

# Variability of Chromosome Structure and Behavior in Southern Pine Hybrids<sup>1/</sup>

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## INTRODUCTION

Interspecific hybridization is a technique of considerable importance to plant breeders. Although hybridization of forest tree species has not received a great amount of emphasis in the past because of the wealth of species in existence and the intra-specific variability they possess, its importance is becoming more evident as our knowledge and sophistication of tree improvement increase.

The value of incorporating the germplasm of one species into that of another species for such purposes as increasing resistance to insects and diseases is being continually documented. In the southern pines, for example, Grigsby (1959) has demonstrated increased resistance of slash x shortleaf and loblolly x slash (var. **densa**) pine hybrids to **Rhyacionia frustrana**. Derr (1966) has shown similarly that longleaf x slash hybrids appear less susceptible than their parents to the brown spot needle blight of longleaf pine and the fusiform rust of slash pine.

Interspecific hybridization also may provide new combinations that are more tolerant to unusual climatic conditions (Hyun and Ahn, 1959). In an attempt to develop strains with such tolerances, several hybridization projects have been initiated recently in the South to produce trees that can be grown commercially on unproductive sites that are extremely wet or dry.

A very important project for forest geneticists, therefore, is to determine how readily species will cross. For those species that cannot be crossed or that cross only with difficulty, it is also of value to determine the barriers. As Critchfield (1962) pointed out, there are two general types of isolating mechanisms operating in the southern pines, those that prevent cross pollination and those that prevent

germination of the seed. Alterations in chromosome structure and genetic make-up are two features of the latter type that may prevent the production of hybrids. They also may cause the hybrids to be infertile once they reach sexual maturity, which causes such trees to be of little or no use for future breeding programs.

The purpose of this project was to study four species of southern pines and their hybrids to determine if chromosome structure and behavior were in any way a barrier to the interspecific exchange of genetic material.

## MATERIALS AND METHODS

Staminate cones with pollen mother cells in various stages of division were collected from 35 trees during a three year period from 1961-1963. The material analyzed included three species and ten different hybrid combinations of **P. echinata**, **P. elliottii** var. **elliottii**, **P. palustris**, and **P. taeda**. Three of the crosses were combinations involving three species.

Most of the trees studied were located in the Fastrill Arboretum of the Texas Forest Service near Alto, Texas, and in the Hodges Gardens at Hodges, Louisiana. The others were located near Raleigh, North Carolina and near Placerville, California in the Eddy Arboretum of the Institute of Forest Genetics. An attempt was made to obtain material from several hybrids located in the Harrison Experimental Research Forest of the Institute of Forest Genetics at Gulfport, Mississippi, but either the trees were not sexually mature or the pollen mother cells were not dividing at the time the collections were made.

Immediately after collection, the staminate cones were sliced in half longitudinally and fixed

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in a 3:1 solution of absolute ethyl alcohol and glacial acetic acid. The material was subsequently changed to 70% alcohol and stored in a freezer. The microsporocytes were stained in aceto-carmin for analysis.

The method of analysis used was similar to that described by Saylor and Smith (1966). Frequencies of meiotic irregularities were determined for each tree in six different stages. Approximately 1500 division figures (250 per stage) were studied for each tree. To facilitate presentation of the data, similar irregularities in the two cycles of division were combined and presented as a single percentage figure.

### RESULTS

Chromosome behavior was entirely regular in a vast majority of the cells studied, and this was true for all stages. Irregularities of some type were found, however, for every tree.

Univalents were the most common irregularity observed. Frequencies of greater than one percent were found in 15 trees for this abnormality. Frequencies of the other irregularities exceeded one percent as follows: lagging chromosomes - 7 trees; fragments - 7 trees; bridges - 8 trees, micronuclei - 5 trees.

The results of this study in conjunction with those of other studies, indicate that the hybrids are slightly more irregular than trees representing parental species. For example, 36.7 percent of the hybrids listed in Table I had total irregularity frequencies greater than one percent. For comparison purposes, irregularity frequencies greater than one percent occurred as follows in a natural population study of loblolly and pond pines: loblolly pine - 16.7%, loblolly x pond pine hybrids - 31.8%; pond pine - 16.7%.

Of the different hybrid combinations studied, the *P. echinata* x *taeda* cross was the most irregular. The reason for this is not understood, and it is possible that several factors may be involved. Tree 1 is the female parent of all of the hybrids of this cross (trees 7-10); it is also the female grandparent of hybrids 22, 23 and 31-35. The level of meiotic irregularity observed in tree 1 is greater than normal for pure species, which indicates the possible presence of genetic or chromosomal aberrations that could be passed on to its progeny.

Environmental conditions also may have been a cause of the unusual irregularities found in the

shortleaf x loblolly pine hybrids. Trees 1, 6-10 and 35 are the only ones located in California, and when ranked according to total irregularity all seven were among the nine most irregular trees studied. The genetic make-up of trees 21-23 (located in Texas) also includes material of slash pine along with that of shortleaf and loblolly pine. This should provide even greater conditions for irregularities to occur, but the total irregularity of these trees was quite low. It seems possible, therefore, that certain climatic conditions, such as temperature fluctuations, occur at the California location that adversely effect meiosis and thereby cause some of the irregularities observed in chromosome behavior.

Shortleaf pine was involved in the formation of 60 percent of the hybrids evaluated in this study. In comparing total irregularity, 70 percent of the 20 most irregular hybrids involved shortleaf pine, which suggests this species may be slightly less compatible than the others. Considerably more information must be obtained, however, before this can be verified.

Perhaps the most significant contribution of this study was the demonstration of a high level of tree to tree variation. For nearly every cross, this was of such a magnitude as to make comparisons between hybrid groups either difficult or impossible. The ranking of individuals within groups frequently differed by as many as 10 of the 30 places, and in some the differences were greater than 20. All but one of the hybrid groups contained at least two individuals that were either half- or full-sibs, and comparable differences were found even among such closely related trees. In the loblolly x slash pine hybrids, for example, trees 11-13 are full-sibs, and they differed in ranking by 13 places; the greatest difference in ranking for all trees of this combination was 21. Trees 16 and 17 (longleaf x slash pine hybrids) similarly are full-sibs, and they differed in ranking by 26 places.

The greatest manifestation of between tree variation occurred among trees 31-35 which were obtained from a three species cross of shortleaf, loblolly and slash pines. These hybrids all have a common seed parent, but the male parent is unknown because a pollen mix was used. The cross was made in California at the Institute of Forest Genetics, but the hybrids included in this section are located in two different areas; trees 31-34 are in the Fastrill Arboretum in Texas and tree 35 is in the Eddy Arboretum in California.

Tree Number	Species or Hybrid	Univalents	Lagging Chromosomes	Fragments	Bridges	Micronuclei	Total Irregularity	Irregularity Rank of Hybrids
1	P. echinata-V22	0	0.71	1.42	1.42	0	1.56	
2	P. palustris-4	1.16	0	0.20	0.26	0	0.39	
3	P. palustris-5	0.79	0	0	0.65	0	0.46	
4	P. taeda-05	1.54	0.57	0.18	0.13	0.20	0.83	
5	P. taeda-84	0.39	0	0.20	0.26	0	0.52	
6	P. echinata x elliottii-10	1.40	6.49	0.84	1.05	12.19	8.30	3
7	P. echinata x taeda-5	5.10	0.39	3.54	1.18	1.20	4.30	5
†	P. echinata x taeda-40	4.17	0.38	0.38	1.14	0.39	2.48	7
9 <sup>a</sup>	P. echinata x taeda-60	1.90	2.95	0.37	2.35	2.11	3.76	6
10a	P. echinata x taeda-67	51.25	14.12	2.47	2.70	3.29	20.53	2
11b	P. taeda x P. elliottii-1	0.50	0	0.74	0	0.24	0.58	21
12b	P. taeda x P. elliottii-2	1.92	0.40	0.40	0.13	0.16	0.69	18
13b	P. taeda x P. elliottii-3	2.71	0.39	1.37	0.65	0.19	1.57	8
14	P. taeda x P. elliottii-4	0.78	0	0	0	0	0.19	29
15	P. taeda x P. elliottii-5	1.21	0	0	0	0.20	0.58	20
16x	P. palustris x P. elliottii-1	0	0	0.20	0	0	0.06	30
17x	P. palustris x P. elliottii-2	0.39	6.99	7.54	0.38	0.97	6.01	4
18c	P. (taeda x elliottii) x elliottii-1	2.44	0.66	0.22	0.98	0	1.48	10
19'	P. (taeda x elliottii) x elliottii-2	0	0.75	0	0.39	0.74	0.91	13
20c	P. (taeda x elliottii) x elliottii-3	0	0	0.78	0	0.20	0.33	24
21	P. (echinata x elliottii) x taeda-1	0.39	0.39	0.20	0.13	0.20	0.33	23
22d	P. (echinata x elliottii) x taeda-2	1.54	0.66	0.22	0.26	0.39	0.79	15
23d	P. (echinata x elliottii) x taeda-3	0	0	0.22	0.93	0.25	0.79	16
24e	P. echinata x sondereggeri-1	0.86	1.70	0.42	0.14	0.21	1.51	9
25e	P. echinata x sondereggeri-2	0.86	0	0.20	1.10	0.43	0.98	12
26	P. (palustris x elliottii) x wind-1	2.31	0	0	0.13	0	0.45	22
27	P. (palustris x elliottii) x wind-2	0.39	0.39	0	0.13	0	0.33	25
28f	P. (elliottii x echinata) x (echinata x elliottii)-1	0	0	1.23	0.33	0.74	0.83	14
29f	P. (elliottii x echinata) x (echinata x elliottii)-2	0.98	0	0.20	0.30	0.25	0.61	19
30f	P. (elliottii x echinata) x (echinata x elliottii)-3	0.39	0.20	0.39	0	0	0.33	26
31°	P. (echinata x taeda) x (echinata x elliottii)-1	0	0.25	0.74	0.65	0	0.76	17
32z	P. (echinata x taeda) x (echinata x elliottii)-2	1.48	1.47	0.49	0.49	0.25	1.23	11
33z	P. (echinata x elliottii) x (echinata x taeda)-3	0	0.20	0	0.33	0.25	0.33	27
34°	P. (echinata x taeda) x (echinata x elliottii)-4	0	0.20	0.25	0.17	0	0.25	28
35z	P. (echinata x taeda) x (echinata x elliottii)-5	(1962) 84.25 (1964) 88.75 (1965) 83.94	50.19 37.99 58.31	3.28 10.07 6.65	13.59 14.95 6.32	25.18 32.41 21.72	47.24 60.38 53.80	1

a-f Individuals with the same superscript are full-sibs

x,z Individuals with the same superscript are half-sibs

The magnitude of meiotic irregularity observed for tree 35 was the greatest ever reported for any pine. On the other hand, meiosis in trees 33 and 34 was no more irregular than normal species representatives. When ranked according to total meiotic irregularity, this group of half-sibs was so variable as to essentially cover the entire range observed in this and other comparable studies of pines. The irregularities, which for each stage were considerably greater in tree 35 than for any other tree, were studied for three different years. The results for each year were remarkably similar, which suggests that a genetic or chromosomal imbalance in this tree may be at least partly responsible for the occurrence of the anomalies. It should be noted, however, that the trees of this cross located in California are growing quite poorly in comparison to those in Texas. They are short, poorly formed, and their foliage is quite sparse. Such poor development indicates that a physiological incompatibility exists with the California environment, and this may be severe enough to also affect meiosis. It is difficult to say which factor is the most important; all quite likely are involved to some degree. A better evaluation of the situation will be possible when the remaining trees at Placerville become sexually mature.

The kinds of irregularities observed in tree 35 are illustrated in figures 1-9. As shown, all stages of the meiotic cycle were drastically disrupted. Univalents ranging in number from 1-24 occurred in more than 80 percent of the late diakinesis and metaphase I cells (Fig. 1 and 2). In fact, unpaired chromosomes occurred so frequently that it was difficult to find a normal metaphase figure in which all of the bivalents were properly aligned on the plate. Evidence of precocious disjunction was found at metaphase I, but in general, it appeared as if desynapsis occurred before the chromosomes reached the metaphase plate.

A very large proportion of the telophase I cells contained lagging chromosomes (Fig. 3 and 4) that very likely resulted from the irregularities that occurred during the earlier stages. Many of these chromosomes (Fig. 4) were single and must have resulted from early centromere division of the normally bipartite anaphase I chromosome.

Additional evidence of the disrupted movement of the chromosomes was found in the second division. In metaphase II chromosomes frequently were not oriented correctly on the equatorial plate (Fig. 6); in telophase II lagging chromosomes, nuclei of

unequal sizes and chromatin bridges appeared (Fig. 7); micronuclei were quite abundant in both the dyad (Fig. 5) and the tetrad stages (Fig. 8); and small aborted and giant pollen grains (Fig. 9) occurred in frequencies greater than 20 percent.

Somewhat surprisingly, chromosome bridges were found to be very abundant in tree 35. Several factors might be responsible for this. If crossing over is partially localized as suggested by Saylor and Smith (1966), a breakdown of the control mechanism might allow more crossing over to occur with a subsequent increase in bridges if heterozygous paracentric inversions exist. Such a development could occur independently among progeny of a given cross. It is also possible that certain physiological processes have been altered in such a way as to make the chromosomes more susceptible to breakage and fusion which in turn produce chromosome bridges.

Variation in morphological traits that is independent of the degree of meiotic irregularity is demonstrated by comparing three of the shortleaf x loblolly hybrids. Trees 8-10 are 27 year old full-sibs that differ markedly in their appearance. Tree 9 is a bushy dwarf about two feet tall; tree 8 is a semi-dwarf approximately 15 feet tall that also is somewhat bushy in appearance; number 10 appears normal in all respects, and is about 60 feet tall. Although all three hybrids were among the seven most irregular trees in this study, there was considerable meiotic variation among them. Surprisingly, the morphologically aberrant trees were the most normal meiotically, while tree 10 was so irregular that it ranked as the second most irregular tree in the entire study. It is interesting to note that the common shortleaf parent (tree 1) has a past history of producing a high frequency of aberrant progeny. Because inversion bridges are noticeably frequent in this tree, it is tempting to speculate that the mutant types are the result of minute deficiencies or duplications in the chromosomes.

## CONCLUSIONS

The results of this study indicate that loblolly, long-leaf, short-leaf and slash pines are compatible enough genetically and chromosomally to allow interspecific hybrids, including three-way crosses, to be formed and to develop normally. Although meiotic irregularities of some type were observed in every tree studied, they were in general only slightly more abundant in the hybrids than in the species representatives. Irregularities frequent enough to

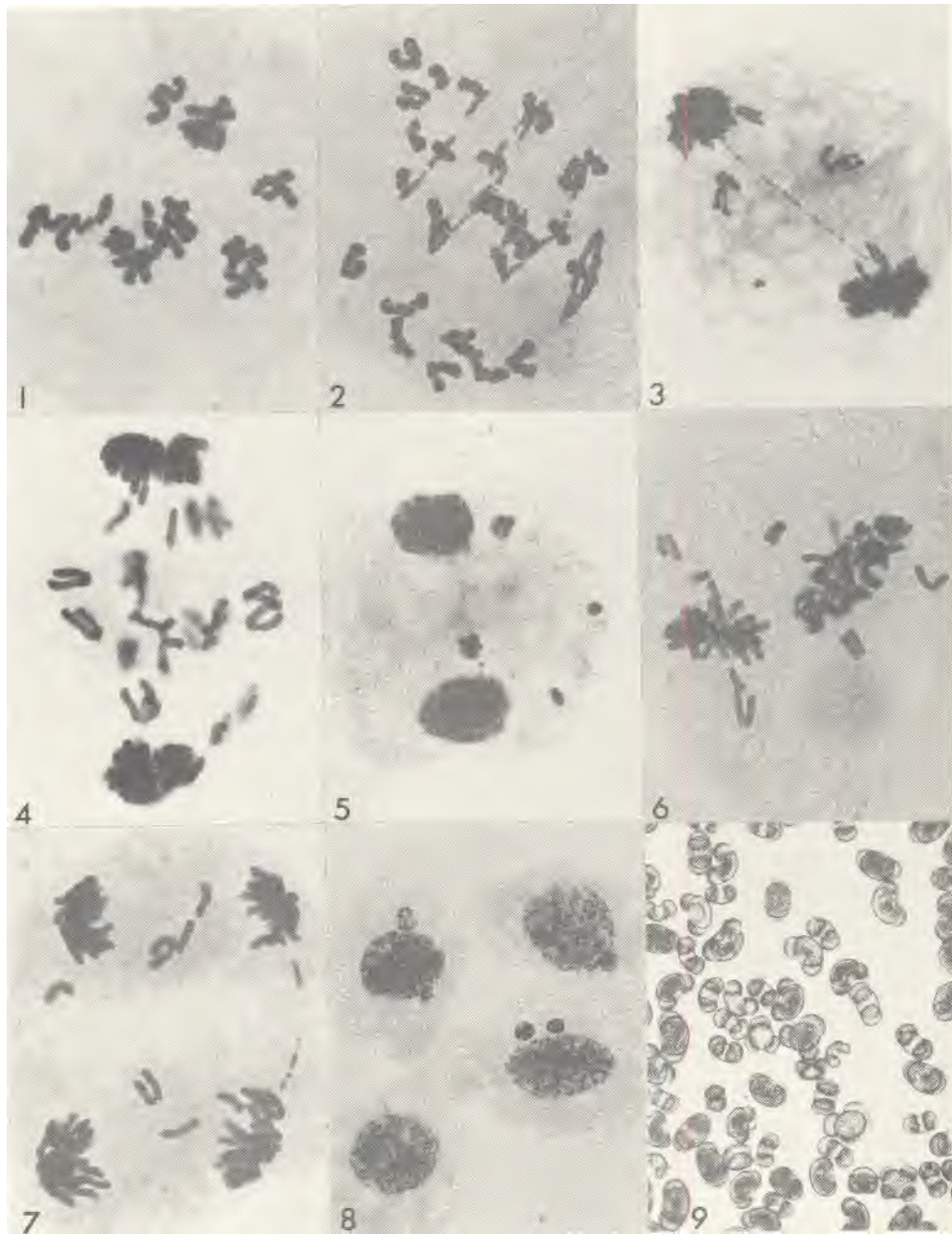


Fig. 1-9. Irregular meiotic divisions in *P. (echinata x taeda) x (echinata x elliottii)*--tree 35.

1. Diakinesis: unpaired chromosomes, X 625.
2. Metaphase I: univalents and nonsynchronous separation of chromosomes of different bivalents, X 625.
3. Telophase I: lagging chromosomes, and inversion bridge with accompanying acentric fragment, X 575.
4. Telophase I: lagging chromosomes, and precocious centromere division, X 625.
5. Dyad: micronuclei, and chromatin bridge, X 575.
6. Metaphase II: lagging chromosomes, X 625.
7. Telophase II: lagging chromosomes, and inversion bridge persisting from the first division, X 625.
8. Tetrad: micronuclei, X 495.
9. Pollen: normal, giant and aborted pollen grains, X 67.

adversely affect gamete formation to any degree were found in only one tree, and this was a three species hybrid that had at least two half-sib relatives that were quite normal. Although somewhat of an extreme case, this exemplifies the high level of tree to tree variation found in all such studies made by this investigator. These results are also in agreement with studies of between tree variation in other characteristics.

It would appear, therefore, that genetically or chromosomally induced meiotic aberrations that lead to inviable gametes are not a major barrier to the exchange of genetic material among these southern pines. The need for care in selecting parents, especially for advance crosses, is demonstrated, however, by the range of variation found among closely related individuals of a given type.

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