directly related to the characters which have been employed for the construction of the identity index.

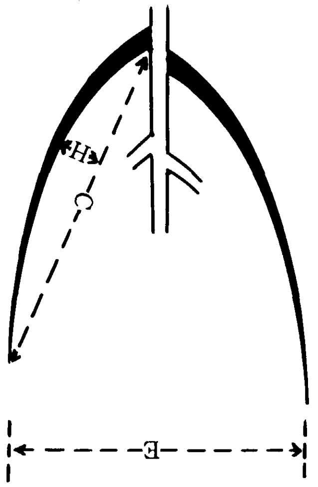

*Figure 2.* — Diagrammatic illustration of needle characters measured in the study described in Part II. — C: chord length of needles; H: height of arched needle; E: expanse of needles on a twig.

### (II-2) *Intraclonal variance and estimation of genetic parameters:* —

Measurement of intraclonal variance was performed with 12 groups which included at least 7 trees per group, since groups with less than six trees were considered to be not suitable for estimating intraclone variance.

*Table 6* represents 12 groups or clones each with more than 7 trees, their intraclonal variances for four characters and a computation of genotypic variance as well as heritability. It is found from *Table 6* that the intraclone variance is in nearly all cases smaller than the total variance: For chord length, 0.0109 $\approx$ 0.0366 with the pooled mean of 0.0198 of intraclonal variance against 0.0383 of total variance, for arch height, 0.0218 $\approx$ 0.1592 with the mean of 0.0581 against 0.2052, for bendedness, 2.1590 $\approx$ 6.6433 with the mean of 3.5302 against 9.6889, and for needle expanse, 0.0109 $\approx$ 0.0510 with the mean of 0.0204 against 0.0322. It happened that in the case of needle expanse, there were two groups which had larger intragroup variance than the total variance, i. e. B with 0.0510 and J with 0.0324. It is hard to explain at present why both groups showed larger variances than the total or the phenotypic variance. It might be due either to genotypically governed high instability in the expression of the character or to the two groups being not single or isogenic clones but genetic mixture. At all events, the heritability values were high for arch height and bendedness, but low for chord length and needle expanse. The heritability thus estimated is considered to represent the efficiency of selection in the forest, though the characters investigated herein are of little economic importance in themselves.

*Table 6.* — Main clonal groups including each more than 7 trees of *Cryptomeria japonica* in the Mumai Natural Forest and their intraclone variances in comparison with total variances, together with estimated values of heritability.

| Clone | Number of trees | Intraclone variance | | | |
|---|---|---|---|---|---|
| | | Chord length | Arch height | Bendedness | Needle expanse |
| A | 28 | 0.0269 | 0.0235 | 3.4116 | 0.0176 |
| B | 8 | 0.0242 | 0.1277 | 2.3200 | 0.0510 |
| C | 18 | 0.0160 | 0.0449 | 4.5826 | 0.0109 |
| D | 19 | 0.0180 | 0.0856 | 5.0333 | 0.0134 |
| E | 18 | 0.0111 | 0.0299 | 2.1590 | 0.0263 |
| F | 11 | 0.0202 | 0.0358 | 2.3700 | 0.0143 |
| G | 13 | 0.0239 | 0.1592 | 4.9017 | 0.0317 |
| H | 12 | 0.0109 | 0.0218 | 2.7146 | 0.0113 |
| I | 9 | 0.0366 | 0.0250 | 2.9511 | 0.0231 |
| J | 7 | 0.0229 | 0.0328 | 3.0500 | 0.0324 |
| K | 7 | 0.0141 | 0.0807 | 4.3350 | 0.0116 |
| L | 7 | 0.0162 | 0.0520 | 6.6433 | 0.0247 |
| Environmental variance, $V_E$ | | 0.0198 | 0.0581 | 3.5302 | 0.0204 |
| Total variance, $V_T$ | | 0.0383 | 0.2052 | 9.6889 | 0.0322 |
| Genotypic variance, $V_G$ | | 0.0185 | 0.1471 | 6.1587 | 0.0118 |
| Heritability, $h^2$ | | 0.4830 | 0.7169 | 0.6356 | 0.3665 |

### (II-3) *Study on genetic variability:* —

The heritability estimated in the last chapter speaks for the efficiency of selection of individual trees in a forest. It is of interest, however, to enquire into the genetic variability maintained in a forest. The problem here is to find what kind of genotypes for a given quantitative character are involved in the forest. The solution is found in Smith (1936), but a brief account of it will be given here.

Suppose that genotypic values (H) are distributed normally as in *Figure 3* with the mean $\bar{H}$ and the variance $V_H$. Transforming H to a variate u, with unit variance and

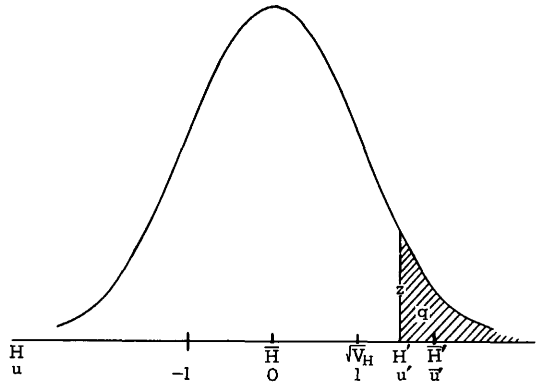

*Figure 3.* — Normal curve with mean, $\bar{H}$, and variance, $V_H$. $\bar{H}'$ is the average value of H's falling in the shaded area, q, or that of H's having greater values than $\bar{H}'$. — (After Smith, 1936 with a slight modification.)

mean at zero, that is, $u = \frac{H - \bar{H}}{\sqrt{V_H}}$, the average value of u falling in the shaded area q is given by

$$\bar{u}' = \frac{1}{q}\int_{u=u'}^{\propto} u \frac{1}{\sqrt{2\pi}} e^{-\frac{u^2}{2}} du = \frac{z}{q}$$

where z is the ordinate of the unit normal curve at the deviate u'.

Then, $\bar{H}'$ or the average value of genotypes in the shaded area is

$$\bar{H}' = \bar{H} + \frac{z}{q}\sqrt{V_H}$$

in which $\frac{z}{q}$ at the 5% point is 2.046 and that at the 1% point 2.64. The $V_H$ can be obtained from

$$V_m = V_H + \frac{V_E}{k}$$

$$\text{or} \quad V_H = V_m - \frac{V_E}{k}$$

where $V_m$ is the variance of mean values of various clonal groups, 52 in total, as given in *Table 5*. $V_E$ can be found in *Table 6* while k is given by

$$k = \frac{1}{a-1}\left[ N - \frac{\sum_{i=1}^{a} f_i^2}{N} \right]$$

in which a is the number of clonal groups or 52, N being the total number of trees in the forest or 266, and $f_i$ is the number of trees belonging to the i-th clonal group: $\Sigma f_i^2$ in the present study is (see *Table 5)*:

$$13 \times 1^2 + 9 \times 2^2 + 7 \times 3^2 + \ldots\ldots\ldots\ldots + 1 \times 28^2 = 2934.$$

Thus, k in this study is 4.98.

In *Table 7*, the results of the computation are given. From this table, we find that in the present forest, the mean value of genotypes of the top 5 and 1% of assumptive distribution is very high in comparison with the mean value of the forest: 2.34 vs. 1.17 for arch height or 21.69 vs. 11.01 for bendedness. In other words, it is theoretically expected that the present forest includes such clones (or single trees) that have so high genotypic values as given in *Table 7*. In this way, prediction of superior genotypes in the forest becomes possible.

*Table 7.* — Overall mean ($\bar{H}$) variance of mean values of 52 clonal groups ($V_m$) ,environmental variance ($V_E$) and variance of genotypic values ($V_H$) together with the mean value of genotypes of top 5 and 1% of the assumptive distribution ($\bar{H}'$).

| | Chord length cm. | Arch height mm. | Bendedness | Needle expanse mm. |
|---|---|---|---|---|
| Overall mean ($\bar{H}$) | 1.0008 | 1.17 | 11.0088 | 0.9179 |
| Variance of mean values of clonal groups ($V_m$) | 0.0297 | 0.2074 | 17.0691 | 0.0168 |
| Environmental variance ($V_E$) | 0.0198 | 0.0581 | 3.5302 | 0.0204 |
| $V_{E/k}$ | 0.0040 | 0.0117 | 0.7089 | 0.0041 |
| $V_H$ | 0.0257 | 0.1957 | 16.3602 | 0.0127 |
| $\sqrt{V_H}$ | 0.1603 | 0.4424 | 4.0448 | 0.1127 |
| The mean genotypic value ($\bar{H}'$) | | | | |
| of top 5% | 1.3288 | 2.0752 | 19.2845 | 1.1485 |
| of top 1% | 1.4240 | 2.3379 | 21.6871 | 1.2154 |

## Discussion

A mathematical approach to estimating resemblances between two organims has been often undertaken in ecology and taxonomy. Sokal and Sneath (1963) have in their book reviewed various indices of similarity forwarded by different authors. Recently, Goodall (1966) and McIntosh (1967) have discussed similar indices from the biometrical viewpoint. Those indices were investigated for the purpose of evaluating the degree of resemblance between populations or organisms in order to detect to what extent they are related with each other. In taxonomy, for example, any two forms are compared on the basis of a number of characters, more than sixty if possible, for detection of closeness or remoteness in the phylogenetic relationship between them.

In the present study, however, the purpose was to find out if two trees are so much alike as to be regarded as sibs from the same clone, or not. Offspring propagated vegetatively from the same trees are without doubt of the same genotype except for infrequent somatic mutations. If we assume that the effect of mutation is negligible, then the comparison between trees for characters which are little affected by environment may allow us to identify trees of the same genotype in the forest. If is needless to say that the more characters are included in the comparison, the more effective is the identification.

If we take n characters each having three classes of +1, 0 and —1, the possible number of distinguishable types theoretically expected on the basis of their combinations is $3^n$. In our study, five characters were used, one having 2 classes while the remaining four had three classes. If we assume that they combine at random, though in fact some were partially correlated, the number of types expected from their combination would be 162. The actual number of different types recognized in the forest was 52 or about 32% of expectation was realized. Increase if possible in number of characters compared is without doubt highly desirable, but it seemed practically difficult: Characters of flowers or cones can not be used for the study, because there is a considerable number of juvenile trees which are too young to bear flowers or cones.

There are a few studies on the reproduction system in a natural forest of *Cryptomeria japonica*. Tihara (1918), Iwasaki (1927), Okimura, Yamane and Ono (1961) and Sato, Kato and Suzaki (1965) observed that *Cryptomeria japonica* in a natural forest propagates sexually as well as vegetatively. Vegetative propagation can, according to them, take place in various ways: Layering or rooting on branches which are still connected to the living or felled tree and cuttings, spontaneously occurring in the forest. They all consider that layering is most common in the natural forest while propagation by seed is of rare occurrence. Arita, Tomita, Hayashi (H.) and Kamiya (1964) have investigated in detail the ecological situation of Mumai natural forest, the same one investigated by the present authors. They observed that in the forest vegetative propagation was occurring around the stumps and along the felled trees.

In the present study, we have succeeded in dividing 266 trees growing in an area of some 750 m² of Mumai natural forest into 52 types, 13 of which represented by single trees, whereas 12 types included 7 to 28 individuals per group and the remaining 27 types included 2 to 6 trees per group. Since groups including more than 7 trees seemed of more interest than groups of smaller size, the twelve groups were submitted to further study. As shown in *Figure 1*, they appeared to form rather clusters, more or less isolated from the other clusters, than being widely dispersed throughout the forest. This distribution pattern of sib-trees leads us to accept as the most probable the view forwarded by the foresters, as described above, that vegetative propagation in natural forests takes place by layering from tree stumps or felled trees layed down by natural or artificial forces.

As to small groups which include only a few trees it is difficult to decide whether they have arisen sexually or vegetatively. If we provisionally assume that groups including more than 7 trees would be most certainly vegetative clones, then there were 12 clones with 157 trees in total.

The method of measurement of environmental variance by the aid of detected clones in the natural forest allows us to estimate genetic variance, heritability and also genetic variability of a forest where trees of different ages are growing in random combination and at various densities. It has been pointed out by SAKAI, MUKAIDE and TOMITA (1968) that heritability estimated from the comparison between seed-propagated and clone forests as was usually performed would probably be an overestimation due to the effect of intergenotypic competition involved in the estimated value of genotypic variance. It is interesting to notice that the environmental variance measured in a natural forest in the way described in the text of this article may involve partly the competitive effect though not to its full extent, since trees of different genotypes are rather randomly distributed in the forest growing side by side. In other words, the heritability values obtained in such a way if they were overestimated, would be so to a lesser extent.

The results of such studies may be useful for various purposes of genetic researches. Heritability would be helpful for the effective selection of individual trees. Of more interest, however, would be to ascertain the genetic value and genetic property of natural forests. We hope, if possible, to learn which of the natural forests of *Cryptomeria* distributed in various districts of Japan could be the source of the most valuable genetic stocks. We want to know what is the extent of genetic variability of a given natural forest, because forest growers quite frequently collect their planting materials from such forests.

Further studies on natural forests of *Crytomeria japonica* on the basis of demonstrated clones may be of interest from various viewpoints.

## Conclusions

Conclusions drawn from the present study are as follows:

(1) With the aid of an identity index constructed from five characters more or less free from environmental influence, it was possible to classify trees of *Cryptomeria japonica* in a natural forest into a number of groups.

(2) The 266 trees growing in Mumai natural forest have been classified into 52 types, 13 of them were represented by single trees, and 27 by 2 to 6 trees; the remaining 12 types were larger groups including each more than 7 trees with the maximum number of 28 trees per group.

(3) Trees of the same group tend to form a cluster, of a linear or circular form, which is more or less isolated from the other groups. Such distribution seems to indicate that they have arisen vegetatively by layering or drooping branches, shooting around a stump or rooting of branches on a felled tree.

(4) Measurements were performed of variances of some quantitative characters which were different from those employed before for the construction of the identity index. The intraclone variances are mostly smaller than the phenotypic variance of the whole forest.

(5) Since the intraclone variance is considered to be nonheritable or environmental, it is possible to estimate the genotypic variance by subtracting the intraclone variance from the total or phenotypic variance. Thus, heritability or the efficiency of selection of individual trees in the forest was estimated.

(6) Genetic variability maintained by the forest could also be estimated.

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## Summary

With the aid of an identity index constructed on the basis of five characters of needle leaf, the writers could divide 266 trees of *Cryptomeria japonica* growing in Mumai natural forest into 52 types. Twelve of them were found to include more than seven individuals and one of them comprised as many trees as 28. Trees included in the same group tended to form a cluster, linear or circular, being more or less isolated from the other groups in the forest (see *Figure 1*). Such a distribution pattern leads us to supposing that they are clones propagated vegetatively by layering or drooping branches, shooting around stumps or branches on the felled trees. That they could be regarded as clone has been evidenced by the apparently small intragroup variance in comparison with the phenotypic or total variance.

By the use of intraclone variance or evironmental variances, genotypic variance and heritability were estimated for a few quantitative characters. Genetic variability or genetic potentiality of the natural forest was also estimated by measuring the distribution of genotypic merits of different types.

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