Acknowledgements

Work by many officers of the Queensland Department of Forestry over a long period of time enabled this study to be completed. We gratefully acknowledge the contribution of Dr D. G. Nikles who planned tnd supervised much of the research and also encouraged us to conduct the wood study. Substantial contributions were also made by several other tree-breeding officers in establishing, maintaining and measuring the progeny trial, and by the staff of the Wood Structure Laboratory in providing wood quality data. Critical review comment on the manuscript by Dr. K. G. Eldridge and Dr. K. P. Ades was appreciated.

References

Becker, W. A.: Manual of quantitative genetics. Student Book Corp., Washington State University (1975). —— Cotterill, P. P. and Jackson, N.: Index selection with restrictions in tree breeding. Silvae Genetica **30**: 106—108 (1981). —— Cotterill, P. P. and Jackson, N.: On index selection. I. Methods of determining economic weight. Silvae Genetica **34**: 56—63 (1985). —— Dean, C. A., Cotterill, P. P. and Cameron, J. M.: Genetic parameters and gains expected from multiple trait selection of radiata pine in eastern Victoria. Aust. For. Res. **13**: 271—278 (1983). —— Dean, C. A., Cotterill, P. P. and Eisemann, R. L.: Genetic parameters and gains expected from selection in *Pinus caribaea* var. *hondurensis* in northern Queensland, Australia. Silvae Genetica **35**: 229—236 (1986). —— Dean, C. A., Nikles, D. G. and Harding, K. J.: Estimates of genetic parameters and gains expected from selection in hoop pine in south-east Queensland. Silvae Genetica **37**: 243—247 (1988). —— Falconer, D. S.: Introduction to quantitative genetics. Oliver and Boyd, Edinburgh. 365 p. (1960). —— Gordon, I. L., Byth, D. E. and Balaam, L. N.: Variances of heritability ratios estimated from phenotypic variance components. Biometrics **28**: 401—415 (1972). —— Harris, J. M.: Non-destructive assessment of spiral grain in standing trees. N. Z. J. For Sci. **14**: 395—399 (1984). —— Heinrichs, J. F. and Lassen, L. E.: Improved techniques for determining the volume of irregularly shaped wood blocks. For. Prod. J. **20**: 24 (1970). —— Nicholls, J. W. P.: Some aspects of the genetic and environmental control of spiral grain in *Pinus* species. 14th IUFRO Congress, Munich (1967). —— Nicholls, J. W. P., Dadswell, H. C. and Fielding, J. M.: The heritability of wood characteristics of *Pinus radiata*. Silvae Genetica **13**: 68—71 (1964). ——Nikles, D. G.: Realized and potential gains from using and conserving genetic resources of *Araucaria*. Proc. IUFRO Meet. on Forestry Problems of the Genus *Araucaria*, Curitiba, Parana, Brazil. p. 87—95 (1980). —— Searle, S. R.: The value of indirect selection. I. Mass selection. Biometrics **21**: 682—707 (1965). —— Shelbourne, C. J. A., Zobel, B. J. and Stonecypher, R. W.: The inheritance of compression wood and its genetic and phenotypic correlations with six other traits in five-year-old loblolly pine. Silvae Genetica **18**: 43—47 (1969). —— Smith, W. J.: The wood utilization potential of the *Araucarias*. Proc. IUFRO Meet. on Forestry Problems of the Genus *Araucaria*, Curitiba, Parana, Brazil. p. 265—285 (1980). —— Steel, R. G. B. and Torrie, J. H.: Principles and procedures of statistics. McGraw-Hill, New York. 481 p. (1960). —— Tallis, G. M.: Sampling errors of genetic correlation coefficients calculated from analyses of variance and covariance. Aust. J. Stat. **1**: 35—43 (1959). —— Zobel, B. J.: Breeding for wood properties in forest trees. Unasylva **16**: 89—103 (1964).

Giemsa C-Banded Karyotype in Quercus L. (Oak)

By D. Ohri[1]) and M. R. Ahuja

Federal Research Centre for Forestry and Forest Products,
Institute of Forest Genetics and Forest Tree Breeding,
Sieker Landstraße 2, D-2070 Grosshansdort,
Federal Republic of Germany

(Received 12th December 1989)

Abstract

A detailed karyotypic investigation by Giemsa C-banding in three species of *Quercus*: *Q. robur*, *Q. petraea* and *Q. rubra* has revealed a general interspecific uniformity with respect to C-banded patterns and karyomorphology. However, the different chromosomes can be identified and paired on the basis of C-bands. A similar constancy in nuclear DNA amounts is also noticed. One B-chromosome has been found in all the three species of oak investigated.

Key words: Quercus, oak Fagaceae, Giemsa C-banding, Karyotype analysis, nuclear DNA, B-chromosome.

Introduction

Quercus belongs to the family Fagaceae which represents the most important source of timber among the broad-leaved species of the northern hemisphere. Oak wood is specially known for its strength, durability and all-round usefulness. A good amount of work has been done in population genetics, hybridization and speciation in North America and Europe. However, cytological studies are restricted to only chromosome numbers. No thorough karyological studies using modern banding methods are available for *Quercus*. Proper identification of chromosomes with the help of C-bands, on the one hand, is essential in the systematics of the species and, on the other hand, for a clear understanding of the chromosomal basis of somaclonal variation in the micropropagated plants. Micropropagation is being increasingly carried out in oak for clonal propagation and conservation of elite genotypes (Ahuja, 1986). The present study deals with a detailed karyotypic analysis of three oak species.

Materials and Methods

Three species of oak, viz, *Q. robur* L., *Q. petraea* Liebl., and *Q. rubra* L. were employed for cytological studies. Root tips were taken from potted plants grown at the Institute of Forest Genetics and Forest Tree Breeding at Grosshansdorf. These were pretreated in 0.002 M aqueous solution of 8-hydroxy-quinoline at 15° to 16° C for three hours and fixed in 1:3 acetic alcohol overnight. After washing twice for 30 minutes each in 0.1 M citrate buffer pH 4.7, root tips were macerated in 1% w/v pectinase at 37° C for two hours, and subsequently in 0.25% w/v cellulase for 15 minutes, again washed in 0.1 M citrate buffer (ph 4.7) for 10 minutes and squashed in 45% acetic

[1]) present address: Cytogenetic Laboratory, National Botanical Research Institute, Rana Pratap Marg, Lucknow-226 001, (U.P.), India

acid. Cover slips were removed by the dry ice method. Slides were stored dry at room temperature following ethanol treatment. These were then transferred to 45% acetic acid at 60° C for 15 minutes, washed in running tap water, then transferred to saturated solution of barium hydoxide for ten minutes at room temperature and washed extensively in running tap water. Slides were then incubated in 2X SSC (0.3 M sodium chloride + 0.03 M trisodium citrate, pH 7.0) at 60 °C for 90 minutes, then rinsed in 0.067 M citrate buffer pH 6.7 and stained in freshly prepared Giemsa stain in 0.067 M citrate buffer (pH 6.7) for 10 minutes. Following staining, slides were rinsed in distilled water, air dried, dipped in xylene and mounted in Euparol.

For the measurement of nuclear DNA amounts, root tips were fixed in 1:3 acetic alcohol for 2 hours. Following a thorough wash the root tips were then hydrolysed in 5 N hydrochloric acid for 30 minutes at room temperature and stained in leuco-basic fuchsin at pH 3.6 for 2 hours. Then they were given three washes of 10 minutes each in sulfur dioxide water, and finally squashed in a drop of glycerol. Four slides of each material were prepared using a single root tip. The relative absorption at 565 nm of individual Feulgen stained nuclei was measured using a Vickers M86 Scanning Microdensitometer. Measurements of at least 10 early telophase nuclei per replication of each taxon was made. The absolute values were calculated using *Allium cepa*, whose nuclear DNA value is 33.55 pg, as a standard.

Results and Discussion

All the investigated species of *Quercus* have a chromosome complement of 2n = 24, which corroborates earlier reports (Fedorov, 1969). This possibly represents a paleopolyploid number. It is conceivable that the karyotype has diploidized during evolutionary history following sev-

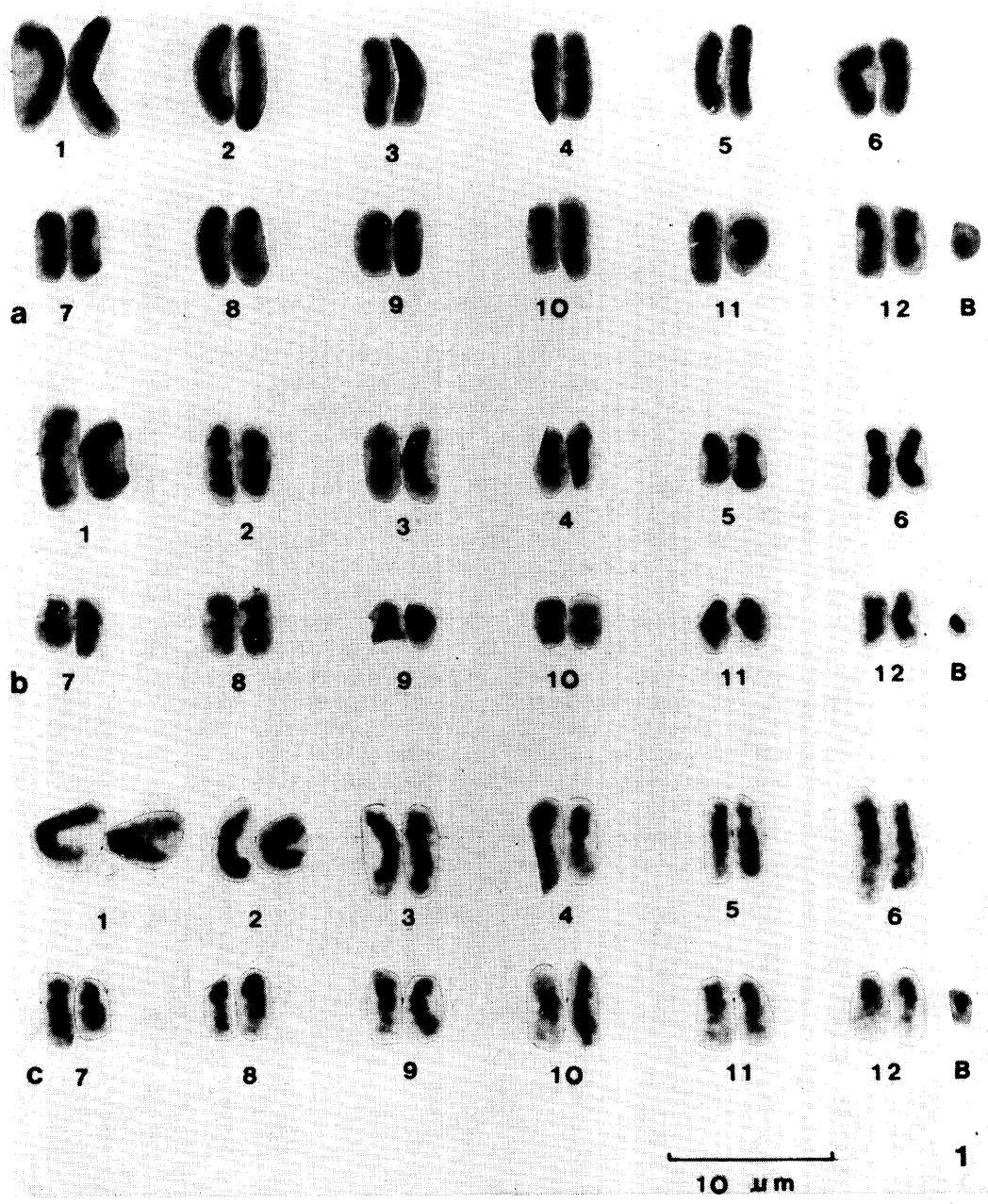

Figure 1. — Giemsa C-banded karyotypes in three species of *Quercus*; (a) *Q. petraea*; (b) *Q. rubra*; (c) *Q. robur*.

eral structural changes. Therefore, it is rather difficult to speculate on a base number in *Quercus*.

Karyomorphology

The chromosome pairs have been identified and arranged on the basis of chromosome length, arm ratio and banding pattern. The different condensing behaviour of chromosome arms from prometaphase to metaphase does not allow a really accurate measurement of arm length and ratio. Nevertheless, some of the chromosomes can be identified easily. The biggest pair is ca. 6.24 μm long and metacentric, the second chromosome pair has secondary constriction on the long arm. The eleventh pair is very characteristic in being sub-telocentric in all the three species. The ratio between the longest and the smallest chromosome is about 2.49 and rest of the chromosomes are of continuously decreasing size. The karyotypes of the three species seem to be identical and are characterised by 5 metacentric (m), 6 submetacentric (sm) and 1 subtelocentric (st) chromosome pairs (*Figure 1*). Accordingly, these karyotypes belong to the 2b category of STEBBINS (1958) which indicates a moderate degree of asymmetry. Even on the basis of Giemsa C-banding patterns, the corresponding pairs of the three species remain indistinguishable. The C-banding reveals that most heterochromatin is localised as proximal heavy blocks and all chromosomes contain heterochromatin. Individual C-banded chromosomes in the three species are characterised as fallows:

Chromosome 1: 6.24 μm to 6.26 μm long; metacentric. It has one centromeric band and one intercalary band on each arm.

Chromosome 2: 4.78 μm to 5.24 μm long; submetacentric. The long arm bears a satellite which is fully heterochromatic. Besides, a centromeric band there is a telomeric band on the short arm.

Chromosome 3: 4.28 μm to 4.31 μm long; submetacentric. It seems to possess much more heterochromatin than euchromatin. Possibly the whole chromosome consists of heterochromatin.

Chromosome 4: 3.57 μm to 3.92 μm long; metacentric. The proximal arm is fully heterochromatic, while the lower arm has a centromeric band and an intercalary band.

Chromosome 5: 3.57 μm to 3.83 μm long; metacentric. This chromosome is characterised by one very prominent centromeric band.

Chromosome 6: 3.57 μm to 3.76 μm long; submetacentric. The short arm is fully heterochromatic while the long arm has a centromeric band.

Chromosome 7: 3.39 μm to 3.45 μm long; submetacentric. The short arm is fully heterochromatic while the long arm has a centromeric and an intercalary band.

Chromosome 8: 3.21 μm to 3.28 μm long; submetacentric. The short arm is fully heterochromatic while one centromeric band is present on the long arm.

Chromosome 9: 2.96 μm to 3.10 μm long; submetacentric. One very thick centromeric band.

Chromosome 10: 2.85 μm to 2.87 μm long; metacentric. One thick centromeric band.

Chromosome 11: 2.78 μm to 2.80 μm long; subtelocentric. It has fully heterochromatic short arm and one narrow centromeric band on the long arm.

Chromosome 12: 2.49 μm to 2.68 μm long; metacentric. It is characterised by one centromeric band.

Nuclear DNA amount

A perusal of *Table 1* shows that 2C DNA amounts among the three species vary insignificantly from 1.58 pg to 1.61 pg. This is quite comparable with the value obtained in *Q. petraea* by GREILHUBER (1988). This shows that the three species investigated have very stable genomes with respect to nuclear DNA amounts, C-band patterns and karyotypic features. This underlines their close affinity. Such interspecific similarity of banding patterns is known in certain woody species (EHRENDORFER, 1982). Furthermore, the presence of 6 sm and 1 st pairs indicates a high degree of structural rearrangements during evolution (c. f. JONES, 1978). It can therefore be argued that structural changes in karyotype, evolution of nuclear DNA amounts, and alteration in position and amount of heterochromatin are much older than actual speciation events among the species studied. It is significant to note here that *Q. rubra* is geographically distinct (distributed in the western U.S.A.) from other two species (distributed in Europe) (RÖHRIG, 1980).

A further point of discussion is the very low DNA values as observed in the three *Quercus* species. Angiosperm trees are in general known to possess very low and much less variable DNA values than herbs (OHRI and

Table 1. — Chromosome number and 2C DNA*) amounts in *Quercus* species.

Taxon	2n	DNA in pg $\bar{X} \pm SE$	DNA/Chromosome in pg
Q. petraea	24+1B	1.58 ± 0.03	0.063
Q. robur	24+1B	1.59 ± 0.04	0.064
Q. rubra	24+1B	1.61 ± 0.03	0 064

*) (*Allium cepa* taken as standard, 2C DNA = 33.55 pg)

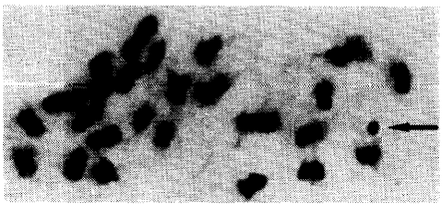

Figure 2. — C-banded metaphase in *Q. robur* showing intensely heterochromatic B-chromosome (arrow) (x 1500).

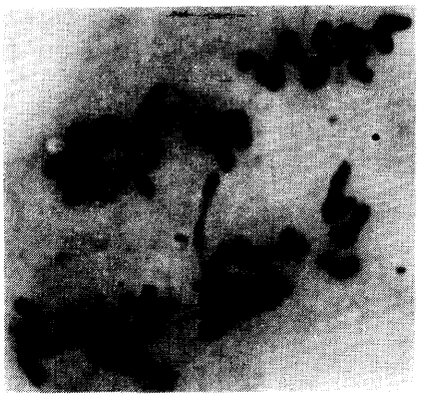

Figure 3. — Anaphase in *Q. robur* showing late dividing B-chromosome (x 1500).

KUMAR, 1986; OHRI and KOSHOO, 1987). Significant interspecific variation as encountered in many herbaceous groups (c. f. OHRI and KHOSHOO, 1986) has not been found in tree species except for *Cassia* (OHRI et al., 1986). Detailed studies are therefore needed in more species of *Quercus* to assess the extent of intra- and interspecific variation in DNA amounts.

B-Chromosomes

One B-chromosome is present in all the three species of *Quercus* investigated. This seems to be the first report of B-chromosomes in any temperate hardwood species. The B-chromosome is much smaller than the smallest chromosome of the complement and is very intensely heterochromatic as revealed by C-banding (*Fig. 1a, b, c; Fig. 2*). This would mean that DNA amount per unit volume for B's is considerably higher than for A chromosomes on average (JONES and REES, 1982). At anaphase the B-chromosome can be seen to be late dividing (*Fig. 3*).

For the first time MEHRA and BAWA (1968) reported the occurence of B-chromosomes in six eastern Himalayan woody species. According to the recent information, B's are represented in about 3,9% of the chromosomally known 862 woody species from the Himalayas and central India (BEDI et al., 1985). Quite contrary to earlier concepts, the percentage (3,9%) of woody species with B-chromosomes is almost double that of the overall percentage (1.9%) for flowering plants in general (JONES, 1981).

In this regard, it is an interesting observation that all three species of oak investigated show B-chromosomes. This would indicate that these chromosomes may actually be very widespread among temperate hardwoods. Therefore, detailed investigations are needed on the population basis to understand relationship, if any, between the presence of B-chromosomes and growth characteristics, phenological factors, and ecological tolerance.

Acknowledgements

D.O. is grateful to Deutsche Forschungsgemeinschaft for providing financial assistance for visiting West Germany under INSA-DFG Exchange of Scientists Programme.

References

AHUJA, M. R.: Perspectives in plant biotechnology. Curr. Sci. **55**: 217—224 (1986). — BEDI, Y. S., GILL, B. S. and BIR, S. S.: Cytological evolution in the woody taxa of Gamopetalae and Monochlamydeae. J. Cytol. and Genet. **20**: 162—203 (1985). — EHRENDORFER, F.: Speciation patterns in woody angiosperms of tropical origin. In: Mechanisms of Speciation. C. BARIGOZZI (Ed.) Alan, R. Liss, Inc. New York, pp. 479—509 (1982). — FEDOROV, A. A.: Chromosome numbers of flowering plants. Reprint by Otto Koeltz Science Publishers, Königstein, West Germany, 1974 (original in 1969). — GREIHUBER, J.: "Self-tanning"- a new and important source of stoichiometric error in cytophotometric determination of nuclear DNA content in plants. Pl. Syst. Evol. *158*: 87—96 (1988). — JONES, K.: Aspects of chromosome evolution in higher plants. Adv. Bot. Res. **6**: 120—194 (1978). — JONES, R. N.: Wide spreed occurrence of B-chromosomes. Bionature **1**: 63—73 (1981). — JONES, R. N. and REES, H.: B Chromosomes. Academic Press, London, New York (1982). — MEHRA, P. N. and BAWA, K. S.: B-chromosomes in some Himalayan hardwoods. Chromosoma **25**: 90—95 (1968). — OHRI, D. and KHOSHOO, T. N.: Plant DNA- contents and systematics. In DNA Systematics, Vol. II, Plants. S. K. DUTTA (Ed.). CRC Press, Florida, pp. 1—19 (1986). — OHRI, D. and KHOSHOO, T. N.: Nuclear DNA content in the genus *Ficus* (LINN.) TOURN. (Moraceae). Pl. Syst. Evol. **156**: 1—4 (1987). — OHRI, D. and KUMAR, A.: Nuclear DNA amounts in some tropical hardwoods. Caryologia **39**: 303—307 (1986). — OHRI, D., KUMAR, A. and PAL, M.: Correlation between 2C values and habit in *Cassia* (Leguminosae: Caesalpinioideae). Pl. Syst. Evol. **153**: 223—227 (1986). — RÖHRIG, E.: Der Wald als Vegetationstyp und seine Bedeutung für den Menschen. Verlag Paul Parey, Hamburg und Berlin (1980). — STEBBINS, G. L.: Longivity, habitat and release of genetic variability in the higher plants. Cold Spring Harb. Symp. Quant. Biol. **23**: 365—378 (1958).

Comparison of Selection Methods for Improving Volume Growth in Young Coastal Douglas-fir[1])

By W. T. ADAMS and D. G. JOYCE[2])

Department of Forest Science, College of Forestry,
Oregon State University, Corvallis, Oregon 97331, U. S. A.

(Received 18th January 1990)

Summary

Data from a Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO var. *menziesii*] open-pollinated progeny test in Oregon were used for evaluating methods of selection for bole volume. Tree height and bole diameter at breast

[1]) Paper No. 2537 of the Forest Research Laboratory, Oregon State University.

[2]) Current address: Ontario Ministry of Natural Resources, Forest Resources Group, P. O. Box 1000, Sault St. Marie, Ontario P6A 5N5, Canada.