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STUDIES ON THE BREEDING STRUCTURE OF TREE SPECIES IN THE TROPICAL RAIN FOREST. I: FAMILY CLUMPS AND INTRAPOPULATION DIFFERENTIATION

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ABSTRACT

Breeding structures of two tropical rain forest tree species, *Altingia excelsa* in Java and *Agathis borneensis* in Kalimantan were investigated. Assuming that similarity in the assortment pattern of the isoperoxidase bands tells genetic relationship between trees, on the one hand, and that inbreeding increases smaller values of the disagreement counts, on the other, it has been concluded that inbreeding occurs considerably in *Altingia excelsa* and to some extent in *Agathis borneensis*. Finding that trees showing very low disagreement counts are located close to each other, they were grouped as an assumptive family. It was found that different families were quite dissimilar with respect to isoperoxide constitution and in several leaf characters as well. The distance between two trees at which they can mate is estimated to be 16 to 18 meters or 16.5 meters and the area one family occupies is 200 to 250 m^, assuming that a family clump can be a breeding unit in *Altingia excelsa,* within which trees mate at random. Some families were distributed mixed with each other within the mating distance, but they were found still genetically differentiated from each other. This reproductive isolation among families is interpreted to be due to genetic differences between families in flowering time.

In *Agathis borneensis,* there was no indication of family clump formation. Related trees may have been widely scattered in the forest, and the inbreeding of the species may be due to self-fertilization of individual trees and not to outcrossing between relatives.

INTRODUCTION

One of the characterisitic features of a tropical rain forest is its high species diversity. It stimulates our interest to inquire into two relevant problems. One is how trees could propagate in such a forest with an extremely low population density for each species, or what the breeding system of tropical tree species is.

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Another problem is how speciation takes place in such a tropical rain forest of high diversity of species. In the meantime, we recollect two hypotheses already advanced concerning the speciation problem: one is the so-called genetic drift hypothesis by Fedorov (1966), and another is the selection hypothesis by Ashton (1969). Fedorov assumes that a very small number of conspecific trees with asynchronous flowering among them should naturally induce each individual tree to propagate by self-fertilization which after many generations results in speciation by genetic drift. Ashton, on the contrary, assumes that notwithstanding more or less difficulty in outbreeding, it would still occur frequently enough to allow gene exchange throughout populations in a continuous habitat causing speciation as a result of allopatric differentiation between populations in response to differential selection pressures.

This paper describes results of an investigation into the breeding structure of two species, *Altingia excelsa* Noronha (Hamamelidaceae) in Java and *Agathis borneensis* Warb. (Araucariaceae) in Kalimantan,with an aim to find a way to pierce an opening to the problem of speciation.

MATERIALS AND METHODS

Two natural forests were investigated. The stand in which *Altingia excelsa* was investigated was located on the outskirts of the village of Ciwidey near Bandung in Western Java. All trees with diameter at breast height (DBH)

exceeding 6 cm, growing in quadrat of 50 x 50 m in the stand were measured for their growth, and their position in the quadrat was mapped on a section-paper. In all, 148 trees of various species were counted, among which 38 were trees of *Aliingia excelsa.* Twigs with mature leaves were collected from each of these 38 trees for laboratory research. The mature leaves from each tree were divided into two parts, one for measurement of several leaf characters while the remaining part for the electrophoretic analysis of the isoperoxidases. Preliminary studies showed that the zymogram patterns for acid phosphatase and esterase were rather simple and thus only peroxidase was used in this study.

Agathis borneensis was sampled from a quadrat of the same size plotted in a natural forest of International Timber Corporation Indonesia in Kalimantan. There were about 230 trees with DBH exceeding 6 cm of various species in the quadrat, of which 21 *were Agathis borneensis.* Growth measurement and mapping of individual trees were made and their leaves were collected for the study of leaf characters and eletrophoretic analysis.

Leaf characters measured in both species were leaf length, leaf width, leaf size (length x width), number of veins, and length and thickness of petioles.

Methods applied for the electrophoretic analysis were as follows: Enzyme extractant M4 containing 10% Triton X-100, 0.5 M NaCl Tris and 0.2 M ascorbic acid, was adjusted with acetic acid to pH 7.5. One hundred mg of finely cut leaf pieces were crushed in a mixture of 0.5 ml of the extractant, 100 mg of quartz sand and 50 mg of polyvinyl-polypyrrollidone (Endo 1981). This system proved to be generally suitable for the extraction of peroxidase in *Altingia* as well as in *Agathis.* Starch gel electrophoresis was subjected to the system modified by Brewer (1970). The gel buffer contains 0.42 g of histidine HC1 and 0.072 g of NaOH per 0.5 1 mixed with 60 to 66 g of hydrolyzed potato starch, with the final pH of the solution around 6.1. The tray buffer was with a pH of about 6.0 and it contained 117.6g(or 0.6 M) trisodium citrate and 9.4 g of citric acid per liter. The gel mold of Toyo Model HA-1 devised by Endo (1968) was used. Electrophoretic run was done under constant 250 voltage per 20 cm for 4.5 hours in a refrigerator with an ice cooling box. Reaction mixture of peroxidase stain contains 4 mM eugenol, 4 mM 3-amino-9-ethylcarbazole in 10% acetone solution at final concentration, and 0.03% hydrogen peroxide, and 0.02M Tris-acetic acid buffer, pH 4.0 (Endo 1978). The

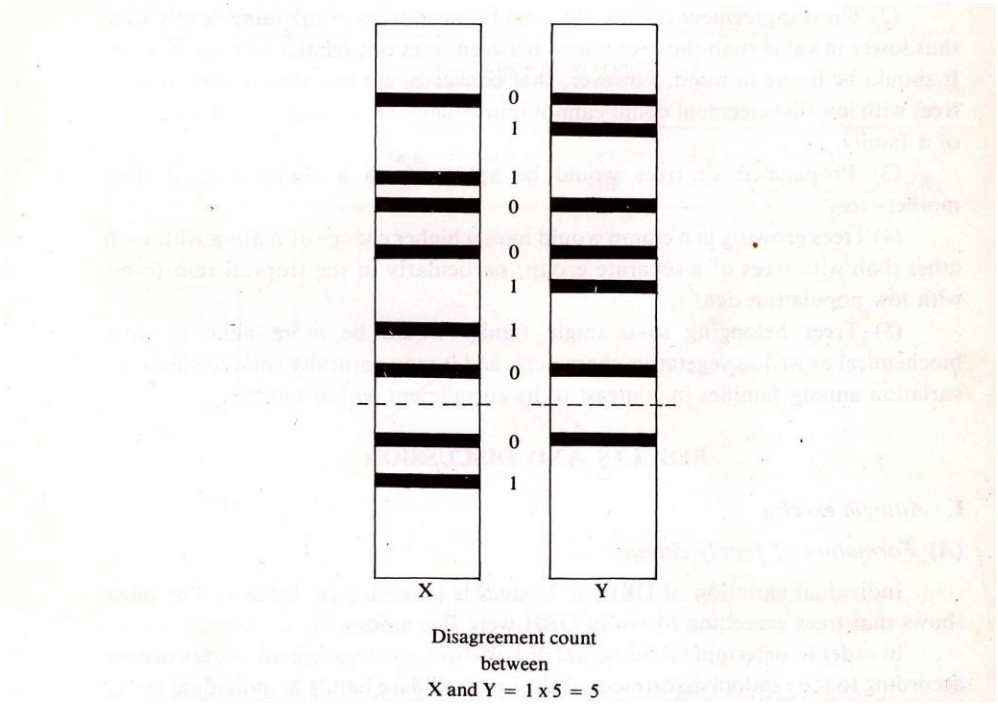

Figure 1. Schematic explanation for measuring the disagreement count between X and Y.

isoperoxidase zymograms of 38 *Altingia excelsa* and 21 *Agathis borneensis* trees are schematically drawn in Appendices 1 and 2.

On the assumption that the pattern of the isoperoxidase bands of a tree would be nothing but the reflection of its genetic make-up, genetic similarity was measured among trees with the aid of the disagreement count which gives the total number of missing mate to each band between a given pair or trees (Sakai & Miyazaki 1972). In practice, there may occur variation in activity of bands, but it was neglected for the present study. Figure 1 explains the method of calculation of the disagreement counts between two zymograms.

Detection of family structure of a tree species in a natural stand with the aid of the disagreement counts was made on the following premises:

(1) It was assumed that the plural isoperoxidase bands appearing in the population would be distributed at random among individual trees if mating occurs at random, while inbreeding, either self-fertilization or mating between relatives, would tend to produce the isoperoxidase combination of the parental type.

(2) The disagreement counts obtained between trees of the same family were thus lower in value than those obtained between trees not related with each other. It should be borne in mind, however, that converses are not always true; that is, trees with low disagreement count cannot immediately be regarded as sib-members of a family.

(3) Propagated sib-trees would be apt to form a clump around their mother-tree.

(4) Trees growjng in a clump would have a higher chance of mating with each other than with trees of a separate group, particularly in the tropical rain forest with low population density.

(5) Trees belonging to a single family would be more alike in their biochemical as well as vegetative characters, and it may naturally enlarge character variation among families in contrast to its curtailment within families.

RESULTS AND DISCUSSION

I. *Altingia excelsa*

(A) Formation of family clumps

Individual variation of DBH of 38 trees is presented in Table 1. The table shows that trees exceeding 61 cm in DBH were five among 38, or 13%.

In order to determine if the actual distribution of disagreement counts occurs according to the random assortment of the isoperoxidase bands in individual trees, we calculate the theoretical expectation of the latter (Appendix 3).

The comparison between theoretical expectations and observed disagreement of bands by the method described is given in Table 2. Data presented in Table 2 show an apparently excess occurrence of observations in the lower classes of 0, 1 and 2, suggesting that the fertilization in *Altingia excelsa* is not panmictic, but a fair amount of inbreeding could be occurring.

Table 1. Frequency distribution of DBH in 38 trees of ***Altingia excelsa*** growing in a 50 x 50 m quadrat in the Ciwidey natural forest.

	DBH in cm										Total
	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	
Number of trees	1	4	10	2	12	4	1	2	1	1	38
Frequency in percent	13.16			73.68				13.16			100

Table 2. Comparison of the actual observations with the theoretical expectations of disagreement counts in ***Altingia excelsa***.

	Disagreement count						Total
	0.1	2	3	4	5	6	
Expectation	18.7	54.6	110.7	152.8	152.1	214.1	
Observation	51	70	95	116	117	254	703

X^2 = i th, μ d.f. = 5 $p < 0.01$

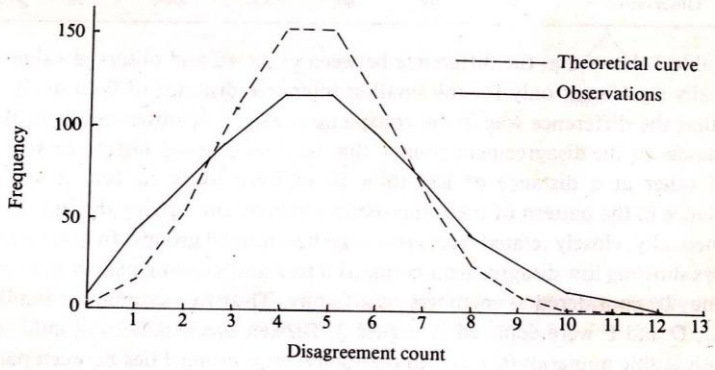

Figure 2. Comparison between observed and expected frequencies of disagreement counts in ***Altingia excelsa***.

In Figure 2 we find that actual observations exceeded the theoretical expectations in such lower classes as 0, 1 and 2, and also in those of higher classes exceeding 8. That disagreement counts of several smallest and largest values appeared more frequently than theoretical expectation suggests that inbreeding on the stand tended to construct two contrary groups of trees, one with very similar isoperoxidase patterns and another with highly dissimilar ones.

Inbreeding in a natural forest can occur in two ways: self-fertilization of individual trees and crossing among relatives. For the latter to occur, trees belonging to the same family growing together in a clump would be more advantageous.

Are consanguineous trees of the same family really growing in a clump?

In order to answer this question, the relationship between inter-tree distance and disagreement counts was investigated (Table 3).

Table 3. Relationship between disagreement counts and inter-tree distances in *Altingia excelsa*.

Inter-tree distance (m)		Disagreement count				Total	X^2	p
		0.1	2.3	4.5	6<			
0-10	Expectation	6.7	21.6	30.5	33.3			
	Observation	15	25	25	27	92	13.17	<0.01
11-20	Expectation	123	39.8	56.3	61.6			
	Observation	11	29	70	60	170	6.43	>0.05
21-30	Expectation	14.3	46.4	65.5	71.7			
	Observation	15	42	58	83	198	3.05	>0.20
31<	Expectation	17.7	57.2	80.8	88.4			
	Observation	10	69	80	85	244	5.90	>0.10

Table 3 shows that the difference between expected and observed value was statistically significant only for the smallest inter-tree distance of 0-10 m. It was found that the difference was in the conspicuous excess of observations in the 0 and 1 classes of the disagreement counts, that is, trees growing nearby or so close to each other at a distance of less than 10 m were likely to bear a striking resemblance in the pattern of the isoperoxidase zymograms, giving the impression that genetically, closely related trees grow together in small groups. In other words, tree pairs showing low disagreement count as 0 to 2 and growing nearby in natural forest may be considered as members of a family. Thus five assumptive families: A, B, C, D and E were depicted in Figure 3. Broken lines connecting individual trees with arabic numerals 0, 1 or 2 in the figure are presumed ties between parent and child trees, the numerals indicating the corresponding disagreement counts. Until trees in question are proven to be related members of genetically separate

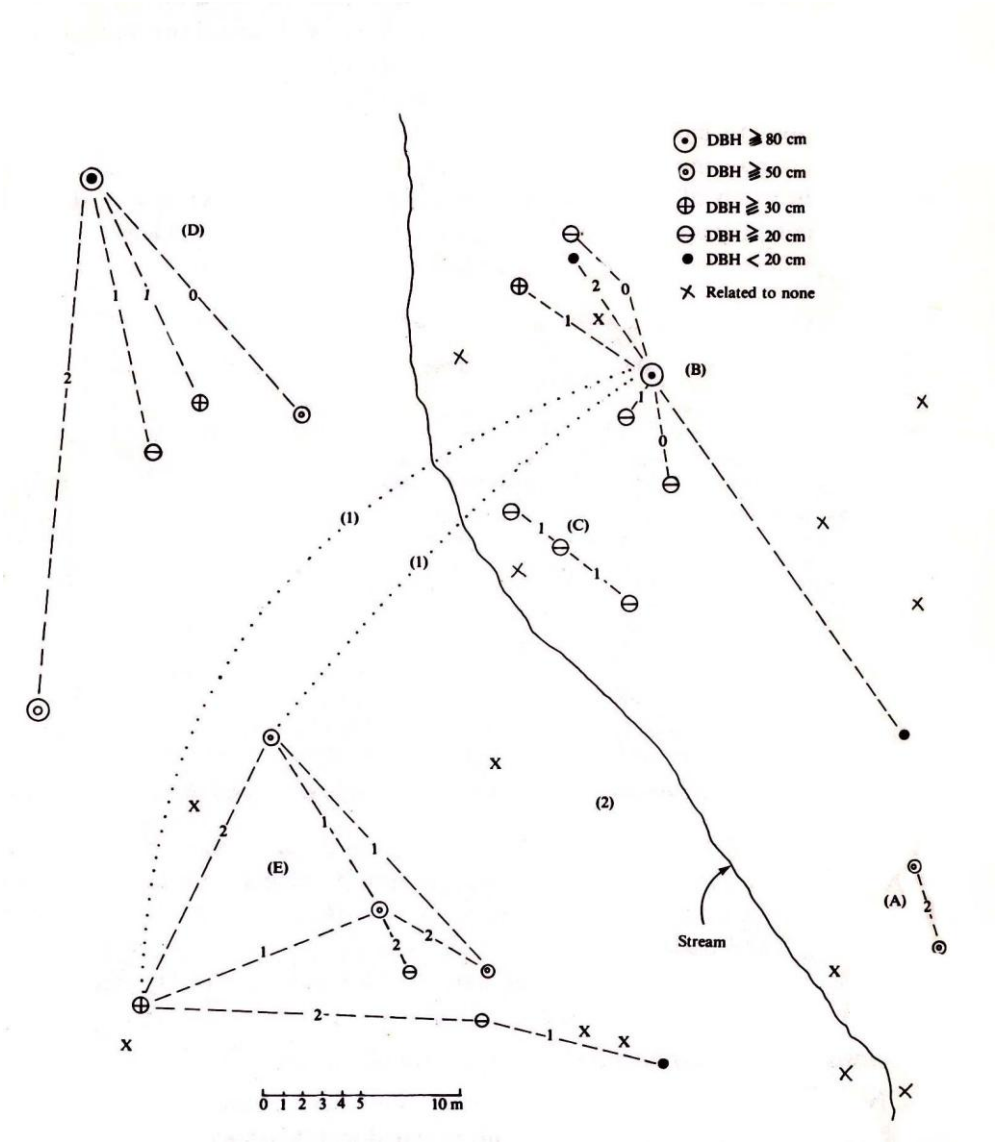

Figure 3. Family clumps *inAltingia excelsa.* Arabic numerals represent disagreement counts, while those in parentheses disagreement counts between trees of presumed relation.

families, the "assumptive families" designated above will be called merely as tree "groups" for the time being. Of the five groups, two, i.e. B and E are worthy of note because both have the largest groups, each with 7 trees. The largest trees of both groups were connected with small disagreement count 1 or 2, indicating that the two groups are more or less related or maybe descendants of a common progenitor. Of more interest is that both groups appeared to show a within-group segregation in width of leaves (Table 5). Although we are not yet in a position to speak definitely, it is supposed that B and E groups might be both heterozygous for a gene probably with major effect on leaf width.

(B) Differentiation in the constitution of isoperoxidase components

The occurrence of various isoperoxidase bands in trees of five groups was examined (Table 4).

Table 4. Interpopulation differentation in distribution of isoperoxide bands

Group	Number of trees	Isoperoxidase band (%)*							
		a	c	d	e	f	g	k	m
A	2	100	50	100	—	100	—	100	—
B	7	100	—	100	—	100	—	—	100
C	3	—	—	—	100	—	100	100	100
D	5	100	100	—	—	100	60	—	100
E	7	43	43	—	—	100	—	—	80

*) The maximum number of isoperoxidase bands per tree in *Altingia excelsa* was 16.

It was found that trees of each group were characterized by specific isoperoxidase bands. For instance, C group is very peculiar in not having such bands as a and / which are rather ubiquitous in *Altingia excelsa.* Instead, it possessed *e, g,* and *k* bands which are more or less uncommon to the other groups. Table 4 shows that those five groups were very variable in their biochemical characteristics demonstrating an intrapopulation genetic differentiation.

(C) Intrapopulation differentiation in vegetative characters

Now we should further inquire into differentiation among groups in some leaf characters. Six leaf characters were investigated and their variation is given in a form of frequency distribution in Table 5. It was noted that in almost all characters, variation among these groups was apparent. B group had the biggest size in many leaf characters, E the next, while C and D groups were the smallest, leaving A intermediate. The analysis of variance of the leaf characters is shown in Table 6.

Table 5. Variation of six leaf characters in five family groups of *Altingia excelsa*

Character	Family	Number of trees	Class value* 1	2	3	4	5	6	7	Mean
Leaf length (mm)	A	2	1	—	—	—	1	—	—	115.8
	B	7	—	—	1	2	2	1	1	134.9
	C	3	1	2	—	—	—	—	—	104.4
	D	5	1	2	2	—	—	—	—	109.8
	E	7	1	2	1	2	—	1	—	117.8
Leaf width (mm)	A	2	—	1	—	—	1	—	—	50.7
	B	7	—		—	2	—	5	—	60.9
	C	3	—	1	1	1	—	—	—	48.1
	D	5	—	2	3	—	—	—	—	47.2
	E	7	—	—	4	—	—	3	—	53.7
Leaf size (mm^2)	A	2	1	—	—	—	1	—	—	60.0
	B	7	—	—	—	2	2	2	1	83.1
	C	3	1	2	—	—	—	—	—	50.3
	D	5	1	2	2	—	—	—	—	52.4
	E	7	1	2	1	1	1	1	—	64.3
Number of leaf veins	A	2	—	1	1	—	—	—	—	13.0
	B	7	—	5	2	—	—	—	—	12.5
	C	3	—	1	2	—	—	—	—	12.9
	D	5	—	2	2	1	—	—	—	13.3
	E	7	—	2	4	1	—	—	—	13.5
Petiole length (mm)	A	2	—	—	—	—	1	1	—	36.0
	B	7	—	—	2	5	—	—	—	27.4
	C	3	—	3	—	—	—	—	—	18.1
	D	5	—	2	2	1	—	—	—	22.5
	E	7	—	3	3	1	—	—	—	23.1
Petiole thickness (1/100 mm)	A	2	—	1	1	—	—	—	—	106.5
	B	7	—	—	1	2	1	2	1	130.9
	C	3	—	3		—	—	—	—	103.3
	D	5	1	1	2	—	—	1	—	110.9
	E	7	1	1	2	1	2	—	—	112.4

* One unit of class value corresponds to 10 mm for leaf length, 5 mm for leaf width and petiole length, 10/100 mm for petiole thickness, 10 mm for leaf size and 2 for vein number.

Table 6. Analysis of variance of some leaf characters of 5 family groups of *Altingia excelsa*.

Source	d.f.	Mean squares					
		Leaf length	Leaf width	Leaf size	Vein number	Petiole length	Petiole thickness
Between families	4	7179*	1708*	9407*	10.5^{ns}	1186**	6073*
Within families	19	2039**	307**	1875**	13.2**	86**	1988**
Within trees	216	118	34	149	1.4	9	172

Significant at the 5%* and 1%** levels, respectively. ^{ns}) Non-significant.

From Table 6 we find that vein number was not significantly variable among groups, but measurements in leaves and petioles were all significantly variable.

From the facts described above, one can conclude that groups A to E defined up to now are clumps of trees of single families or family clumps, each of which is being genetically differentiated from others.

For genetic differentiation among clusters within a population to occur, it is necessary that propagation is by mating within the same, but not between different families. If one assumes that trees of a single family form a breeding group within which mating occurs at random, then what would be the area occupied by a group in a natural stand? Of the five families depicted in Figure 3, families A and C are not included in the following discussion because of too small number of trees in them. One notices in Figure 3 that in each of the three remaining families, one tree always stood relatively far from the others of the same family. Thus, the inter-tree

Table 7. Average inter-tree distances in five families of *Altingia excelsa*.

Family	All trees are considered				The farthest tree is excluded			
	Number of		Distances (m)		Number of		Distances (m)	
	Trees	I.D.*	Mean	SD	Trees	ID	Mean	SD
A	2	2	4.14	—	—	—	—	—
B	7	21	12.42	9.56	6	15	7.09	8.71
C	3	2	3.50	—	—	—	—	—
D	5	10	13.97	7.00	4	6	10.10	5.27
E	7	21	12.57	6.61	6	15	10.79	5.71
Mean (B, D and E)	6.33	17.33	12.99	7.75	5.33	12.00	9.33	4.90

* Inter-tree distance.

distance was measured in two ways: one for all trees, and another for all trees but one standing apart. The results are presented in Table 7 and Figure 4.

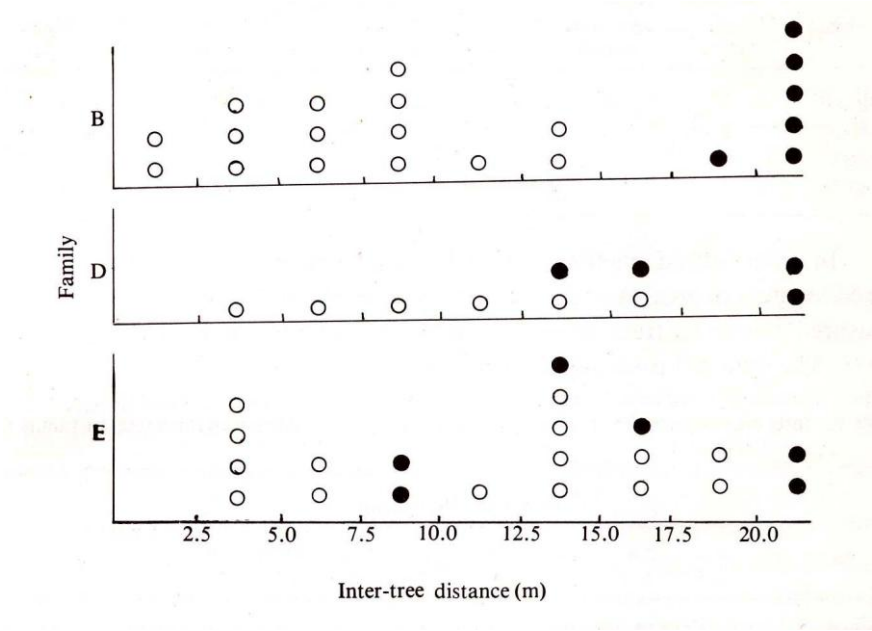

Figure 4. Distribution of inter-tree distances in three families of *Altingia excelsa.* Black circles are those between the farthest tree and the remaining ones in each family.

The distribution of inter-tree distances in three families is shown in Figure 4, wherein distances involving the farthest tree are depicted in black circles. Excluding the black circles, one may state that the mean distance of the mean plus one standard deviation as the family distance for E = 10.79 + 5.71 = 16.5 m (Table 7). This distance is expected to include 84% of cases in a theoretical normal distribution and could be taken as the mating distance in *Altingia excelsa,* if we assume 16 to 18 meters as the mating distance, then the area as a circle of a family clump in the species is roughly estimated to be $\pi r^2 = 200$ m^2 to 250 m2 with r = 8 to 9 meters.

If 16 to 18 meters is the mating distance, then why do B and C families look genetically isolated from each other in spite of growing mixed together in the forest (Figure 3)?

We have already concluded that the two families B and C are very different biochemically as well as morphologically. Table 8 shows the distribution of the disagreement counts obtained between trees within and between B and C families, based on which we have distinguished B from C. It indicates that both families are very dissimilar in the assortment pattern of the isoperoxidase bands.

Table 8. Variation in disagreement counts between trees within and between B and C families.

Families	Number of trees	No. of disagreement counts	Disagreement count									Mean
			0	1	2	3	4	5	6	7	8	
Within B	7	42	8	20	10	4	—	—	—	—	—	1.26
Within C	3	6	—	4	2	—	—	—	—	—	—	1.33
Between B and C	—	21	—	—	—	—	—	5	8	6	2	6.24

In order to find whether trees of both families could be regarded as growing mixed together in an area of the mating distance, the inter-tree distances have been measured among six trees excluding the farthest one of the B family and three of the C. The data are presented in Table 9.

Table 9. Inter-tree distances of six trees* in family B and distances between three trees of family C and six of family B.

	Inter-tree distance (m)								Total	Mean	SD
	0.1-2.5	2.6-5.0	5.1-7.5	7.6-10.0	10.1-12.5	12.6-15.0	15.1-17.5	17.6-20.0			
Within B	2	3	3	4	1	2	0	0	15	7.09	3.71
Between B and C	0	0	3	3	3	4	4	1	18	12.25	3.98

* One farthest tree was discarded.

The inter-tree distance between the two families are from 5 to 20 meters, but 13 of 18 or 72% of the inter-tree distances was less than 15 meters, and 17 of 18 or 94% was less than 17.5 meters, which are for the present understood as within the range of the mating distance for *Altingia excelsa.* Why are trees of both families isolated sexually in spite of growing within the range of 16 to 18 meters? At present we guess that the two families may not flower at the same time inducing sexual isolation between them. Details of the argument is given in the discussion.

II. *Agathis borneensis*

A comparison was made between theoretical distribution of disagreement counts and the actual observations (Table 10).

From Table 10 one notes that the number of actual observations appeared to exceed the expectations of the disagreement counts in the respective classes of 0 and 1, and that of 6 or more although the excess is not enough to reach the level of statistical significance. In Figure 5, we find that the general situation of the curve is

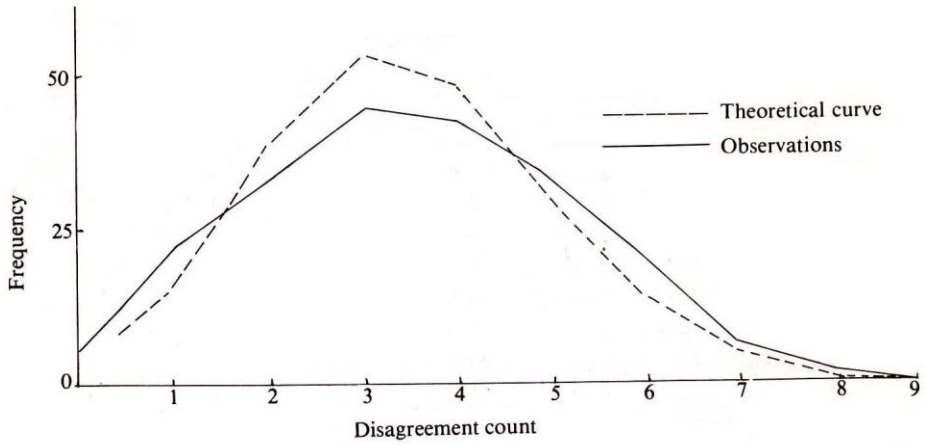

Figure 5. Comparison between observed and expected frequencies of disagreement counts in *Agathi.s borneensis.*

Table 10. Comparison between theoretical expectations and actual observations of disagreement counts in *Agathis borneensis*

	Disagreement count						Total
	0.1	2	3	4	5	6 ≦	
Expectation	20.4	39.5	53.7	48.1	29.8	18.3	—
Observation	28	34	45	42	33	28	210

$X^2 = 11.01$ d.f. = 5 p = 0.05—0.10

similar with that of *Altingia,* and it is considered that inbreeding occurs to some extent in *Agathis borneensis,* too.

The next step is to examine if the occurrence of low disagreement counts was related to small inter-tree distances (Table 11).

Disagreement counts in *Agathis borneensis* looked to be randomly distributed and quite independent of inter-tree distances.

Thus, one can conclude that in *Agathis borneensis* parental and child trees do not form the clusters of a single family group in a natural stand. Occurrence of inbreeding in the species may have been due to self-fertilization of individual trees.

Table 11. Distribution of disagreement counts in relation to inter-tree distances in *Agathis borneensis*.

Inter-tree distance (m)		Disagreement count 0.1	2.3	4.5	6 ≦	Total
9-10	Expectation	4.50	13.00	12.84	4.67	
	Observation	5	16	8	6	35
11-20	Expectation	6.56	18.94	18.71	6.80	
	Observation	10	18	16	7	51
21-30	Expectation	5.66	16.34	16.13	5.86	
	Observation	3	22	16	3	44
31 ≦	Expectation	10.29	29.72	29.34	10.67	
	Observation	9	22	37	12	80
	Total	27	78	77	12	210

$X^2 = 12.34$ d.f. = 9 p = 0.10—0.20

DISCUSSION

Plants growing in a population naturally involve related and nonrelated individuals more or less intermingled with each other. In fact, there is an indication that a plant population can be divided into these two groups. For instance, Levin (1977) gave a brief description in his paper dealing with the distribution of genetic variation in the annual herb *Phlox drummondii* Hook that crossing between plants growing within 2 meter distance gave about three times more degenerative embryos than those from plants growing farther than 15 meters. Price and Waser (1979) in their crossing experiment with plants of *Delphinium nelsonii* in wild populations collected pollen from plants growing at different distances of 0 (self), 1, 10, 100 and 1000 meters. It was found that the cross between plants growing at an intermediate outcrossing distance of 10 meters gave the best results in seed set and survival rate of seedlings, while cross with plants growing nearby seemed to show inbreeding depression. Coles and Fowler (1976) found in *Picea glauca* that seeds obtained from crossing between trees growing within a radius of 100 meters showed some inbreeding effect in seed set, germination and seedling growth.

How do parental and child trees distribute in a forest? Hubbell (1979) found in a tropical dry forest that there were three groups of tree species with respect to the distribution pattern of the adult and juvenile densities around given adult trees. Of the 30 species examined, 15 belonging to the first group showed the highest mean juvenile density in the 0 to 5 meters annulus, closest to the adult. The ten

species of the second group showed horizontal density curves, while the remaining 5 species constituting the third group showed the maximal juvenile density in the area between 5 and 15 meters from the adult.

There are few papers describing that seedlings are not likely to grow in the neighborhood of their mother trees. For instance, Janzen (1970, 1971) described the effect of herbivores in preventing growth of saplings around their mother trees in tropical forests. Webb, Tracy and Haydock (1968) found an autoallelopathic effect of subtropical rain forest trees that produced a substance toxic to seedlings of the same species. Watkinson (1978) observed that in an annual grass species *Vulpia fasciculata* (Gramineae) the seed drops first around the mother plant (first phase), but later they migrate or are buried in the soils (second phase).

The present study aims at detection of family clumps, if any, in two tree species, *Altingia excelsa* Noronha and *Agathis borneensis* \Varb. Both are the tropical rain forest tree species having not a few conspecific trees in the same stand. The number of trees *of Altingia* in the 50 x 50 m quadrat in the Ciwidey natural forest that was investigated was 38 among a total 140 trees. This seems to be not exceptional for the species, because Federov (1966) wrote in his paper that *Altingia excelsa* was one of those rare species which could attain a relatively large population density in the tropical rain forests in East Java. Another species, *Agathis borneensis* also had a relatively large population density, i.e. 21 among 230 trees in the 50 x 50 m quadrat in Kalimantan.

For detection of familial structure in these two species, the results of isoperoxidase analysis have been used. Two of the present authors, Sakai and Miyazaki (1972) published a paper reporting the analysis of family groups in the natural forests of *Thujopsis delabrata* Sileb. et Zucc. by means of the so called disagreement counts which measures the degree of dissimilarity in the assortment pattern of isoenzyme bands between individual trees. Based on the accepted views that the behavior of isoenzymes is monopolitically controlled by genes, and that isoenzymes have nothing to do with adaptability and are thus free from natural selection, it is interpreted that the degree of dissimilarity in the assortment pattern of isoenzymes could tell the genetic dissimilarity.

As a matter of fact, Schwartz and Armitage (1983) have hypothesized in the study of wild marmots that related individuals should have a higher average electrophoretic genetic similarity than unrelated individuals, although their case is a little different from ours.

This disagreement counts of isoenzymes described above have thenceforth been employed for detecting familial structure in *Fagus crenata* Blume by Hashizume and Sugimoto (1980), *Cryptomeria japonica* D. Don by Hashizume and Sugimoto (1982), *Chamaecyparis obtusa* Endl. by Hashizume, Watanabe and Ookita (1983) and *Abies sachalinensis* Mast, by Matsuura (1983).

All these studies depend on a few premises, among which is that random mating between trees of a population would give rise to random assortment of individual bands of the isoenzymes in individual trees, while inbreeding would induce resemblance in the combination pattern of their bands.

It was found in the present study that in *Altingia,* the disagreement counts of such a low value as 0, 1 or 2, i.e. trees with very high similarity, were more significant than the theoretical expectation calculated on the basis of random assortment of isoenzyme bands. In other words, trees similar in the assortment pattern of the isoperoxidases were more than randomly expected, indicating that propagation by inbreeding has most probably taken place in the stand. Further analysis of the distribution of the disagreement counts in relation to the inter-tree distances has shown that the lowest counts were found mostly in the distances of 0 to 10 meters between trees. This means that trees with similar assortment patterns of isoperoxidase bands are located very close to each other, or that related trees form a small subgroup or clump in the stand. In this connection, it is interesting to remember the work of Hashizume and Sugimoto (1980) on *Fagus crenata* Blume. They found that the disagreement counts measured between mother-trees and their respective offspring were mostly so small as 0 and 1.

Thus presumptive families have been depicted for *Altingia excelsa* in Figure 3, where five groups, A, B, C, D and E were considered.

In some papers concerning formation of small groups of trees in a forest, Roe (1967) reported that in *Picea engelmannii,* the seed dispersal occurs in the vicinity around the mother trees. Fedorov (1966) wrote that in the tropical rain forest, "not only rare species, but dominant species too, usually form small populations". Ashton (1969) wrote that "contagious distribution of individuals is in fact general among rain-forest trees". According to Ashton, the clumping is related to the means of dispersal on the one hand, and to the chance of saplings of a single species to take their place in a gap, on the other hand.

What role would these small groups of trees in a forest play with respect to the reproduction and propagation of trees?

Sarvas (1967) divided pollen dispersal of tree species into 4 levels: (1) within individual trees, (2) between trees within a subpopulation, (3) between sub-populations within a population and (4) between populations. He investigated the problem in wind-pollinating *Pinus silvestris* by the pollen catch technique and found that 50% of pollen affecting fertilization came from outside the population. Contrary to Sarvas, however, Langner (1953) made an experimental study on the fertilization problem in *Picea abies* (L.) Karst. He made use of the "aurea" mutant and found that pollination occurred mostly between immediately neighboring trees in the stand.

Ashton (1969) described that in the tropical forest, group or clump is likely to be the principal breeding group in most tree species although outcrossing with other groups also occurs. Chan (1980) observed that in *Shorea leprosula,* trees in a cluster produced more fruits than isolated ones. Appanah (1980) described that in the tropical rain forest, inter-tree movements of pollinators occurs mainly between neighboring trees in a clump, suggesting short distance pollen transfer.

If reproduction occurs within a clump, or the effective breeding group is very small and with little exchange of genes between clumps, genetic differentiation should be great among them (Wright 1946). In a perennial herb *Liatris cylindracea,* Schaal (1975) and Schaal and Levin (1978) found striking variation in gene frequencies of isoenzymes and in plant growth characters among subpopulations. Levin (1977) investigated distribution of genetic variation in wild populations of *Phlox drummondii* Hook., a complex annual species consisting of six subspecies. The isoenzyme studies showed that most genetic variation was found within populations, less between populations within subspecies and least among subspecies.

On the contrary, Schaal and Smith (1980) investigated isoenzymes in populations of a leguminous allogamous species *Desmodium nudicaule* and found that no genetic differentiation within the population or no genetic substructuring was found in the species.

In tree species, Curies and Ledig (1982) found in 11 populations of *Pinus rigida* that 97% of genetic diversity was among individual trees within populations and only 3% was between them. A similar result was obtained by Hiebert and Hamrick (1983) in *Pinus longaeva* Bailey. Linhart, Mitton *et al.* (1981) investigated genetic variation at seven isoenzyme loci in six clusters of trees in a population of *Pinus ponderosa* laws. They found that the tree clusters differed significantly from each other in the isoenzyme loci as well as in several gross morphology. In *Pinus monticola* Dougl., Rehfeldt (1979) grew 8 full-sib families from each of 12 populations to investigate variation in several growth characters. He found significant variation among families within populations, but little or no differentiation among populations. In *Pseudotsuga menziesii* (Mirb.) Franco, Yeh and O'Malley (1980) investigated 21 isoenzyme loci in 11 populations. They found that almost all genetic variation was found within populations and very little among them. In the tropical rain forest, Gan, Robertson *et al.* (1977) have found spatial heterogeneity in several isoenzymes as well as in some leaf characters in a population of *Shorea leprosula* and *Xerospermum intermedium.* They attributed this heterogeneity to the short-range pollen flow and fruit dispersal causing spatial isolation between subpopulations. In *AItingia excelsa,* it was found that each of the five presumptive families or groups was apparently different from others in several respects. As seen in Table 4, every group possesses its specific bands which other

groups do not. The groups were also variable in several leaf characters. These facts tell us that the five groups were genetically differentiated, suggesting that each group had been genetically separated from others. In other words, reproduction may have occurred mainly within each group without exchanging genes with others. Thus, the five groups are admitted to be separate as breeding groups, and because of very low values of disagreement counts within each group, each of them is certainly considered to be a single family.

Assuming that trees within a single family clump could mate freely, we are able to estimate the mating distance in the species as 16 to 18 meters or 16.5 meters. For estimating the area occupied by the family clump as a breeding group, it would be a circle of 200 or 250 m^2 with a radius of 8 or 9 meters.

A panmictic unit of breeding or a group of plants in which their gametes may come together has been defined by Wright (1946) as a neighborhood in a plant population. Schaal and Levin (1978) estimated the area of the neighborhood of a perennial herb, *Liatris cylindracea* to be 33 m^2 on the basis of flying distance of insects and the distance of seed dispersal. The family clump in the present paper may correspond to the neighborhood in some respects, because the two concepts equally emphasize an occurrence of random mating among members of a group. A remarkable difference between these two is breeding in the family clump, mating occurs among related individuals, while in the neighborhood it occurs among all individuals.

Attention should be paid to the two families B and C (Figure 3). Granting that 16 to 18 meters are the mating distance of *AItingia excelsa,* a question arises as to why trees of both families could be so much differentiated from each other in electrophoretic as well as in morphological characters, in spite of their mixed distribution in the forest.

In nature, there may be various kinds of reproductive barriers. An extreme case may be found among provenances from a wide region. For instance, Dogra (1981) found in *Pinus wallichiana* A.B. Jacks native to Himalayas that flowering time was different from provenance to provenance, particularly between low and high elevation. The mountain ridge could also be a very effective barrier. In the present case of *Altingia,* however, this geographical barrier was not present in the population.

Flowering time of a plant is of course dependent on environmental conditions, on the one hand, and genetic constitution of the plant, on the other. For the genetic control on flowering of plants, Stern and Roche (1974) have given forcible discussions in their book, "Genetics of Forest Ecosystems". Stam (1983) took a view of natural selection of environments on within-population differentiation of flowering time in plants.

It is often stated that the lack of seasonality in the tropical forest leads to irregularity and lack of coincidence in flowering among not only related species, but also among individual trees of the same species (Fedorov 1966, Ashton 1969, Frankie 1975, Chan 1980). This maybe due to shortage in the tropics of environmental effect like day/night length or vernalizing temperature which serves as an incentive to flower induction. Thus, in the tropical rain forest where climatic control is absent, genetic make-up or genotype should be responsible for the initiation of flowering in each tree. Here we can recollect that trees of the same family are likely to have many of the same genes in common. So, it is to be expected that they will show synchronized flowering so far as they belong to the same family, flowering time of which, however, may be different from trees of the other families.

The two families B and C would have been sexually isolated from each other though their trees grow mixedly distributed within the same area of the mating distance. Thus different families in *Altingia excelsa* would be subjected to the effect of genetic drift. (Additional investigations are needed).

As described previously, two opposite views have been proposed for reproductive system of trees in the tropical rain forest. One is that of Fedorov (1966) who thinks that in the tropical rain forest, tree species reproduces predominantly by inbreeding, probably by self-fertilization due to scarcity of conspecific individuals combined with asynchronous flowering among them, leading to genetic drift and finally to speciation.

Another view is that of Asthon (1969) who looks that the mode of speciation in the tropical rain forest is essentially not different from other terrestrial plant ecosystems, the speciation being expected to occur as a result of outcrossing and gene recombination combined with natural selection.

Data obtained from the present study on *Altingia excelsa* seem to favor Fedorov although we take a view of intra-family propagation instead of Fedorov's self-fertilization hypothesis.

In *Agathis borneensis,* inbreeding occurs to some extent but there is no sign of clustering of a group of trees or formation of family clumps as detected in *Altingia excelsa.* It is assumed that related trees are separated widely in the forest without forming a small group per family. Effect of the inbreeding observed may be attributed to self-fertilization of individual trees.

CONCLUSION

From the present study it has been found that a population of *Altingia excelsa* involved several breeding groups, each of which consisted of related trees of a single family. Families were very variable biochemically as well as

morphologically, suggesting that they were genetically differentiated subgroups within a population. The mating distance, that is, the distance within which trees can mate at random has been estimated to be 16 to 18 meters in *Altingia excelsa.* If trees of different families were mixedly growing within the mating distance, they still maintained their peculiarities suggesting the presence of inter-familial sexual isolation due perhaps to genetic difference in flowering time among families.

In *Agathis borneensis,* formation of no family clump was noticed, though there was an indication of occurrence of inbreeding to some extent. Inbreeding in this species may probably be due to self-fertilization in individual trees.

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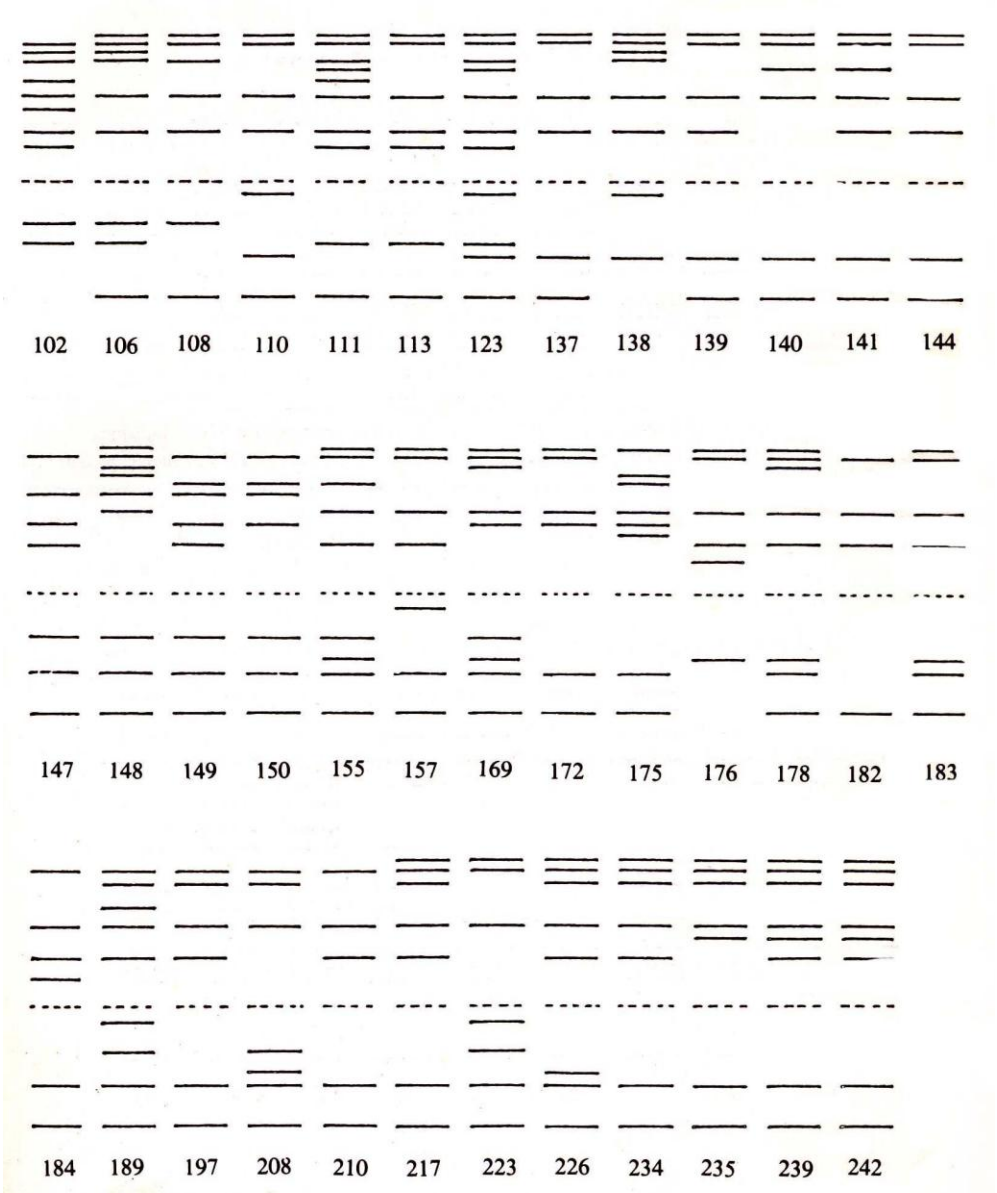
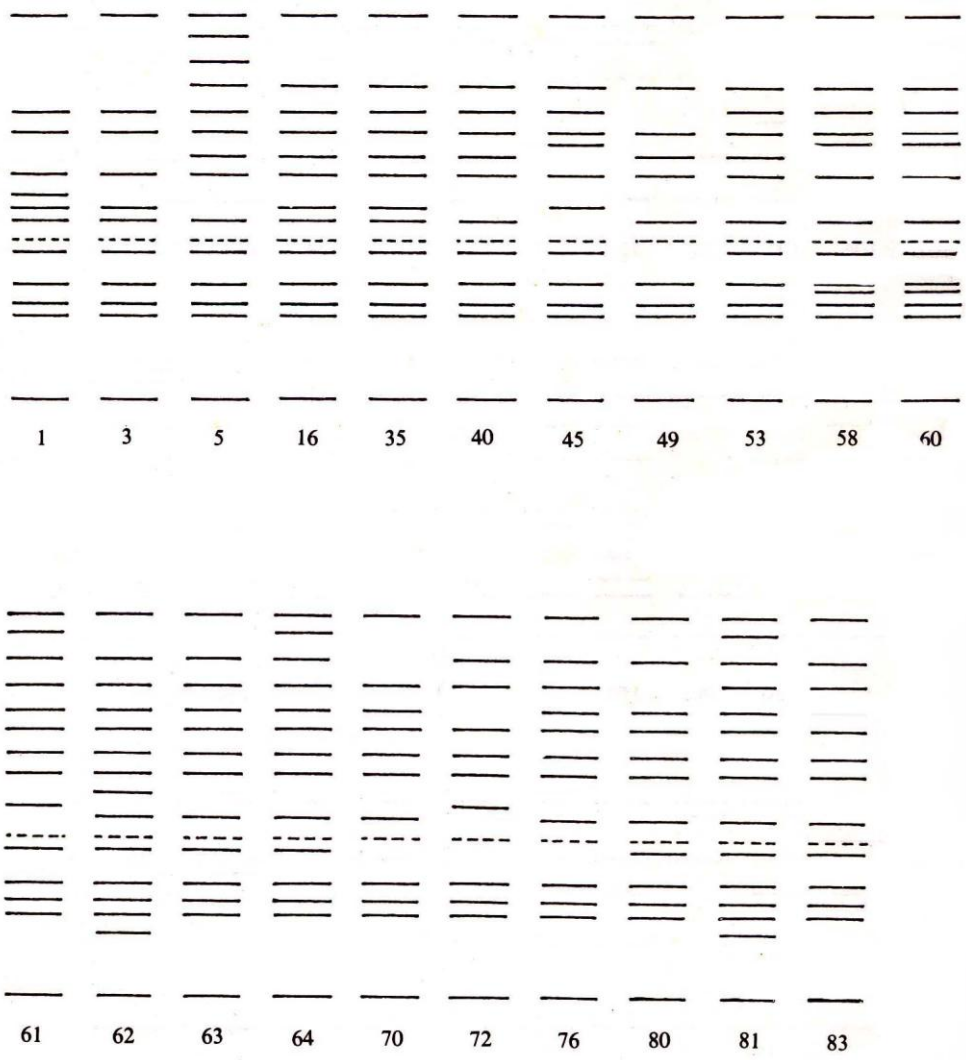
Appendix 1



Isoperoxidase zymograms of 38 trees of *Altingia excelsa* in the Ciwidey forest near Bandung, West Java.

Appendix 2.



Isoperoxidase zymograms of 21 trees of *Agathis borneensis* in the K-1 forest of Kenangan, Kalimantan.

Appendix 3

Calculations of the theoretical expectations of disagreement counts according to the random assortment of isoperoxidase bands in individual trees

Let the frequency of occurrence of i-th band in the population be designated as Xj. The probability that the disagreement count will be either zero or one for the band is given in the following:

Probabiliy that the ppulation will give zero or one disagreement count for the i-th isoperoxidase band

Tree X	Tree Y	(+) : X_i	(—) : 1—X_i
(+) : X_1		(+/+) : X_i^2	(+/—) : $X_i(1-X_i)$
(—) : 1 = X_1		(—/+) : $(1-X_i)X_i$	(—/—) : $(1-X_i)^2$

The probability of getting zero disagreement count for the i-th band is given as $(Xj + (1 — Xj)^2)$, and that of getting one is given as $2X_i(1 — X_i)$, since plus meeting with plus or minus meeting with minus (+ /+ or — / —) gives zero, while minus meeting with plus or the inverse (+ / — or — / +) gives disagreement count of value one. Let the total number of isoperoxidase bands which are assumed to be genetically independent with each other be p, then the probability of getting zero disagreement count in the population will be $(_{X1}{}^2 + (1— X_1{}^2)$ x ..... x $(X^2_i + (1— X_i)^2)$ x ..... x $(X^2_p + (1-X^2_p) = \prod_{i=1}^{p} (X_{2_i} + (1-X_j)2)$

The probability that the disagreement count gets the value of one in the population with p bands will be given by:

$$\sum_{i=1}^{P} [2Xi\,(-Xj)] \prod_{j=1}^{P} (X2j + (1-Xj)2)]$$

The probability that the disagreement count may take the value larger than 2 can be calculated in the similar way.