

Chromosome Inversions in *Pinus radiata*

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Introduction

Variation between the individuals of a species is the basis upon which the tree breeder is able to improve the quality of future crops. Variation of characteristics has been observed in all well studied species of pine, not only between geographically isolated populations but also between individuals within populations. The variation is exhibited in different aspects, i. e. morphological, physiological, disease resistance, etc. There is keen interest among forest geneticists in learning more about the fundamental factors controlling the amount and inheritance of variation.

Most cytological studies of tree species as yet have been limited in the value of information obtained. This may be partly due to limitations in the size or techniques of handling the chromosomes of woody tree species. In some other organisms, however, detailed studies of chromosome structure have yielded much fundamental knowledge, including the nature and extent of chromosomal rearrangements. Rearrangements have shown a profound influence on the genetic system of some species possessing them, e. g. translocations in *Oenothera* (CLELAND 1936).

Inversions are particularly interesting cytogenetically, since recombination is suppressed within the inverted region of structural heterozygotes (provided the inversion is not long) due to the inviability of gametes bearing crossover chromatids. The genes present in a particular inversion are therefore isolated from their homologues in the normal sequence. Over a long period of time different mutations may become established in the two blocks of genes, so eventually the alleles present in the two sequences may become quite different. If one sequence were superior to the other, it would gradually spread through the population by natural selection. Natural selection would also act against heterozygotes according to the amount of crossing over within the inversions. This would tend to favour the elimination of one of the sequences. However, under some conditions both sequences may be maintained in the population as a balanced polymorphism, e. g. when one sequence has an advantage in only part of the species range, or when the heterozygous condition has a selective superiority or overdominance effect. The existence of such polymorphisms has been demonstrated in two members of the insect kingdom, the fly *Drosophila pseudoobscura* (DOBZHANSKY 1951) and the grasshopper *Moraba scurra* (WHITE and ANDREW 1962). Similar evidence has not been obtained from any plant group, possibly because their chromosomes are more difficult to study, although it is possible that polymorphisms may not be of comparable importance in plants. However, inversion heterozygotes are extremely common in some genera, e. g. *Paeonia* (STEBBINS 1938), and it seems reasonable to suggest that they probably have an adaptive advantage of some kind.

The presence of inversions in hybrids can have quite different significance from their presence in the species

itself. In hybrids they indicate that different inversions have arisen and become fixed in each of the parental species and contribute to part of the species differentiation. In fact, it is quite common among plants for interspecific hybrids to have inversions (STEBBINS 1950). In contrast, inversion bridges found in individuals of a species are evidence that both sequences of one or more inversions are still present in the population, either because the inversions are of recent origin or they are established as polymorphisms. In either case they are a genetic source of variation.

This paper will discuss the characteristics and significance of chromosome inversions in *Pinus radiata* and also in *P. radiata* X *attenuata* hybrids.

Inversions in Conifers

At the pachytene stage of microsporogenesis conifer chromosomes appear in the form of a closely packed ball of threads, making the stage unsuitable for observation of the loops which form in plants heterozygous for inversions. At anaphase I, paracentric inversions can be detected by the occurrence of chromatin bridges, together with their accompanying acentric fragments which form whenever crossing over occurs within an inverted region of a heterozygote. Pericentric inversions do not form bridges, but result in duplications and deficiencies in the crossover chromatids which usually produce inviable gametes. They can be detected in studies of chromosome dimensions only in cases when the inversion leads to a significant shift in the position of the centromere. No pericentric inversions have yet been reported in the literature relating to coniferous species.

There have been several reports of paracentric inversions based on the presence of chromatin bridges at anaphase. However, at least one of these may have been erroneous due to confusion with temporary chromatin bridges formed as a result of slow terminalisation of chiasmata. The report by GOPAL-AYENGAR (1942) of up to 5% bridges in *Pinus nigra* var. *austriaca* is suspect, as there was no mention of accompanying acentric fragments, and in the light of other data the frequency of bridges seems rather high for a widely distributed *Pinus* species.

SAX (1960) studied microsporogenesis in certain soft pine hybrids but did not record any inversions in this group of plants. On the other hand, SAYLOR (1962) conducted an extensive survey in both the hard and soft pine groups and reported inversion bridges in all but two trees out of a total of 61 plants of 45 different species and hybrids. The frequency of inversion bridges ranged from less than 1% of cells examined in most plants to a maximum of 18% in a particular three-species hybrid, *P. (echinata* X *taeda*) X (*echinata* X *elliottii*). In six of the trees (hybrids) more than 4% of cells contained inversion bridges. Photographs clearly showed the presence of acentric fragments together with the bridges. LUNA (1965) reported the presence of infrequent paracentric inversions in *Pinus pinceana*.

In other genera of the *Coniferae*, ANDERSSON (1947) recorded bridges and fragments in a cytologically atypical *Picea*

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Table 1. — The frequency of bridge-fragment configurations indicative of paracentric inversions in *P. radiata* and *P. radiata* × *attenuata*.

Species	Tree No.	Anaphase I — Telophase I				Inversion bridges observed at Anaphase II
		Total Cells Examined.	Bridge plus Fragments.	Bridge Only	Total Inversion Bridges	
<i>P. radiata</i>	ACT38 ^A	793	2	1	3	1
	ACT956 ^B	757	3	1	4	
	ACT419 ^A	676	3	—	3	
	CA 4	300	2	—	2	
	CR 7	194	—	—	—	1
	CR 51	1109	3	1	4	1
	NR 10	934	1	2	3	
	NZ 7	1613	5	3	8	
	NZ 55	733	4*)	1	6	
	NZ 96	721	2	2	4	
	MY 2	606	2	1	3	
	MY 7	796	5	—	5	
		9232	32	12	45	0.49% (1 per 210 cells)
<i>P. radiata</i> × <i>attenuata</i>	1	816	9	—	9	
	2	606	9	—	9	
	3	165	—	—	—	
	4	33	1	—	1	
		1620	19		19	1.2% (1 per 81 cells)

*) one of these 4 cells contained two bridges (see fig. 12).

abies, and Ross and DUNCAN (1949) recorded them in *Juniperus virginiana* × *horizontalis* hybrids. QUINN (1965) recorded an interesting case in a hybrid *Dacrydium laxifolium* × *intermedium* in which inversion bridges were observed in 77% of cells at anaphase I. Two different inversions were easily distinguishable by the size of the fragment, one being approximately half the size of the other.

Apart from the work of SAYLOR and QUINN the reports have merely mentioned the observation of infrequent bridges with little attempt to provide definitive information. This study on *P. radiata* was therefore conducted to obtain quantitative data which would define the extent and importance of inversions, if any, in the species.

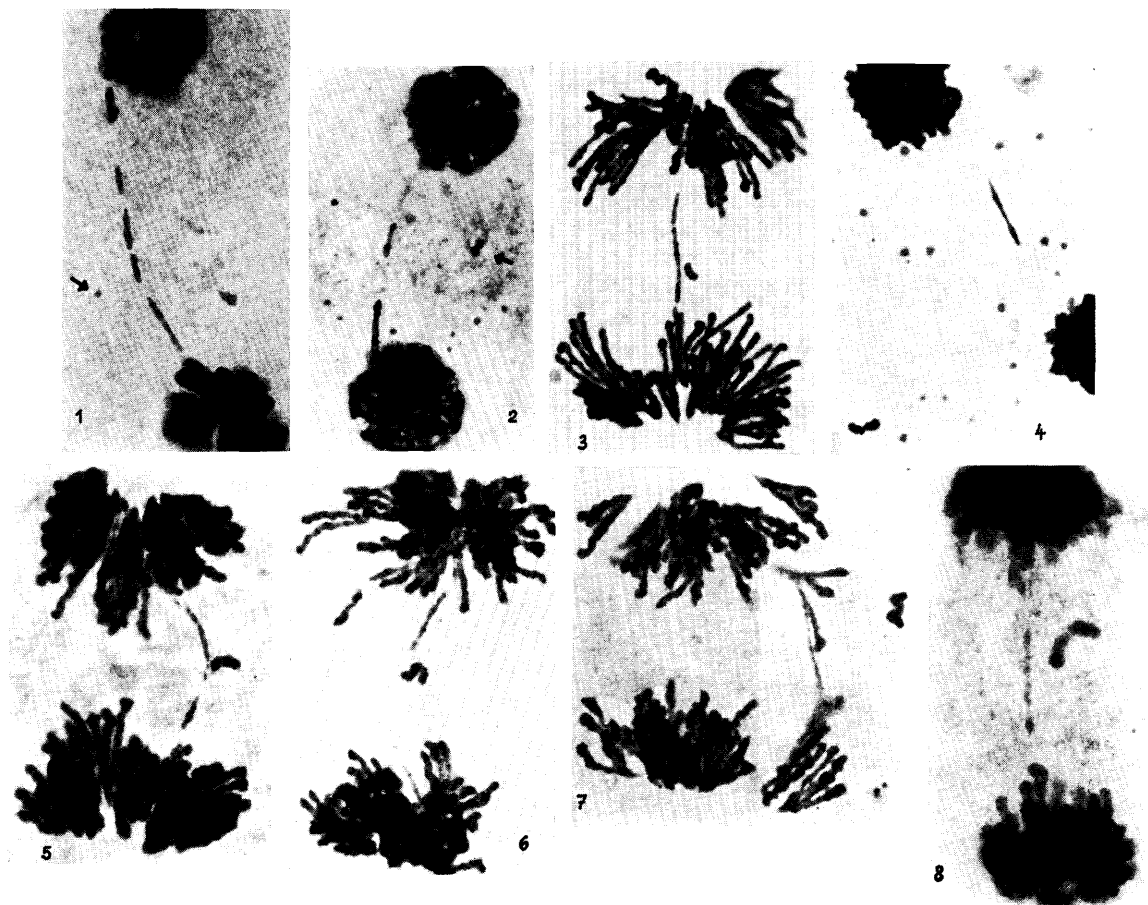
Methods

The following plants were studied: —

- (a) twelve *P. radiata* plants.
- (b) four related (full-sib) *P. radiata* × *attenuata* hybrids.

The twelve *P. radiata* trees were widely distributed in plantations throughout Australia and New Zealand, therefore they are unlikely to be closely related. Ten of them were originally selected for tree breeding work, being phenotypically superior. The other two were mature trees randomly selected during a cytological study of *P. radiata*.

Shoots bearing staminate strobili collected from a clone arboretum or from the original trees were kept in the laboratory with their bases in water and examined daily



Figs. 1—8. — Anaphase I — telophase I bridge and fragment configurations observed in various *Pinus radiata* trees, arranged in a series with increasing fragment size. Arrows point to fragments (× 1000).

Fig. 1 — tree MY2
Fig. 2 — tree ACT38^A
Fig. 3 — tree NR10
Fig. 4 — tree NZ7

Fig. 5 — tree ACT419^A
Fig. 6 — tree CR51
Fig. 7 — tree NZ7
Fig. 8 — tree CA4



Fig. 9. — Inversion bridge at anaphase II, found in tree CR7. The origin of this bridge is probably as shown in fig. 11 B ($\times 500$).

till anaphase I of microsporogenesis commenced (during mid-June). Strobili at the desired stage were fixed in freshly mixed absolute alcohol (3 parts): glacial acetic acid (1 part) to which a little ferric chloride had been added to improve the subsequent staining. Several days later acetocarmine preparations were made. It was found preferable to study the cells on each slide before squashing because many chromatin bridges were very thin and attenuated and were sometimes ruptured when squashed. Counts were made of all cells, from the stage when anaphase separation of chromosomes had been completed, up to and including those telophase cells in which the shape of some chromosome arms could still be detected.

In view of the difficulties associated with the diagnosis of paracentric inversions, particularly the possible confusion with cases of slow terminalisation of chiasmata, the following criteria were adopted for acceptance as inversion bridges.

1. The bridge should be continuous across the cell from pole to pole, and there should not be any swelling in a central region which would indicate non-disengagement of a terminalised chiasma.
2. An acentric fragment must accompany the bridge (although in a small proportion of cases the fragment can become obscured by other chromosomes).
3. Acentric fragments alone do not denote inversions since fragments may arise by breakage of stretched chromosomes, e.g. those in which chiasmata were slow to terminalise.

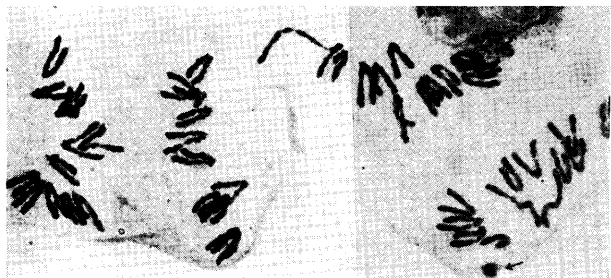


Fig. 10. — Inversion bridge at anaphase II, found in tree CR51. The fragment is visible in the lower right of the picture. In this case the bridge is residual from anaphase I, derived as in fig. 11 A ($\times 1000$).

Inversion bridges were recorded in two classes — those with the accompanying fragment and those without a fragment. Twelve cases without visible fragments were accepted as inversion bridges (see criterion 2).

Results

Evidence of inversions was found in all the twelve *P. radiata* trees examined (see table 1). Bridge-fragment configurations (as in figs. 1–8) were observed in eleven trees, and a bridge at anaphase II was observed in the twelfth (fig. 9). The average frequency of bridges was rather low i.e. 1 per 210 cells.

Variation in the thickness or density of the chromatin of some bridges was noticed. They were often observed to contain short sections which were a little narrower and less densely stained. These narrow sections were usually distributed regularly along the bridge and are considered to indicate the position of constrictions, and to be heterochromatic in structure. (Refer to discussion of chromosome structure, PEDERICK, 1967).

Some cells contained bridges which were very narrow and attenuated. In certain cells observed both before and after squashing the bridge sometimes broke during squashing. For this reason examination of cells before squashing

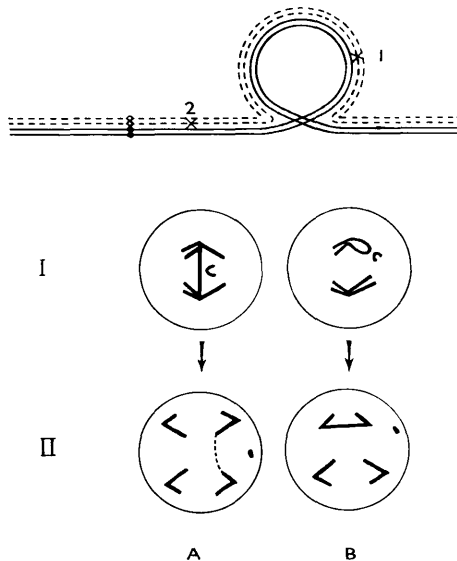


Fig. 11. — Diagram to illustrate the methods of origin of recognisable types of anaphase I and II configurations. —

- A, from a single crossover at position 1 or any position within the inversion. Sometimes the bridge may persist through to anaphase II as indicated by the dotted line.
- B, from two crossovers within and outside the inversion at positions 1 and 2, with one chromatid common to both crossovers.

was preferred. In some such cases considerable displacement of the broken portions of the bridge occurred, so that some cells with inversion bridges would probably not have been recognized as such if examined after squashing, therefore it is likely that the observed bridge frequency may be a little low. It is suggested that breakage takes place at a heterochromatic region because these regions often appear thinner or attenuated and give an impression of weakness.

Although most bridges probably break naturally during telophase, some are thick and persist well into interphase. No attempt was made to determine the frequency of the latter types. A small proportion persist right through to the second division and were observed at anaphase II. There are two types of anaphase II bridge depending on whether

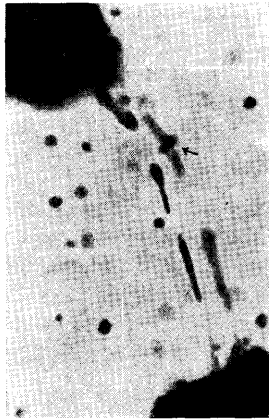


Fig. 12. — Telophase I with double bridge configuration, found in tree NZ55. One fragment appears to be behind a bridge (see arrow), but the other fragment can not be located. The spots in the cytoplasm are the telophase globules (containing RNA but no DNA) commonly observed at this stage ($\times 1000$).

the crossover chromatids segregate at the first or second division (figs. 10, 9). Their origin is illustrated in figure 11. The more common type (first division) originate from bridges which have persisted intact since anaphase I (two of these were found, fig. 10).

The second type only occurs when two crossovers take place in one chromosome arm, one crossover in the inversion and the other proximal to it, one chromatid being involved in both cross overs (one of these was found, fig. 9).

In some of the trees it was observed that the fragments in different cells varied considerably in size (figs. 2–9). Fragment size can sometimes be used to indicate the relative position of an inversion within its chromosome arm. Therefore, the fragments were studied with special interest.

Two bridges were observed in one cell of tree NZ 55 (fig. 12).

Inversions were also found in three of the four hybrid trees at an average frequency of 1 per 81 cells. This frequency is greater than that in *P. radiata*. The difference between the two is highly significant ($\chi^2_1 = 12.94^{***}$, $P < 0.001$).

Discussion

Fragment size corresponds to the length of the inverted section plus twice the length of chromosome arm distal to the inversion. A similarly sized fragment could therefore be produced from two inversions possessing a different combination of length and position. All fragments relating to a given inversion are the same size although their appearance can vary depending on the amount of condensation which has taken place. Fragment size therefore provides an uncertain basis for analysis. For this reason only a very simple classification of fragment size was attempted. However, in the majority of cases observed the fragments were somewhat rod-shaped and of a thickness which indicated that condensation had remained similar to that present during the previous metaphase. In such cases a comparison of length between fragments of similar thickness was valid. It was also noticed that some fragments were curved or bent at their centre point. This was probably a residual effect of the original position occupied by the two chromatids prior to anaphase. Some fragments appeared to be divided into two equal halves by a central heterochromatic zone (figs. 2, 4, 8). It is suggested that this could indicate that crossing-over occurred at or near a constriction.

Distinctly different fragment sizes observed in some trees e. g. ACT956B, ACT419A, MY2, NZ7, MY7, indicate that more than one inversion is present in these trees. Indisputable evidence of this was found in tree NZ55 with a cell containing two bridges (these were not considered to be caused by a four strand double crossover within one inversion because there is good evidence that the inversions are very short). Although it is not possible to determine accurately the number of different inversions in a tree, it does seem likely, on the basis of fragment size, that some trees probably contain even more than two inversions.

Unfortunately there is no means available to determine whether a similar sized fragment found in two trees is derived from the same inversion sequence. Also it is not possible to determine the chromosome on which the inversion is located. However, a considerable variation in fragment size has been found among the twelve trees (figs. 1–8), which points to the likelihood of a considerable number of different inversions being present in the species population. On statistical grounds the observation that all twelve trees were heterozygous for one or more inversions further supports this conclusion.

There is some evidence to indicate the length of the inverted sequences. Some of the fragments are very small (figs. 1, 2), and in such cases it is obvious that the inversion must be a very short one as well as occupying an almost terminal position. Further evidence is provided by the very low frequency of bridges. If the different fragment sizes found in each tree are distinguished, it appears that crossing-over within a particular inversion may occur less frequently than one per thousand cells. Bridge frequency depends on the length of the inversion as well as the likelihood of crossing-over occurring in that part of the chromosome. Other studies by the author (not yet reported) have indicated that although crossing-over may be more frequent in the centre region of chromosome arms it seems to occur in all regions. On the basis of this evidence it appears that in all inversions — even those producing the largest fragments — the lengths of the inversion are quite short.

If this conclusion is correct, the longest fragment found (figs. 7, 8) would indicate an inversion located somewhere in the proximal part of a chromosome arm. The evidence therefore suggests that an inversion can form in any portion of a chromosome arm.

Unfortunately it is not possible to develop the data much further. Although it has been demonstrated that there are a number of different inversions present in the species population it has not been possible to identify trees carrying the same inversion sequence. Therefore, any attempt to relate particular inversions to morphological characters or to adaptive value will not be possible until a new technique is developed to make identification possible. However it is most unlikely that the group of genes in *P. radiata* inversions would exert influences as marked as those recorded in *Drosophila pseudoobscura* (DOBZHANSKY 1951) or *Moraba scurra* (WHITE and ANDREW 1962) because the inversions in these insects are relatively much longer than appears the case in *Pinus radiata*.

The characteristics of bridge and fragment formation in the hybrid *P. radiata* \times *attenuata* were similar to those described for *P. radiata* except that their frequency was significantly higher, i. e. 1 in 81 compared with 1 in 210. MASON (1932) concluded from a study of fossil material that *P. radiata* and *P. attenuata* have evolved from an ancestor *P. masoni* common in the Pleistocene age. Some possible reasons for the difference in bridge frequency include (1)

that a number of different inversions have become established in each of the two species, (2) that some of these may have become fixed and constitute a karyotypic structural difference between the two species, and (3) that the hybrid genic environment has induced more crossing-over in regions where inversions are present.

Finally, one must consider the genetic function of these short inversions. Inversions appear to be present in most if not all *Pinus* species and therefore are a characteristic of the genus. It seems likely, then, that individual inversions are of great antiquity rather than of recent origin. The argument then follows that, as outlined in the introduction, inverted sequences of genes would contain a different allelic composition from their non-inverted homologues, and herein exists a source of variation for the species since some trees have been found to carry two (and possibly more) inversions. It appears likely that individuals without an inversion may be uncommon.

It seems unlikely that inversions have a neutral effect and have just been accumulated by chance, although selection against the heterozygote condition, due to crossing over within the inversion and formation of inviable gametes, is weak in *P. radiata* and unlikely to have much effect. The genus *Pinus* is a very old one in evolutionary history and can be assumed to have a stabilised and proven genetic system. From this study and other published data, it appears that paracentric inversions are a characteristic of the genus *Pinus* and therefore are likely to confer some genetic benefit. In *P. radiata*, whether this is potential variability due to different alleles present in the inverted and normal sequences, or to some other effect such as heterozygote overdominance, remains an intriguing question.

Acknowledgements

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Conclusions

Chromosome inversions (paracentric) were found in all of twelve *Pinus radiata* trees examined, as evidenced by

the presence of bridge-fragment configurations at anaphase I.

Fragments of markedly different size were observed in some of the trees, indicating the presence of two or more inversions in those trees. A double bridge provided additional evidence of at least two inversions in one tree.

Many different fragment sizes were observed in cells from the twelve trees indicating the existence of many different inversions in the species population.

The low frequency of bridge formation suggests that all the inversions are very small.

A similar range of bridge-fragment configurations was observed in *P. radiata* × *attenuata* hybrids but at a greater frequency.

It has been argued that inversions are a characteristic of the *Pinus* genetic system and therefore are likely to have some positive genetic value, although no data to this effect can yet be presented. While inversions may form only a small proportion of the total length of *P. radiata* chromosomes, they nevertheless are a likely source of hidden variability in the species.

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Versuche zur Stimulation der Blüte an Douglasien-Pfropflingen

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Einleitung

Seit Beginn der forstlichen Züchtung werden Anstrengungen unternommen, um Waldbäume zu erhöhtem Blütenansatz und größerer Saatproduktion anzuregen. Die angewendeten Methoden reichen von Versuchen zur Änderung der ökologischen Bedingungen (JOHNSON *et al.* 1955, MILAN *et al.*, VIDAKOVIC 1963, LOWRY 1966, BÁNÓ 1966) bis zur Direktbe-

handlung des Individuums, wie beispielsweise Düngung, mechanische Eingriffe oder die Applikation von Wachstums- und Hemmstoffen (Zusammenfassung bei MATTHEWS 1963). Davon scheinen in Saatguterntebeständen und Samenplantagen die Bodenbearbeitung (NILSSON 1955, THÜMLER 1963 a) sowie Düngung und Formschnitt (HOFFMANN 1959, THÜMLER 1963 b) einen festen Platz eingenommen zu haben. Behandlungen wie Wurzelschnitt, Ringelung, Strangulation und Herunterbinden der Zweige werden dort im Hinblick auf die erhöhte Bruch- und Windwurfgefahr mit Recht nur sehr vorsichtig und vereinzelt angewendet. Um Kreuzungsprogramme mit bestimmten Mutterbäumen durchzuführen, bei

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