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SHORT COMMUNICATION

Inference on the Possible Causes of Segregation Distortion from Open Pollination Progenies of Merkus Pine (*Pinus merkusii*)

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Methods of analysis to infer the possible causes of segregation distortion were carried out using single tree progenies of *Pinus merkusii*. Tests on the progenies of the seed trees showing significant segregation distortion were performed at six gene loci, i.e. GOT-C, GOT-D, PGM-B, SKDH-A, NDH-A, and FDH-A. Results indicated that most fertilisation combination between female and male gametes occurred randomly. This study revealed that 11 out of 17 cases of distortion were detected in both gametes, while the other six were detected only in female gamet. The primary sources of such segregation distortions are discussed regarding to linkage relationships system of mating and post-zygotic viability selection.

Key words: *Pinus merkusii*, segregation distortion, system of mating, viability selection

INTRODUCTION

Basically, segregation distortion is defined by significant deviations among the frequencies of gametic types produced by an individual from the proportions expected under regular meiosis. Operationally, the definition is applied more appropriate to successful gametes in place of any stage prior to fusion, since the majority of experiments concerned with studies of segregation distortion were based on observations obtained for post-zygotic stages.

Segregation distortion has been observed the most in the "traditional" model organisms of genetics like *Drosophila*, and the elucidation of the various causal mechanisms in these organisms has long been the objective of intensive study (Crow 1988). In conifers, distortion in the segregation of alleles has also been observed, although Mendelian segregation was the rule (Neale *et al*. 1984; Cheliak *et al*. 1984; Boyle & Morgenstern 1985; Strauss & Conkle 1986; Perry & Knowles 1989; Beaulieu & Simon 1994; Hussain 1995).

Along the pathway extending from meiosis to the fusion of gametes, three phases can be distinguished in which forces leading to segregation distortion may become active (Gillet & Gregorius 1992), i.e. (i) The process of meiosis may be disturbed resulting in unequal representation of complementary gametic types (meiotic drive); (ii) The gametic types present in the meiotic products differ in their ability to survive until fertilisation (gametic viability selection); (iii) After gametic viability selection, differential fertilisation success of gametic types may occur as a consequence of specific fusion relations determined by mating system (gametic mating success).

Information on the degree of segregation distortion in pine is available. However, results of further examination to determine the possible causes is not well reported. This information on the degree of the distortion as well as its causes is important for consideration in analysis of population genetics. This is due to the pattern of this behaviour which will drastically alter frequencies of particular alleles in population. Therefore, this study was aimed at at inferring the possible causes of segregation distortions using population from open pollination progenies of *Pinus merkusii*.

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MATERIALS AND METHODS

Seeds. Seeds were extracted from cones mostly collected from a *P. merkusii* seedling seed orchard (SSO) in Sempolan, Jember, East Java. This orchard was established in 1980 and located at 600 m above sea level within a approximate geographic range of 113°52' E and 7°67' N.

Electrophoresis. Haploid megagametophytes (endosperms) of 10 single tree seed-lots (mother trees) were analysed according to the procedure largely used by Changtragoon and Finkeldey (1995) with a slight modification by Siregar (2000). Seeds were immersed overnight in water, dissected and the embryo carefully separated from the megagametophytes (endosperms). The megagametophytes were ground in two drops of homogenising (extraction) buffer (0.1 M Tris-HCl pH 7.3, containing 0.03% DTT and 2.5% PVP).

These crude homogenates (extracts) were then subjected to horizontal starch gel electrophoresis, using the buffer system of Ashton and Bradon (1961), pH 8.6 for GOT (*glutamate oxaloacetic transaminase*, E.C 2.6.1.1) and PGM (*phospoglucomutase*, E.C 2.7.5.1) as well as Tris-Citro for SKDH (*shikimate dehydrogenase*, E.C.), NDH (*NADH dehydrogenase*, E.C.1.6.99.3), and FDH (*formiate dehydrogenase*, E.C. 1.2.1.2). Further details of electrophoretic and staining procedures were given by Liengsiri *et al*. (1990).

Segregation Distortion Analysis. Mendelian segregation is assumed under the following requirements (Gillet &

Hattemer 1989): (i) regular meiotic segregation during egg production, (ii) random fertilisation of the eggs by each pollen (haplo) type, (iii) absence of differential viability selection in the offspring prior to the investigation. Progenies from open pollination are resulted from the following fertilisation (Table 1). The homogeneity of the distributions in at least one gamete is equivalent to the product structure of the genotype (Hussain 1995). Three possible cases as described by Gillet and Gregorius (1992) could be categorised to determine the possible causes of segregation distortion, as follows:

Case#1. The distribution of A_j^{σ} gametes (j=1,2) for both alleles is homogeneous. In this case, segregation distortion in ♀ gametes is excluded, if there is no significant departure of A_j^{σ} gametes (j=1,2) from 1:1 ratio in the fertilisation success of (A_1^{φ} A_j^{σ}) and (A_2^{φ} A_j^{σ}).

Case#2. The distribution of A_j^{σ} gametes (j=1,2) is homogeneous for only one allele. In this case, segregation distortion presents as a result of viability selection on ♀ gametes provided that there is a significant departure of A_j^{σ} gametic types (j=1,2) from 1:1 ratio in the fertilisation success of (A_1^{φ} A_j^{σ}) and (A_2^{φ} A_j^{σ}).

Case#3. The distribution of A_j^{σ} gametes (j=1,2) is heterogeneous for both alleles. In this case, segregation distortion presents as a result of haplo-homophasic system of mating and post-zygotic viability selection provided that there is a significant departure of A_j^{σ} gametes (j=1,2) from 1:1 ratio in the fertilisation success of (A_1^{φ} A_j^{σ}) and (A_2^{φ} A_j^{σ}).

Table 1. Progenies resulted from open polination

Gametes	A_1^{σ}	A_2^{σ}
A_1^{φ}	A_1^{φ} A_1^{σ}	A_1 A_2^{σ}
A_2^{φ}	A_2^{φ} A_1^{σ}	A_2^{φ} A_2^{σ}

RESULTS

The first test product structure on trees that showed significant segregation at gene loci GOT-C, GOT-D, PGM-B, SKDH-A, NDH-A, and FDH-A are presented in Table 2. Inheritance analysis of these loci has been described in details by Changtragoon and Finkeldey (1995). Examples of schematic patterns of five isozymes (zymogram) are presented in Figure 1-5.

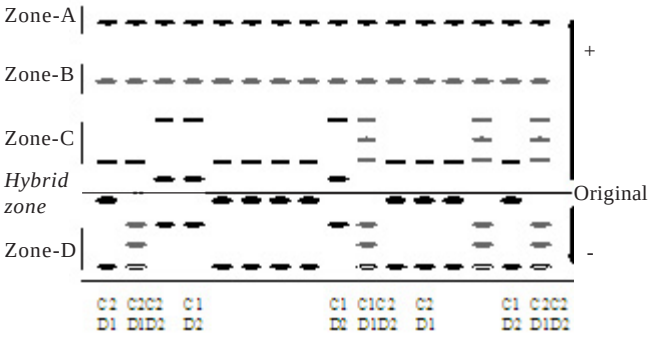

Figure 1. Schematic GOT banding patterns of megagametophytes (*1n*) and their corresponding embryos (*2n*).

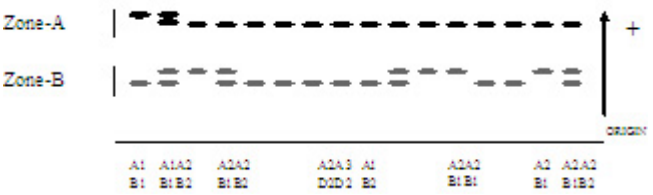

Figure 2. Schematic PGM banding patterns of megagametophytes (*1n*) and their corresponding embryos (*2n*).

Table 2. Summary of the results of product structure tests from trees showing significant segregation distortion at six gene loci

Tree	Genotype	Sample size (N)	Test#1: Product structure	Test#2: N (F_i^{φ} M_i^{σ}) N (F_j^{φ} M_i^{σ})	Test#2: N (F_i^{φ} M_j^{σ}) N (F_j^{φ} M_j^{σ})	Test#2: N (F_i^{φ} M_k^{σ}) N (C_j^{φ} C_k^{σ})
			G-test		χ^2-test	
			Locus GOT-C			
J26	C2C3	62	1.949	10.522**	1.282	-
J54	C2C3	43	2.528	50.97***	4.000*	4.000*
J47	C2C4	36	5.906	8.963**	0.667	12.000**
			Locus GOT-D			
J12	D1D2	28	1.941	0.222	38.000**	-
J27	D1D2	54	2.033	19.565*	28.452**	-
			Locus PGM-B			
J16	B1B2	34	1.406	0.500	10.000**	-
J27	B1B2	58	0.435	41.143**	52.267**	-
A39	B1B2	17	0.253	5.333*	4.545*	-
			Locus SKDH-A			
J16	A1A2	36	1.348	9.000**	7.143**	-
J27	A1A2	58	0.628	1.778	10.316**	-
J36	A1A2	40	2.134	1.778	17.818***	-
J51	A1A2	24	4.708*	12.000***	4.000*	-
			Locus NDH-A			
J12	A1A2	41	2.373	18.62***	12.51***	-
J27	A1A2	46	1.01	1.778	10.316**	-
J47	A1A3	22	1.174	10.67**	0.667	14.00***
			Locus FDH-A			
J51	A1A2	24	9.405**	0.182	26.000***	-
AI08	A1A2	24	0.603	18.615***	8.909**	-

Significant levels are 0.05 (*), 0.01 (**), 0.001 (***); F: Female gametes; M: Male gametes, -: not further tested due to limited number of genotypes at these particular loci.

Figure 3. Schematic SKDH banding patterns of megagametophytes (*1n*) and their corresponding embryos (*2n*).

Figure 4. Schematic NDH banding patterns of megagametophytes (*1n*) and their corresponding embryos (*2n*).

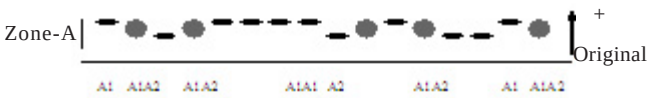

Figure 5. Schematic FDH banding patterns of megagametophytes (*1n*) and their corresponding embryos (*2n*).

The second tests conducted to define the possible causes of distortion revealed that 11 (65%) out of 17 cases of distortion (Table 2) were detected in both male and female gametes, while the others were detected only in female gametes.

DISCUSSION

Test results indicate that most combination between female and male gametes (fertilisation) occurred at random. Only one tree, coded J51, showed no random combination between gametes at gene locus SKDH-A and FDH-A.

There are two important findings from the experiment in *Pinus merkusii* progenies, namely: (i) random fertilization mostly occurred between female and male gametes, (ii) segregation distortion occurred in both gametes were much higher than that in single sex, i.e. female. According to Ledig (1998), in some cases of segregation distortion, alleles may have aberrant segregation ratios across the entire population, and in other cases, distortion is apparent only in a single tree. In addition, there are two possible explanations for the segregation distortion frequently observed in forest trees.

First, the presence of selfish alleles or segregation distorters, which are linked to the allozyme loci and affect meiosis, increasing the frequency with which they are transmitted. According to Stadler (1996), segregation distorters are genetic elements that disturb the meiotic segregation of heterozygous genotypes. The effect is hence often referred to as meiotic drive. The driving chromosome destroys its partner, provided the latter is not resistant against the "killer." The dynamic behavior of a meiotic drive system is in general determined by the interaction of several alleles at different gene loci. The corresponding genes are "ultra-selfish" in that they force their own spreading in the population without contributing positively to the fitness of the organisms carrying them.

Secondly, selection against one of the alleles during gametogenesis or embryogenesis. Equal frequencies of alternative alleles in heterozygous trees are most likely to be true at meiosis. However, studies of segregation using mature conifer seeds do not assess this parameter at meiosis, but at a time considerably later. In *P. merkusii* pollination and fertilisation of ovule are separated by approximately 11 months (Danarto 1983), so there is an ample opportunity for intervening gametic selection. After fertilisation, maturation of a viable seed depends upon embryo survival. Thus, the ascertaintment of a megagametophyte ultimately depends on the fitness of the genotype of the embryo to which it has contributed. The phenomenon could be due to many causes such as the selective differences between isozymes, in this case the excess of the most common allele may be due to its superior fitness when haploid (Strauss & Conkle 1986). It is well known that abortion of female gametophytes may arise at different developmental stages, but it generally occurs during meiosis (Owens & Molder 1984); the seed then continues to develop but it is small and flat or empty. In *P. merkusii* the proportion of empty seed was considerable. It was observed also that the most frequent alleles was in deficit in some seed trees, while all other trees fitted expectations of a 1:1 segregation ratio. Therefore, it was believed that these observations do not support the hypothesis of selection in the haploid phase.

Another cause could be a selection against the rare allele due to a linkage with a lethal or semi-lethal allele affecting the production of maternal gametes (Cheliak *et al*. 1985). When the pine female gametophyte is generated, up to five archegonia are produced following cell divisions (Lill 1976). This high number of eggs, each of which has the potential to produce an embryo, makes it possible to produce an array of zygotes within each ovule. Hence, polyembryony provides a mechanism that facilitates indirect selection through embryo competition (Willson & Burley 1983), increases the quality of the progeny and decreases inbreeding depression by eliminating embryonic lethals (Lindgren 1975). The presence of lethal genes has already been observed in conifers (Fowler 1965). So, if the analysis of segregation of the alleles of a given gene that is linked to a lethal one is made after the abortion of the zygotes, an apparent segregation distortion may arise. The almost same hypothesis of the causes of segregation distortion was proposed by Gillet and Gregorius (1992) using an example of the AP-A gene locus of sweet chestnut (*Castanea sativa* Mill).

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