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The G-banded Karyotype of *Pinus resinosa* Ait.

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Summary

Characterization of the individual chromosomes of *Pinus resinosa* ($2n = 24$) by means of G- and Q-banding techniques is presented. This is the first report of a G-banded karyotype in a plant species and reasons for lack of previous success are discussed. The similarity of the banding patterns and secondary constrictions in apparently non-homologous chromosomes indicates that polyploidy may have played a role in the evolution of *Pinus*.

Key words: *Pinus resinosa*, G-banding, Q-banding.

Zusammenfassung

Durch G- und Q-Bandmuster werden einzelne Chromosomen bei *Pinus resinosa* ($2n = 24$) charakterisiert. Dies ist der erste Bericht über die Anwendung von G-Banden zur Charakterisierung des Karyotypes bei Pflanzen. Gründe für frühere Mißerfolge werden diskutiert. Die Ähnlichkeit der Bandmuster und sekundäre Einschnürungen in offensichtlich nicht-homologen Chromosomen zeigen, daß möglicherweise Polyploidie eine Rolle bei der Evolution von *Pinus* gespielt hat.

Introduction

Identification of individual chromosomes of plants belonging to the genus *Pinus* has not been possible on the basis of such morphological characteristics as size and arm ratio since most of the chromosomes are metacentrics of similar size. In addition there exists a remarkable degree of similarity of chromosome morphology between species of the genus *Pinus*, whose members possess $2n = 24$ chromosomes (SAYLOR, 1964). Since *Pinus* is well represented by fossils over 100 million years old (FLORIN, 1963) the genus provides an opportunity to study unusually slow evolution.

Karyotype comparisons between *Pinus* species with conventional stains have been previously limited by the lack of identifying characteristics for individual chromosomes. Similar problems in human cytology have been solved by the use of fluorochromes and G- and C-banding. In this report G- and Q-banding have been used to identify the individual chromosomes of *Pinus resinosa* AIT. As far as

this author is aware, this is the first plant in which clear G-banding has been demonstrated (DREWRY, 1982).

Materials and Methods

Seeds of red pine *Pinus resinosa* were germinated following stratification. When the primary roots were 1 to 2 cm long, the seeds were placed overnight on filter paper moistened with a 1% solution of colchicine. Root tips were fixed in 3:1 ($C_2H_5OH:CH_3COOH$). The fixed roots were refrigerated (4°C) until they were made into slides 1 to 7 days later.

The fixed roots were rinsed in 45% acetic acid and squashed in the same solution. The coverslips were removed with dry ice.

For Q-banding the slides were briefly air-dried and then stained in 0.5% Atebrin (Gurr) (quinacrine HCL) solution for 5 to 10 minutes. The preparations were passed through a flame and rinsed in water. Coverslips were mounted with 40% sucrose solution. Thereafter the slides were kept on a hot plate at 45 to 60°C until banded (15 to 60 minutes).

For G-banding squashed root tips were air-dried at room temperature and then kept at 45 to 60°C for 1 to 12 hours. Thereafter the slides were treated with trypsin solution (Gibco: Trypsin-EDTA 10X 5.0 ml, Hanks BBS 4.5 ml, H_2O 40.5 ml) at room temperature for 20 to 40 minutes. Staining was accomplished in a filtered Giemsa solution (1 part Gurr's R66 Giemsa to 3 parts buffer from Gurr's buffer tablets adjusted to pH 7.9 with sodium bicarbonate). After 3 to 6 minutes in the stain the slides were briefly rinsed in a pH 6.8 buffer solution (Gurr's buffer tablets). As mounting medium Permount was used. Complete banded metaphase spreads were obtained for both G and Q-banded (Fig. 1) chromosomes. Individual examples from each pair of chromosomes were selected from G- and Q-banded partial metaphase spreads and assembled for Figure 2. Since the two arms of many of the chromosomes are of similar size, the arm with a secondary constriction is up in the karyotype.

Results

The significance of the various types of bands is far from clear even in human chromosomes for which these

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techniques were developed. What is known is that the bright Q-bands and the dark G-bands correspond. There is also evidence that the Q-bright bands have fewer genes than the Q-dark bands (KUHNS *et al.* 1985). Human heterochromatin is either very brightly or not at all fluorescent. In the pine chromosomes, certain bands, especially around the centromere, but also elsewhere, are as Q-bright as for instance the distal heterochromatin in the human Y chromosome (Fig. 1). They also stain very darkly with G-banding (Fig. 2). In analogy with human chromosomes, it is assumed that they represent some type of heterochromatin, and they are tentatively called C-bands. However, in the present context their significance does not really matter since they are used here only as markers to distinguish the different chromosomes.

Most of the G-bands in *Pinus* are very thin. With quinacrine they are only faintly visible (Fig. 2). The majority of the chromosomes can be distinguished on the basis of their C-bands and the location of secondary constrictions. Below is a brief list of the main features, which distinguish the different chromosomes.

Chromosome 1. C-bands are asymmetric with respect to the centromere; one C-band is immediately adjacent to the secondary constriction.

Chromosome 2. One C-band is in the arm containing a secondary constriction; the opposite arm has a tertiary constriction and numerous G-bands.

Chromosome 3. C-bands are situated on either side of the secondary constriction; one C-band is in the opposite arm.

Chromosome 4. Two C-bands in the arm containing the secondary constriction; one C-band in the opposite arm, which also has a thin tertiary constriction.

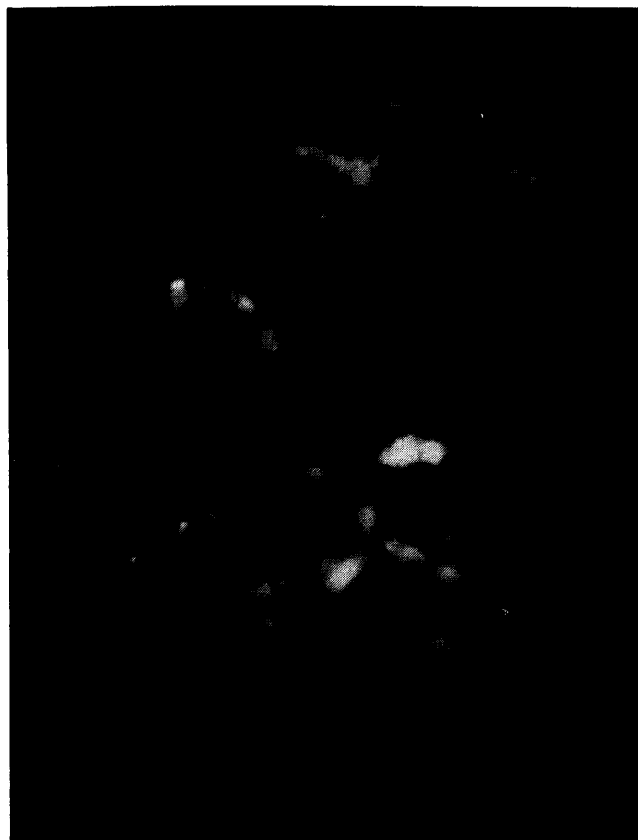


Figure 1. — Quinacrine-stained metaphase of *Pinus resinosa* ($2n = 24$).

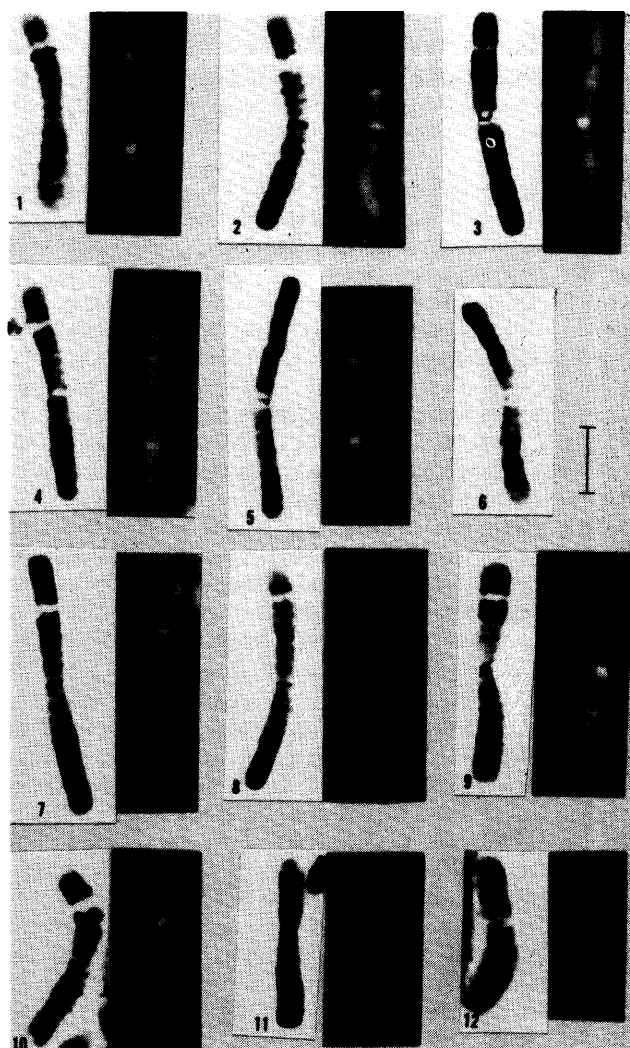


Figure 2. — Haploid karyotype of the red pine. For each chromosome the G-banding pattern is on the left with the Q-banded chromosome on the right. The Q-brightest (G-darkest) bands around the centromere and elsewhere are tentatively assumed to represent C-bands.

Chromosome 5 and 6. C-banding patterns are very similar, with symmetrical C-bands on both sides of the centromere. No secondary constriction is present in either arm. Q-banding patterns are indistinguishable.

Chromosome 7. One C-band is in the arm containing a secondary constriction; the opposite arm shows a tertiary constriction. Centric heterochromatin has a bipartite appearance.

Chromosome 8. Distinguishable most easily by the position of the secondary constriction in one arm showing two thin C-bands; the opposite arm has one thin C-band.

Chromosome 9. Symmetrical C-bands are situated on both sides of the centromere; secondary constriction in one arm. Centric heterochromatin has a bipartite appearance.

Chromosome 10. A C-band is situated adjacent to the secondary constriction; opposite arm shows tertiary constriction.

Chromosome 11. A C-band is localized in the long arm; the chromosome is distinguishable also on basis of size. Centric heterochromatin has a bipartite appearance.

Chromosome 12. This chromosome is distinguishable on basis of size; no C-bands except one at the centromere are present.

Discussion

The genus *Pinus* ($2n = 24$) is fascinating both from cytogenetic and evolutionary standpoints. The genus is ancient; it is well represented in fossil records for more than 100 million years and during this time its morphological evolution has been very slow (FLORIN, 1963). Several different approaches have been used to study the evolution of the genus *Pinus*. Using an immunologic comparison of seed proteins PRAGER (1976) concluded that the evolution of structural genes has occurred at roughly the standard rate. On the other hand karyotypic evolution has been remarkably slow. As early as 1933 (SAX and SAX, 1933) it was reported that the different species of the genus *Pinus* are very similar in regard to chromosome number, length, and centromere position. More recently SAYLOR (1983) has completed a study of the unbanded karyotypes of 87 *Pinus* species, confirming the nearly uniform karyotypes in this genus. This similarity is even more remarkable in light of the high nuclear DNA content and great chromosome length in *Pinus*, factors which should increase the frequency of structural changes and make their discovery easy. Thus the genus *Pinus* poses to the cytogeneticist a challenge of distinguishing between chromosomes which are virtually identical not only within a species, but also within the genus.

In the present study, G- and Q-banding have been employed to identify the individual chromosomes of *Pinus resinosa* (Fig. 2). Interestingly most, possibly all, chromosomes show a resemblance to another member of the haploid complement (2 and 7, 5 and 6, 4 and 8, 3 and 9). The presence of such pairs strongly suggests that the chromosomes of each of these pairs are originally homologous and hidden polyploidy has played a role in the evolution of *Pinus*. This may be the reason for the extreme rarity of naturally occurring polyploids in this genus (DELEVORYAS, 1980), since polyploidization in plants past an optimal level results in decreased vigor.

On the basis of unbanded chromosomes the genus *Pinus* is unique in regard to karyotype conservation. Summarizing the available data from *Drosophila*, *Chironomus*, grasshoppers, and other animals, WHITE (1973) concludes that karyotypic differences exist between even the most closely related species and suggests that chromosome rearrangements play a direct causative role in speciation. The animal model systems which allow the analysis of chromosomes with high resolution techniques are generally, but not totally, consistent with this idea. YUNIS and PRAKASH (1982) compared the prophase banding patterns of man and the great apes and derived probable karyotypes for their presumed common ancestors. They also determined the necessary structural changes to account for the banding pattern differences between these species. Most of the chromosomal differences between the species could be accounted for by inversions, most commonly of the pericentric type and alterations in constitutive heterochromatin. In *Drosophila*, whose polytene chromosomes permit the study of high resolution banding, a relatively large amount of chromosome polymorphism, particularly inversions, exists even within a species. The vast majority of *Drosophila* species differ in the structure of their polytene chromosomes; however, there are examples among the 700 species of Hawaiian *Drosophilidae* that have identical banding patterns. Among the 87 species of *Pinus* analyzed, the subsection *Sylvestris* differs in having two rather than one chromosome pair with a submedian centromer position (SAYLOR, 1983). Preliminary work indicates that this difference represents a pericentric inversion.

Apart from this, an analysis of nonbanded *Pinus* chromosomes shows this genus to be more conservative than either *Hominidae* or *Drosophila*. Determination of whether this conservation extends to G-banding patterns would provide a unique model system for studying the role of chromosome structural changes in speciation.

Pinus resinosa is the first plant for which G-banded chromosomes have been reported (DREWRY, 1982) and, as far as the author is aware, this is the first report of any G-banded plant karyotype. Several ideas have been put forward to explain the lack of success in inducing G-banding in plant chromosomes. GREILHUBER (1977) pointed out that plant chromosomes are generally more contracted at metaphase than mammalian chromosomes and concluded that this would make it impossible to resolve G-banding patterns. The chromosomes of *Pinus resinosa* do show G-banding despite being more contracted than human chromosomes, possibly because of the large size of the chromosomes; however, smaller chromosomes of other plant species may be even more highly contracted. The degree of chromosome contraction is partially dependent upon length of exposure to colchicine; if the chromosomes are too contracted to allow G-banding, adaptation of recent methodological advances in human cytogenetics might prove useful. Observations made using these methods, which include cell synchronization together with shortened exposure to colcemid (YUNIS, 1976) and use of agents which inhibit metaphase contraction (MATSUBARA and NAKAGOME, 1983; RYBAK *et al.*, 1981) show that chromosome length can be increased with various prefixation techniques. Another factor which adds to the difficulty of G-banding plant chromosomes is that swelling of cells with hypotonic solution is prevented by the cell walls. Acid hydrolysis is commonly used to allow squashing of plant cells for chromosome spreading; however this technique prevents G-banding (DREWRY, 1982).

Employment of banding techniques in mammalian cells has had an enormous impact in many fields from human medicine to evolutionary biology. While early human cytogeneticists borrowed techniques and personnel from the field of plant cytogenetics, now the methodology for human cytogenetics, particularly banding techniques are more advanced than plant cytogenetics. Since chromosome structure must be similar in plants and animals it seems that plant cytogenetics could benefit from adaptation of human techniques. Development of G-banding techniques for plant chromosomes could have as great an impact on plant cytogenetics as they have had on animal cytogenetics.

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Provenance Trials of *Eucalyptus grandis* and *E. saligna* in Australia

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Summary

The growth of provenances of *E. grandis* was examined in four trials, and *E. saligna* in two of these, in eastern Australia and compared with results from a number of countries also growing these species. In two Australian trials the performance of the commonly-used Coffs Harbour provenance was not outstanding and there was much variation between provenances collected from the broad Coffs Harbour area. Overall the better provenances of *E. grandis* came from a wide range of collection sites from the furthest north to the furthest south. There is an apparent lack of a geographic pattern associated with the growth of *E. grandis* provenances. Implications for future provenance introduction strategies are discussed. *E. saligna* provenances performed well at one trial but not at the other.

Key words: *E. grandis*, *E. saligna*, provenance trials, breeding strategy.

Zusammenfassung

Der Zuwachs von *Eucalyptus grandis*- und *E. saligna*-Provenienzen wurde in vier bzw. in zwei Feldversuchen in Ostaustralien gemessen, und die Ergebnisse wurden mit Messungen aus anderen Ländern verglichen. In zwei australischen Versuchen zeigte das gewöhnliche, von Coffs Harbour stammende Provenienz kein außergewöhnliches Wachstum. Außerdem zeigten diese Provenienzen aus dem Großraum Coffs Harbour eine höhere Zwischenvariabilität. Die *E. grandis* Provenienzen, die insgesamt eine höhere Zuwachsleistung zeigten, stammen aus einer weitrangigen Sammlung von den nördlichsten bis zu den südlichsten Vorkommen.

Weitere Folgerungen für die zukünftigen Strategien zur Auswahl von Provenienzen wurden diskutiert. Gute Ergebnisse über den *E. saligna* wurden nur in einem Versuch erzielt, nicht jedoch in dem anderen.

Introduction

Flooded gum (*E. grandis* HILL ex MAIDEN) is among the most widely planted eucalypts in the world. It appears that about two million hectares have been established as industrial plantations but it is difficult to be exact because of the uncertainty of some records. South Africa had just under 300,000 ha planted by 1984 (Anon., 1985) while Brazil has probably more than 1 million ha (GALVAO and COUTO, 1984). *E. grandis* occurs between latitudes 17° S and 32° S within about 100 km of the coast of eastern Australia (Fig. 1). *E. saligna* SM. has not been so widely planted but is considered to be of importance in Hawaii (KING, 1983). The natural distribution of *E. saligna* overlaps that of *E. grandis* and ranges from about 25° S to 36° S (Fig. 1). A number of countries have provenance studies in progress, e.g. Australia (ADES and BURGESS, 1983), Brazil (PIRES *et al.*,

1983), India (DEO *et al.*, 1986), Madagascar (RAKOTOMANAMPISON, 1983), South Africa (DARROW and ROEDER, 1983; DARROW, 1983), USA (KING, 1983; LEDIG, 1983; BAILEY and LEDIG, in preparation) and Zimbabwe (BARRET *et al.*, 1975; MATHESON and MULLIN, in press). Also PEDERICK (1983) organised an extensive series of trials involving 15 countries under the auspices of the International Union of Forestry Research Organisation's working party on eucalypt provenances.

In general the results of these trials have indicated that the most productive provenances came from the northern part of New South Wales (NSW) centered around Coffs Harbour e.g. BAILEY and LEDIG (in preparation), DARROW (1983), LEDIG (1983), MATHESON and MULLIN (in press), PIRES *et al.* (1983), RAKOTOMANAMPISON (1983) and DEO *et al.* (1986). Within Australia, CLARKE (1975), demonstrated that Coffs Harbour provenances grew better at Coffs Harbour than the range extremes from Atherton and Minmi. ADES and BURGESS (1983) also reported that the Coffs Harbour district provenances together with a provenance from southern Queensland were the most productive, when planted in the Coffs Harbour area, but this trial did not include the more northern Queensland populations.

The apparent superiority of this provenance is questioned only when disease problems arise, particularly *Cryphonectria cubensis* (BRUNER) HODGES. CAMPHINOS and IKEMORI (1978)

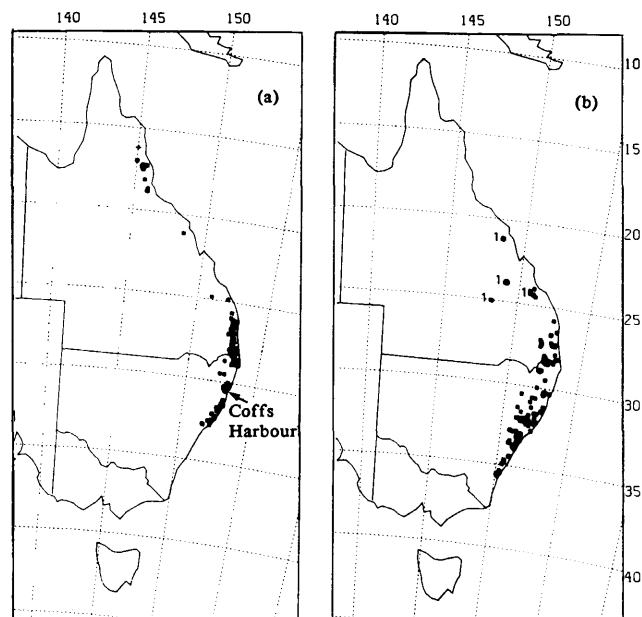


Figure 1. — Distribution map *E. grandis* (a), *E. saligna* (b) and *E. grandis*/*E. saligna* complex 1.