

diameter measured at 8 and 11 years. There is reasonable consistency for stem straightness.

Reciprocal effects reach significance in a number of later height assessments at Bush and are estimable at most height assessments. They normally account for smaller proportions of phenotypic variance than the other genetic effects and close examination of the data reveals no consistency when comparing the performance of male and female parentage at either the array of individual cross level.

It is reasonable to conclude in general that height and stem straightness are under stronger genetic control than diameter and that again for height and straightness there is at least as much non-additive as additive variation present. Non-additive variation has little effect on the expression of diameter.

These results have implications for the strategies adopted in the improvement of Sitka spruce in Britain. Reliable data on the amount of additive variance have already indicated the likely achievements from programmes of plus-tree selection combined with clonal orchards. It is now clear that further substantial gains can be anticipated from the use of breeding techniques that specifically exploit non-additive as well as additive variation. These include such methods as the use of biclonal orchards composed of parents with proven specific combining ability or, more importantly, the mass propagation by vegetative means of any good material derived from the exploitation of non-additive variation. Perhaps the most practical among the latter is the propagation of seedling material derived from bulked mixtures of full-sib families proven for sca; the techniques for this are already established (MASON, 1986) and plant material has been raised in commercial quantities from mixtures of proven half-sib families in Britain.

Whilst the results from the experiments reported here point strongly to the use of both additive and non-additive variation in the improvement of Sitka spruce, it would be unwise to attempt to derive anything but broad estimates of genetic gains on the basis of this work. Furthermore, the presence of non-additive variation for height may point to the need for more detailed investigation on this subject. BARNES *et al* (1987), for example, found evidence among full-sib families of *Pinus patula* SCHIEDE and DEPPE of a single gene with possible overdominance controlling diameter growth. In the present study there is also a considerable measure of disagree-

ment between the two sites used and there are some clear trends in the estimates of variance components with time. In particular the fall in the proportion of the total phenotypic variance attributable to gca has also been noted by FRANKLIN (1979) in a number of species, although the present data only encompass the earlier part of the age range he considered. This could also be a consequence of the very small plot size used and the low number of plants per family, notably at Bush. At the later assessments, interaction between adjacent plots could be having a serious influence. A set of crosses comprising an 8 x 8 diallel among parents drawn at random from those contributing to the half-sib population study previously referred to (SAMUEL and JOHNSTONE, 1979) is now complete and should provide sufficient material for progeny testing in larger plots with greater replication on more sites. The results of these further tests should provide more detailed substantiation of those basic conclusions established from the work reported here.

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ALAN FLETCHER initiated work on making these crosses in 1968. Many past and present colleagues have been involved in establishing, monitoring and measuring these experiments over 15 years; in particular GEOFF WEBB has been responsible for all work at Tywi. IAN WHITE has provided helpful discussion in the presentation of statistical analysis.

References

- BARNES, R. D., MATHESON, A., C., MULLIN, L. J. and BIRKS, J.: Dominance in a metric trait of *Pinus patula* SCHIEDE and DEPPE. *Forest Science* 33, 809–815 (1987). — BECKER, W. A.: Manual of procedures in Quantitative genetics. 3rd edition. Washington State University (1975). — FAULKNER, R.: Procedures used for progeny-testing in Britain with special reference to forest nursery practice. Forestry Commission, Forest Record 60 HMSO, London (1965). — FRANKLIN, E. C.: Model relating levels of genetic variance to stand development in four North American conifers. *Silvae Genetica* 28, 207–212 (1979). — GRIPPING, B.: Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 9, 463–493 (1956). — HAYMAN, B. I.: The analysis of variance of diallel tables. *Biometrics* 10, 235–244 (1954). — LEE, S. J.: Tree breeding in Britain. — 2. *Forestry and British Timber* 15 (7), 31–32 (1986). — MASON, W. L. and GILL, J. G. S.: Vegetative propagation of conifers as a means of intensifying wood production in Britain. *Forestry* 59, 155–172 (1986). — SAMUEL, C. J. A. et al.: A diallel cross in Sitka spruce. Assessment of first year characters in an early glasshouse test. *Theor. Appl. Genet.* 42, 53–61 (1972). — SAMUEL, C. J. A. and JOHNSTONE, R. C. B.: A study of population variation and inheritance in Sitka spruce. I. Results of glasshouse, nursery and early forest progeny tests. *Silvae Genetica* 28, 26–32 (1979).

Giemsa C-Banding in *Fagus sylvatica* L., *Betula pendula* Roth and *Populus tremula* L.

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Abstract

A detailed karyotypic study by Giemsa C-banding has been done in *Fagus sylvatica* L., *Betula pendula* Roth,

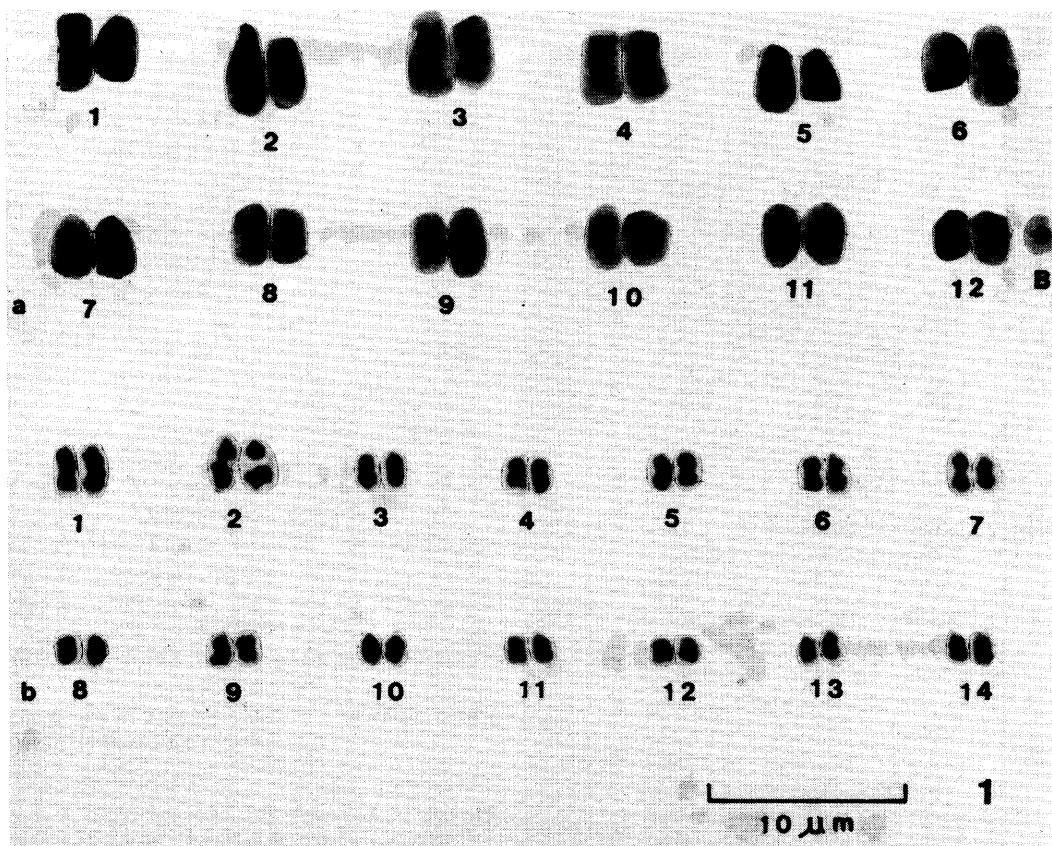


Figure 1. — Giemsa C-banded karyotype in: (a) *Fagus sylvatica*, (b) *Betula pendula*.

and *Populus tremula* L.. In addition to various other characteristics, C-banding has facilitated chromosome identification and pairing in *F. sylvatica* and *B. pendula*. A peculiar feature of *P. tremula* karyotype is the presence of a distinctly large, almost completely heterochromatic pair. Rest of the chromosomes have C-bands adjacent to centromeres.

Key words: *Fagus*, *Betula*, *Populus*, Giemsa C-banding, Karyotypic analysis.

Introduction

Recent developments in genetic engineering techniques have opened up possibilities of genetic manipulation of forest trees through cytogenetic-tissue culture procedures. Various areas of research to be considered are: chromosome mediated gene transfer, microcell mediated gene transfer and cytometric fractionation of chromosomes (SCHLARBAUM, 1987). However, a specific identification of small chromosomes of hardwoods by proper banding techniques is a prerequisite for such manipulations. Work on forest trees has been minimal in this respect. However, C-banding has been successfully applied in some hardwood species. One earlier report dealt with three species of *Quercus* (OHRI and AHUJA, 1990), while the present report is on *Fagus sylvatica*, *Betula pendula* and *Populus tremula*.

Materials and Methods

Three hardwood species, namely *F. sylvatica*, *B. pendula* and *P. tremula* were employed for cytological studies. Root tips were taken from potted plants grown in the Institute of Forest Genetics and Forest Tree Breeding at

Grosshansdorf. For details of methods see OHRI and AHUJA (1990).

Results and Discussion

The chromosome counts for the three species i. e., *F. sylvatica* ($2n = 24$), *B. pendula* ($2n = 28$) and *P. tremula* ($2n = 38$) confirm earlier reports (FEDOROV, 1969; GOLDBLATT, 1981).

Karyomorphology

F. sylvatica: All the chromosomes can be identified and paired according to the chromosome length, arm ratio and banding pattern. The ratio between the longest and shortest chromosomes is about 1.75, the rest of the chromosomes show gradual difference in size. The karyotype can be characterised as 3 M (median point), 3 m (median region), 3 sm (submedian) and 3 st (subterminal) (Fig. 1a) (cf LEVAN et al, 1964). While the ratio between the longest and shortest chromosomes is not very substantial, the structural alterations have occurred to produce chromosome asymmetry as depicted by 3 sm and 3 st pairs. One B-chromosome has also been revealed (Fig. 1a, 2 arrow). It is smaller than the smallest chromosome of the complement and is fully heterochromatic (Fig. 1a, 2 arrow). This is a significant observation as B-chromosomes have also been found in three species of *Quercus* studied (OHRI and AHUJA, 1990).

The C-banding reveals that the heterochromatin is present in all chromosomes and is mostly located adjacent to centromeres. Individual C-banded chromosomes can be characterised as follows; r represents ratio between long/short arm of respective chromosomes:

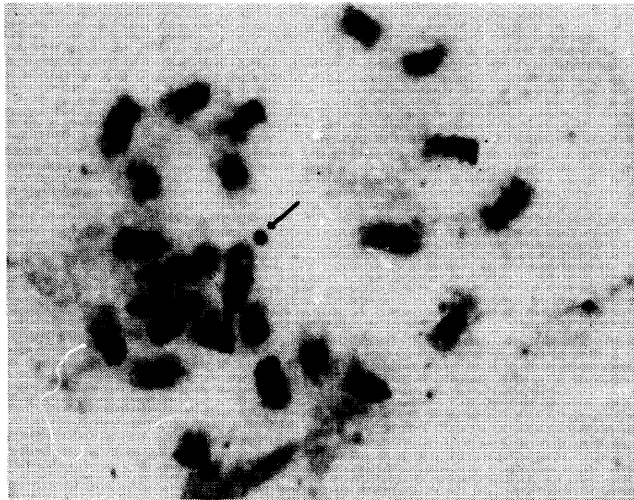


Figure 2. — C-banded mitotic complement of *Fagus sylvatica* showing one B-chromosome (arrow). X2300.

- Chromosome 1: About 3.43 μm long; median (M; $r = 1.0$). It seems to possess much more heterochromatin than euchromatin. Possibly the whole chromosome is heterochromatic.
- Chromosome 2: About 3.12 μm long; subterminal (st; $r = 6.6$). The short arm is fully heterochromatic while long arm has a centromeric and a faint intercalary band.
- Chromosome 3: About 3.02 μm long; median (M; $r = 1.0$). One band adjacent to centromere on both sides gradually merging with the surrounding euchromatin.
- Chromosome 4: About 2.93 μm long; submedian (sm; $r = 2.54$). Short arms densely heterochromatic and a centromeric band on long arm.

- Chromosome 5: About 2.80 μm ; subterminal (st; $r = 8.0$). Fully heterochromatic short arm, a centromeric and a faint intercalary band on long arm.
- Chromosome 6: About 2.68 μm ; submedian (sm; $r = 1.8$). A band adjacent to centromere on both sides.
- Chromosome 7: About 2.49 μm ; subterminal (st; $r = 3.5$). Short arm fully heterochromatic and a centromeric band gradually merging in to euchromatin on the long arm.
- Chromosome 8: About 2.34 μm ; median (m; $r = 1.3$). Faint heterochromatin adjacent to centromere.
- Chromosome 9: About 2.18 μm ; submedian (sm; $r = 2.01$). One centromeric band on both arms merging gradually in to surrounding euchromatin.
- Chromosome 10: About 2.15 μm ; median (m; $r = 1.2$). A prominent band adjacent to centromere on both sides merging gradually into euchromatin.
- Chromosome 11: About 1.96 μm ; median (M; $r = 1.0$). One centromeric band on each arm.
- Chromosome 12: About 1.94 μm ; median (m; $r = 1.31$). One centromeric band on each arm.

B. pendula

The ratio between the longest (2.21 μm) and smallest (1.06 μm) chromosomes is 2.08. The karyotype is expressed as 2M (1st and 5th pair) and 12 m (Fig. 1b), therefore belonging to 1b class of STEBBINS (1958). Compared to that of *F. sylvatica*, the complement is very rich in heterochromatin as very big blocks of C-bands are present adjacent to centromere in each chromosome with a small distal portion revealed as euchromatin. The heterochromatic blocks are so big as to constitute 70 to 90 per cent of each chromosome. The second longest pair is peculiar

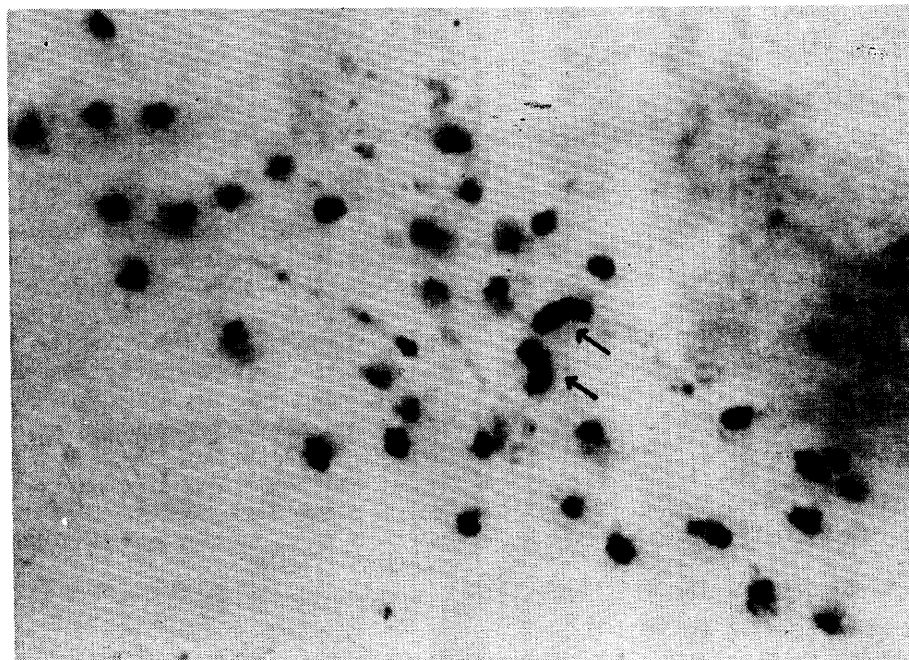


Figure 3. — C-banded mitotic complement of *Populus tremula* showing two long chromosomes (arrows). X2300.

in showing very large fully heterochromatic satellites attached to the short arm.

P. tremula

The chromosomes are m to sm and have mostly proximally located C-bands. Some small chromosomes appear to be devoid of heterochromatin. The distinct feature of the complement is the presence of a very large pair (3.27 μm) (Fig. 3 arrows), which is 1.74 times longer than the second longest pair (1.87 μm). This pair is sm, the short arm is fully heterochromatic as is also the long arm except for two narrow intercalary regions of euchromatin. This shows that during the course of evolution this particular pair has gained C-band material disproportionate to that in rest of the chromosomes. It might be that it is concerned with sex determination! However, its exact nature and significance can only be ascertained by the detailed karyotypic studies in some more species.

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References

- FEDOROV, A. A.: Chromosome numbers of flowering plants. Reprint by Otto Koeltz Science Publishers, Königstein, West Germany, 1974 (original in 1969). — GOLDBLATT, P.: Index to Plant Chromosome Numbers 1975 to 1978. Missouri Botanical Garden, Braun-Brumfield, Inc., Ann. Arbor, Michigan (1981). — LEVAN, A., FREDGA, K. and SANDBERG, A. A.: Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220 (1964). — OHRI, D., and AHUJA, M. R.: Giemsa C-banded karyotype in *Quercus L.* (oak). *Silvae Genet.* 39: 216–219 (1990). — SCHLARBAUM, S. E.: Cytogenetic manipulation in the forest trees through tissue culture. In: Cell and Tissue Culture in Forestry. Vol. 1. General Principles and Biotechnology. J. M. BONGA and D. J. DURZAN (eds.). Martinus Nijhoff Publishers, Dordrecht. pp. 330–352 (1987). — STEBBINS, G. L.: Longevity, habitat and release of genetic variability in the higher plants. Cold Spring Harb. Symp. Quant. Biol. 23: 365–378 (1958).

Genetic Structure and the Mating System in an Old Stand of Polish Larch

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Summary

The genetic structure and the mating system were investigated in a 250 year old stand of Polish larch (*Larix decidua subsp. polonica* [RACIB.] DOMIN) using six allozyme marker loci (Gdh, Mdh-1, Mdh-2, Mdh-3, Shdh, Srdh). The average expected heterozygosities, based on these loci were 0.189 and 0.187 for the parental and filial generation respectively, and the mean expected heterozygosity for the mature stand based on 18 gene loci was calculated to be 0.193. An excess of heterozygotes was found in the mature forest population. Single-locus estimates of outcrossing rate (\hat{t}_s) ranged from 0.776 to 1.132 (minimum variance mean 1.048), while the multilocus estimate (\hat{t}_m) was 0.943. Detected amount of selfing and heterogeneity of single-locus estimates were statistically significant. The calculated ratio of genetically effective male individuals to receptive female individuals was only 0.22 in this population.

Key words: Isozymes, heterozygosity, inbreeding, outcrossing, variance effective population size.

Introduction

Polish larch (*Larix decidua subsp. polonica* [RACIB.] DOMIN), is of high wood quality, fast growth and relative high resistance to air pollution (LATOCHA and HAWRYS, 1976), thus it belongs to valuable coniferous trees in Europe. However, despite the economic value of this species, little is known about its biology and especially about its genetic properties.

Larch in Poland occurs in small groups or even as individual trees within stands of pine, spruce or fir. It is suggested that such a structure of populations and heavy

pollen create significant barriers for the free passage of genes between and within populations (MEJNARTOWICZ and BERGMANN, 1975). Larch produces many empty seeds and it is supposed that self-fertilization plays an important role in this process. KOSINSKI (1986) studying the causes of empty seed formation in larch has shown, that embryo degeneration took place in 85% to 100% of the pollinated ovules, after controlled self-pollination. However, there is no information about the rates of natural self-fertilization. A significant level of self-fertilization was found for natural populations of another larch (*Larix laricina* (Du Roi) K. KOCH) (KNOWLES et al., 1987). Thus it is very interesting to study the mating system of Polish larch, which could be helpful in explaining empty seed formation in natural stands and could extend our knowledge about this species.

The mating system, being part of the whole genetic system of the species, defines the mode of transmission of genetic information from the parental to the filial generation (STERN and ROCHE, 1974), and is an important determinant of plant population structure (CLEGG, 1980). In recent years the mating system was studied in many conifer trees in natural populations, however statistically efficient multilocus estimates of mating system parameters have been obtained only for a relatively small number of conifers (see reviews: MITTON, 1983; ADAMS and BIRKES, 1988; MUONA, 1989).

Coniferous forest tree species are wind pollinated and genetically highly variable (HAMRICK et al., 1981). Many of them display strong inbreeding depression (KOSKI, 1973; PARK and FOWLER, 1982), which following selfing results in reduced seed set, decreased survival and growth of prog-