New Forests **6**: 373–390, 1992.

Review paper

Nuclear, chloroplast, and mitochondrial DNA polymorphisms as biochemical markers in population genetic analyses of forest trees

DAVID B. WAGNER
Department of Forestry, University of Kentucky, Lexington, Kentucky 40546–0073, USA

Received 1 October 1990; accepted 15 July 1991

Key words: copy number, genome size, RFLP, PCR, DNA sequencing

Application. Study of DNA polymorphisms in forest trees has provided new insights into basic biology. Chloroplast, mitochondrial, and nuclear DNA polymorphisms are expected to play increasingly important roles for forest population geneticists, and further investigation of these polymorphisms may lead to applications in germplasm improvement and conservation programs.

Abstract. DNA analyses have been used only occasionally to investigate genetic polymorphisms in forest tree populations. Nonetheless, these analyses have already contributed to significant discoveries, such as paternal chloroplast and maternal mitochondrial DNA inheritance in Pinaceae. DNA polymorphisms will be increasingly exploited in the future by forest population geneticists, because available technology permits large sample sizes and yields excellent resolution. The utility of chloroplast, mitochondrial, and nuclear DNA polymorphisms is expected to be greatest when less expensive genetic markers are unavailable, insufficiently numerous, or ineffectively polymorphic. For example, DNA fingerprinting may permit the unambiguous elucidation of genetic relationships within and among populations of woody species.

Introduction

A major goal of population geneticists is to understand evolutionary mechanisms (Hartl and Clark 1989), but applications of population genetics transcend evolutionary inference. For example, germplasm conservation strategies have benefited from knowledge of population genetic principles (e.g., Brown 1978; Millar and Westfall 1992). Also, studies of population variation have led to discovery of important and useful genes, such as "quantitative trait loci" (QTLs), which may increase gains in genetic improvement programs (e.g., Paterson et al. 1991).

Regardless of specific goals or applications, empirical population genet-

ics requires genetic variation (polymorphism). The most useful genetic polymorphisms for population genetic study vary qualitatively, permitting one-to-one mapping of phenotype onto genotype in large samples. Polymorphisms that provide this type of specific genotypic information can be considered "genetic markers." Population geneticists have traditionally employed unusual morphological phenotypes, proteins, or secondary compounds as genetic markers, but DNA analyses are now providing new markers (Leigh Brown 1989; Kreike et al. 1991; Szmidt and Wang 1991).

One type of DNA variation is copy number polymorphism, which is due to differences in the number of genomic occurrences of a particular DNA sequence (e.g., Strauss and Tsai 1988). DNA variation can also occur in base sequence, length, and arrangement. These latter types of variation are often detected as restriction fragment length polymorphisms (RFLPs), using enzymes (restriction endonucleases) that cut DNA molecules into restriction fragments of varying lengths. RFLP methodologies are technically straightforward and permit analyses of large numbers of samples (e.g., Govindaraju et al. 1989a).

DNA variation can also be studied by direct determination of DNA base sequences in a genomic region of interest. In principle, comparison of individuals is then possible at each base pair, which is a level of resolution not attainable with copy number variation or RFLPs (e.g., Kreitman 1983). Collection of sequence data from population samples now appears feasible because of the efficiency of the polymerase chain reaction (PCR) in DNA amplification (Saiki et al. 1988; Leigh Brown 1989).

In general, DNA markers are especially powerful for several reasons, including the following:

1) Potentially, a large number of polymorphisms (some of which are selectively neutral and some of which are under selection) can be identified in any taxon.
2) Widely differing levels of polymorphism can be studied.
3) DNA analyses allow investigation of not only coding, but also noncoding, variation.
4) Both Mendelian and non-Mendelian markers can be identified, because DNA resides in chloroplasts and mitochondria as well as in the nuclei.
5) It is usually possible to determine the mutational differences among DNA variants (e.g., point mutations, insertions/deletions, or rearrangements), which strengthens population analyses.

These advantages have been convincingly demonstrated on occasions when traditional genetic markers were deficient (Leigh Brown 1989; Hartl and Clark 1989; Strauss et al. 1991; Szmidt and Wang 1991). However,

DNA polymorphisms are not a panacea: other genetic markers are sometimes preferable because of their lower costs in personnel, reagents, laboratory facilities, and time.

DNA polymorphisms of forest tree populations have received only limited attention, in comparison with such variation in other organisms. Nevertheless, these new genetic markers have already permitted important insights into fundamental biological processes in forest trees. The purpose of this article is to describe the significant results and likely future roles of nuclear, chloroplast, and mitochondrial DNA analyses in population genetic studies of forest trees. This review will not address methodological choices or details of laboratory techniques, because these topics have been discussed previously (e.g., Gustafsson and Sitbon 1986; Strauss and Tsai 1988; Strauss et al. 1989; Neale and Williams 1991; Szmidt and Wang 1991).

Nuclear DNA polymorphisms

The first direct analyses of DNA variation in forest trees were reported more than twenty years ago and detected variance in DNA content (e.g., Burley 1965; Mergen and Thielges 1967; Miksche 1968, 1971; El-Lakany and Sziklai 1971; Dhir and Miksche 1974). Nuclear DNA presumably contributes to this variance, but organellar DNA may also play a role.

Copy number variation of specific nuclear sequences is related to variance in DNA content but is more precise and informative. For example, the observed 20-fold range in ribosomal (r) RNA gene copy number in radiata pine (*Pinus radiata* D. Don) is due to 10 or more nuclear loci (Cullis et al. 1988a, b). Ribosomal RNA gene concentration appears stable within individuals of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Strauss and Howe 1990), but exhibits geographic trends in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Strauss and Tsai 1988) and red spruce (*P. rubens* Sarg.) (Bobola et al. 1992b).

In addition to variation in copy number and genome size, nuclear RFLPs are available in forest trees. Scotch pine (*P. sylvestris* L.) (Sitbon and Gustafsson 1988), *P. radiata* (Cullis et al. 1988a), black spruce (*P. mariana* (Mill.) B.S.P.), *P. rubens* (Bobola et al. 1992a; Bobola et al. 1992b), poplars (*Populus* L.) (Smith and Sytsma 1990), and several oak (*Quercus* L.) species (Whittemore and Schaal 1991) provide examples of RFLPs in or near rRNA genes. Nuclear RFLP genotypes are variable in trembling aspen (*P. tremuloides* Michx.) and appear to distinguish this species from largetooth aspen (*P. grandidentata* Michx.) (G. R. Furnier,

pers. comm.). RFLPs of highly-repeated sequences, possibly nuclear in origin, differentiate half-sib cell lines of yellow poplar (*Liriodendron tulipifera* L.) (Merkle et al. 1988).

Nuclear restriction fragments differ between two cottonwoods (*P. fremontii* Wats. and *P. angustifolia* James) and can be used to identify natural hybrids and hybrid derivatives. Joint analyses of RFLP and other data from these two species revealed that introgression is unidirectional and that susceptibility to aphid infestation is associated with repeated backcrossing to *P. angustifolia* (Keim et al. 1989a, b).

The greatest contribution of nuclear DNA markers to forest population genetics will likely be the large number of polymorphisms that can be studied. Nuclear genomes can be saturated with RFLPs (Paterson et al. 1991; Neale and Williams 1991), which would permit population genetic analyses of virtually any region of a forest tree genome. Random amplified polymorphic DNA (RAPD) markers, which are based on PCR amplification of DNA from arbitrary oligonucleotide primer sequences (Williams et al. 1990), may be more efficient and may ultimately become even more useful than RFLPs for the saturation of nuclear genomes with genetic markers. Saturated linkage maps, once available, can be used to resolve quantitative variation into Mendelian factors (Paterson et al. 1991). This would eventually allow direct study of allele frequencies of economically important traits. However, the cost of saturating a linkage map is high at present, and the benefits of such an investment are untested in forestry applications (e.g., Neale and Williams 1991).

In addition to the large number of polymorphisms that are identifiable by DNA analyses, each polymorphism is potentially highly variable. DNA fingerprinting now appears possible in woody taxa, including *Polyalthia glauca* (Hassk.) Mueller, orange (*Citrus sinensis* Osbeck), Torrey pine (*P. torreyana* C. Parry ex Carrière), *P. radiata*, eastern cottonwood (*P. deltoides* Marsh.), *P. tremuloides*, apple (*Malus* x *domestica* Borkh.), black cherry (*Prunus serotina* Ehrh.), blackberries and raspberries (*Rubus* L.), and box elder (*Acer negundo* L.) (Rogstad et al. 1988a, b; Ryskov et al. 1988; Nybom et al. 1989; Zimmerman et al. 1989; Nybom 1990; Nybom et al. 1990; Nybom and Schaal 1990a, b; Nybom and Rogstand 1990; Nybom and Hall 1991; Rogstad et al. 1991). DNA fingerprinting may eventually permit unambiguous elucidation of genetic relationships in forest tree populations.

Chloroplast (cp) DNA polymorphisms

The power of DNA analyses to identify polymorphisms extends beyond

the nuclear genome to the organellar genomes. In fact, cpDNA variation in forest trees has received greater attention than nuclear DNA polymorphisms for several reasons. Chloroplast DNA is readily detected because of its occurrence in multiple copies per cell. Also, the cpDNA molecule is smaller and structurally simpler than nuclear DNAs, which allows straightforward molecular interpretations of polymorphisms (e.g., Palmer et al. 1988).

Inheritance tests provided some of the first reports of cpDNA variation in forest trees, because knowledge of the mode of inheritance is important for any new polymorphic marker. This knowledge is critical for chloroplast polymorphisms, which do not obey the rules of Mendelian inheritance. These polymorphisms are inherited maternally in most angiosperms (Sears 1980), but paternal contributions can be variable in taxa with biparental chloroplast inheritance (e.g., Chiu et al. 1988).

Chloroplast DNA inheritance appears predominantly paternal in conifers, including *P. menziesii* (Neale et al. 1986), larch (*Larix* Mill.) hybrids (Szmidt et al. 1987), pine (*Pinus* L.) hybrids (Wagner et al. 1987; Neale and Sederoff 1989; Wagner et al. 1992), spruce (*Picea* A. Dietr.) hybrids (Szmidt et al. 1988; Neale and Sederoff 1988; Stine et al. 1989; Stine and Keathley 1990), coast redwood (*Sequoia sempervirens* D. Don Endl.) (Neale et al. 1989), jack pine (*P. banksiana* Lamb.) (Wagner et al. 1989b), incense-cedar (*Calocedrus decurrens* [Torr.] Florin) (Neale et al. 1991), and arbor-vitae (*Thuja* L.) hybrids (A. E. Szmidt, pers. comm.). The coniferous mode of cpDNA inheritance contrasts sharply with the norm in angiosperms, but is consistent with the inheritance of a plastid mutant in Sugi (*Cryptomeria japonica* D. Don) (Ohba et al. 1971) and with ultrastructural evidence (Chesnoy 1987; Owens and Morris 1990; Owens and Morris 1991). Unfortunately, cpDNA inheritance data has not been reported for non-coniferous forest trees.

It would be premature to conclude that chloroplast inheritance is strictly paternal in conifers, because the available sample sizes are usually small and do not exclude the possibility of maternal leakage. In fact, progeny with non-paternal cpDNA genotypes were observed in *C. japonica* (Ohba et al. 1971), *P. menziesii* (Neale et al. 1986), *Larix* hybrids (Szmidt et al. 1987), *P. banksiana* (Wagner et al. 1989b), and *C. decurrens* (Neale et al. 1991). The causes of these non-paternal cpDNA genotypes are unresolved in most cases, but germplasm contamination is at least partially responsible in *P. banksiana* (Wagner et al. 1989b).

Most attempts to identify intra-specific cpDNA polymorphism in forest trees have succeeded, even with small sample sizes (e.g., Ali et al. 1991). In addition to the examples represented by inheritance tests (cited above), cpDNA polymorphisms are also known in lodgepole pine (*P. contorta*

Dougl.) (Wagner et al. 1987), *P. sitchensis* (Szmidt et al. 1988), white spruce (*Picea glauca* (Moench) Voss), (Szmidt et al. 1988; Stine et al. 1989), *P. densata* Mast. (Wang and Szmidt 1990), western white pine (*P. monticola* Dougl. ex D. Don) (White 1990b), Engelmann spruce (*P. engelmannii* Parry ex Engelm.) (Stine and Keathley 1990), eastern hemlock (*Tsuga canadensis* (L.) Carr.) (Wang 1990), *Liriodendron tulipifera* (Parks and Wendel 1990), white oak (*Q. alba* L.), bur oak (*Q. macrocarpa* Michx.), swamp chestnut oak (*Q. michauxii* Nutt.), post oak (*Q. stellata* Wang.) (Whittemore and Schaal 1991), sessile oak (*Q. petraea* (Matt.) Liebl.), pedunculate oak (*Q. robur* L.) (Kremer et al. 1991), loblolly pine (*P. taeda* L.), slash pine (*P. elliottii* Engelm.) (Wagner et al. 1992), Rocky Mountain juniper (*Juniperus scopulorum* Sarg.), eastern red cedar (*J. virginiana* L.) (S. G. Ernst, pers. comm.), Hartweg pine (*P. hartwegii* Lindl.) (J. A. Matos and B. A. Schaal, pers. comm.), and pinyon (*P. edulis* Engelm.) (P. Keim, pers. comm.).

Identification of cpDNA polymorphisms is encouraging, but the actual utility of these new genetic markers can only be evaluated in population surveys. Although such surveys are rare in forest trees, cpDNA polymorphisms are already clearly informative. For example, cpDNA variants provide markers of hybridization in *Picea* and *Pinus* (Szmidt et al. 1988; Sigurgeirsson et al. 1991; Wang and Szmidt 1990; Wagner et al. 1992). These markers provide evolutionary insights and also may reduce economic costs resulting from inappropriate deployment of cryptic hybrid seedlots (El-Kassaby et al. 1988, Wang and Szmidt 1990; Sutton et al. 1991a).

In contrast to the *Picea* and *Pinus* examples, cpDNA polymorphisms fail to distinguish closely related *Quercus* species in either North America or Europe, possibly because of inter-specific hybridization in *Quercus* (Whittemore and Schaal 1991; Kremer et al. 1991). This result indicates that cpDNA diversity within taxonomic groups has the potential to confound cpDNA phylogenies (Whittemore and Schaal 1991). Nonetheless, the *Quercus* cpDNA variability is geographically structured on both continents and may be informative for seed zone delineation.

The largest population survey of DNA variation in forest trees has examined cpDNA insertion/deletion polymorphisms in more than 1300 individuals from 152 populations of *P. banksiana* and *P. contorta*. Chloroplast DNA variability differentiates these two species and also resides within subspecies of *P. contorta*, within allopatric and sympatric populations, and even within chimeric individuals (Wagner et al. 1987; Govindaraju et al. 1988). Chloroplast DNA is also variable within individuals in *P. monticola* and *Picea*, and the hypothesis of occasional biparental inheritance has been advanced to account for these observa-

tions (White 1990b; Sutton et al. 1991a; but see Stine et al. 1989). This hypothesis is consistent with the reports of infrequent non-paternal cpDNA genotypes in coniferous progeny arrays (e.g., Wagner et al. 1989b). Although the significance of within-individual genetic variation has been debated at length (e.g., Painter 1966; Libby et al. 1969; Klekowski 1988), cpDNA markers may now facilitate further analyses of this type of variation.

One of the *P. banksiana — P. contorta* cpDNA polymorphisms has at least 27 distinct genotypes, 19 of which are associated with a region of natural hybridization (Govindaraju et al. 1989a), and mutation mapping experiments have revealed even greater levels of variation (Govindaraju et al. 1989b). Formally, cpDNA recombination is a possible cause of the unusual chloroplast genotypes that occur in sympatric populations of *P. banksiana — P. contorta* (Govindaraju et al. 1989a). These unusual genotypes are of special interest, because recombination between parental chloroplast genomes has not been observed in controlled matings of any land plant (e.g., Chiu and Sears 1985). If cpDNA recombination between parental genotypes is extremely rare, one would predict that recombinants might be detectable only in populations that have persisted for many generations. Thus, geographic regions of natural hybridization could permit detection and rigorous tests of cpDNA recombination, if an adequate number of parental markers can be identified in the chloroplast genome.

Several *P. banksiana — P. contorta* cpDNA genotypes are spatially patterned within sympatric populations (Wagner et al. 1991b). This pattern would ordinarily be unexpected for paternally-inherited factors in outcrossers (Epperson and Allard 1984; Cheliak et al. 1985; Epperson and Allard 1989), but effective gene flow between *P. banksiana* and *P. contorta* may be limited (Saylor and Smith 1966; Righter and Stockwell 1949). Inter-specific gene flow constraints could also explain the observed concordance within sympatric individuals of morphological taxonomic classification and cpDNA genotype (Wagner and Govindaraju 1988; D. B. Wagner and D. R. Govindaraju, unpubl. data). However, such constraints would not simultaneously explain the predominantly random association of allozymes and cpDNA genotypes in the same individuals (Wagner et al. 1989a; D. B. Wagner and B. P. Dancik, unpubl. data). Although several questions will remain unanswered until more data is available, it is clear that cpDNA variation is a significant component of a complex population genetic architecture in sympatric populations of *P. banksiana — P. contorta.*

The evolutionary conservatism of the chloroplast genome (Palmer et al. 1988; Clegg et al. 1991) appears to contradict the numerous examples of intra-specific cpDNA polymorphisms in forest trees. However, coniferous

chloroplast genomes lack an inverted repeat that is typical of other plants (Strauss et al. 1988; Lidholm et al. 1988). This physical characteristic may contribute to the occurrence of DNA rearrangements in conifers (Ali et al. 1991). Moreover, cpDNA variability may be restricted to only a few mutation hotspots within a particular species (Ali et al. 1991), in which case there would be no contradiction.

Hotspots of chloroplast polymorphism are associated with the presence of cpDNA repeat units in several plants, including *P. menziesii* (Blasko et al. 1988; Strauss et al. 1988; Tsai and Strauss 1989; Ali et al. 1991). Theory predicts that within-individual organellar variation may be frequent when DNA repeats are responsible for polymorphism (Clark 1988). Therefore, the duplicated and repeated DNA sequences that occur in the chloroplast genomes of *P. banksiana—P. contorta* and *P. monticola* (Govindaraju et al. 1989b; White 1990a; Lidholm et al. 1991) are especially intriguing, because these taxa harbor cpDNA variation within individuals (Govindaraju et al. 1988; White 1990b).

Although most attempts to identify intra-specific cpDNA RFLPs in forest trees have succeeded, failure have been reported for European larch (*Larix decidua* Mill.), Japanese larch (*Larix leptolepis* Sieb. and Zucc.) (Szmidt et al. 1987), blue spruce (*P. pungens* Engelm.) (Stine et al. 1989), *P. taeda* (Neale and Sederoff 1989; Ali et al. 1991), Chinese pine (*P. tabulaeformis* Carr.), Yunnan pine (*P. yunnanensis* Franch.), Masson pine (*P. massoniana* Lamb.) (Wang and Szmidt 1990), *Liriodendron chinense* (Hemsl.) Sarg. (Parks and Wendel 1990), *P. deltoides*, black poplar (*P. nigra* L.) (Smith and Sytsma 1990), *Q. virginiana* var. *fusiformis* (Small) Sarg. (Whittemore and Schaal 1991), longleaf pine (*P. palustris* Mill.) (Wagner et al. 1992), and Michoacán pine (*P. michoacana* Martínez) (J. A. Matos and B. A. Schaal, pers. comm.). Within shortleaf pine (*P. echinata* Mill.), the only observed cpDNA variation is explainable by inter-specific hybridization (Wagner et al. 1991a). Despite their failure to detect intra-specific cpDNA polymorphism, most of the above investigations identified distinctions among closely-related species. For example, Szmidt et al. (1987) found three cpDNA variants in a total sample of only 11 individuals from species crosses of *Larix*.

Additional study of the above examples would be appropriate prior to concluding that cpDNA is monomorphic intra-specifically, because sample sizes were usually small in terms of numbers of trees and numbers of cpDNA base pairs. A priori, one would expect to screen a very large number of base pairs in the chloroplast genome before finding any polymorphism, because cpDNA evolves slowly (Palmer et al. 1988; Clegg et al. 1991).

The role of the long-lived, woody-perennial life form in producing and

maintaining cpDNA polymorphisms is entirely unknown. Similarly, we understand little of the similarities and/or differences in gymnosperm vs. angiosperm cpDNA polymorphisms and in inter- vs. intra-specific cpDNA polymorphisms. Expanded investigation would be enlightening on these topics.

Mitochondrial (mt) DNA polymorphisms

Male sterility is one of the best known effects of plant mitochondrial polymorphisms (Pring and Lonsdale 1985). Consequently, current understanding of mitochondrial population genetics in plants arises primarily from analyses of male sterility polymorphisms (i.e., gynodioecy) (e.g., van Damme 1986).

Maternal inheritance of mitochondrial genomes predominates in angiosperms (Conde et al. 1979; Soliman et al. 1987), but conifers differ in this regard. Ultrastructural evidence indicates that mitochondria are contributed solely by the pollen donor in Cupressaceae and Taxodiaceae, while both paternal and maternal mitochondria are present at fertilization in Pinaceae and Taxaceae (Chesnoy 1987; Owens and Morris 1990, 1991). Mitochondrial restriction fragments are predominantly maternally inherited in *Pinus* species hybrids (Neale and Sederoff 1988; Wagner et al. 1991c), *P. taeda* (Neale and Sederoff 1989), *P. contorta* (Wagner et al. 1991c), and *Picea* species hybrids (Sutton et al. 1991b; M. Stine, pers. comm.). In contrast, mtDNA RELPs appear paternally inherited in *S. sempervirens* and *C. decurrens* (Neale et al. 1989, 1991). These RFLP results are consistent with the ultrastructural evidence, because they represent assays of "net" outcomes after reproduction and development. Mitochondrial inheritance data from non-coniferous trees are unavailable for comparison.

Despite the availability of mtDNA polymorphisms within at least six conifer species (see articles cited above), we know little about the genomic, taxonomic, or geographic distributions of mitochondrial variation. The molecular nature of mtDNA polymorphism has been inferred in only one forest tree species, *C. decurrens* (Neale et al. 1991), and the use of mitochondrial markers in population surveys is just beginning (e.g., Sutton et al. 1991b). However, mtDNA polymorphisms are under investigation in several laboratories (e.g., S. H. Strauss et al., pers. comm.; J. Dong, pers. comm.; T. Li, pers. comm.), and we can expect substantive new information in the near future.

Conclusions

Despite the limited resources directed toward population surveys of DNA polymorphisms in forest trees, DNA as a biochemical marker has advanced our understanding of biology and provides additional opportunities to enhance the quantity and quality of genetic markers. The new markers are expected to be of greatest value when other polymorphisms are inadequate, such as in studies of taxa that appear genetically depauperate. For example, although isoenzyme variation is unknown within *P. torreyana* populations (Ledig and Conkle 1983), DNA polymorphisms revealed two genotypes in a sample of only three individuals of this species (Rogstad et al. 1988b)!

Future searches for DNA polymorphisms are likely to benefit from a variety of technical advances, such as DNA amplification by PCR and the use of RAPD technology. The polymerase chain reaction has already been used successfully in forest trees (Bousquet et al. 1990) and could be combined with DNA sequencing to reveal polymorphism in genomic regions that appear monomorphic by less sophisticated approaches (Leigh Brown 1989). A practical consideration is that PCR permits production of virtually unlimited quantities of DNA from very small amounts of stable materials (e.g., seeds), thereby reducing or eliminating the need to collect and transport large quantities of comparatively unstable tissues (e.g., leaves).

The potentially large number and widely varying diversities of nuclear DNA polymorphisms will be useful for solving a variety of problems in forest genetics. Identification and analysis of QTLs (e.g., Neale and Williams 1991) may be one of the most significant applications of these polymorphisms. Nuclear DNA markers could also increase the resolution of parentage analyses (Adams 1991) and permit more thorough investigation of linkage disequilibrium (Bush and Smouse 1992). However, the future roles of nuclear DNA polymorphisms should themselves be considered critical topics for current research. The most appropriate roles will likely depend on important details regarding cost and distribution of polymorphism.

Recombinant DNA technology has provided the first markers suitable for organellar population genetics in trees. Organellar polymorphisms may now be identifiable in nearly any species of choice, and these markers promise much information. For example, we should expect gene flow studies to benefit from organellar markers, because current gene flow estimators often fail to distinguish gene flow by pollen vs. gene flow by seed in natural populations (Ellstrand 1992). In particular, the contrasting modes of cpDNA and mtDNA inheritance in Pinaceae may enhance

simultaneous analyses of paternal and maternal lineages (Neale and Sederoff 1989).

Multilocus population genetics will benefit substantially from the availability of organellar markers. Cytonuclear disequilibria have been defined recently and can be more enlightening than the corresponding nuclear parameters (Asmussen et al. 1987; Schnabel and Asmussen 1989). Forest trees may be among the most informative of all organisms for empirical applications of the new cytonuclear theory, because they represent three inheritance patterns in a single life-form, i.e., mtDNA paternal, cpDNA paternal, nuclear loci Mendelian (as in *S. sempervirens* and *C. decurrens*); mtDNA maternal, cpDNA paternal, nuclear loci Mendelian (as in *Pinus* and *Picea*); and mtDNA maternal, cpDNA maternal, nuclear loci Mendelian (assuming that angiospermous forest trees behave as most other flowering plants).

Organellar polymorphisms should not be overlooked as a potential source of economically useful variation (McDaniel 1984; Pring and Lonsdale 1985; Börner and Sears 1986). Indeed, reciprocal effects occur in forest trees, although it is not yet known if these effects are due to cytoplasmic factors (e.g., Perry 1976; Wilcox 1983). Identification and detailed molecular investigation of organellar DNA polymorphisms, in concert with classical genetic experiments, may elucidate the causes of these reciprocal effects.

Clearly, DNA polymorphisms have enhanced the wide array of methodologies available to forest geneticists. It now appears possible to choose appropriate genetic markers based on questions at hand, rather than the reverse.

Acknowledgments

I am grateful to many teachers, colleagues and students for numerous discussions and for their generous willingness to share unpublished information. The manuscript was improved substantially by the enormously careful reading and suggestions of D. E. Harry, D. B. Neale and D. L. Copes. Support was provided by Kentucky Agricultural Experiment Station Research Funds (McIntire-Stennis Project KY00640), USDA Grants 85-FSTY-9-0149 and 90-37290-5681, USDA Forest Service Cooperative Agreements 19-88-032 and 19-88-033, and L'Institut National de la Recherche Agronomique. This is Journal Paper Number 90-8-40 from the Kentucky Agricultural Experiment Station.

References

Adams, W. T. 1992. Gene dispersal within forest tree populations. New Forests 6: 217—240.

Ali, I. F., Neale, D. B. and Marshall, K. A. 1991. Chloroplast DNA restriction fragment length polymorphism in *Sequoia sempervirens* D. Don Endl., *Pseudotsuga menziesii* (Mirb.) Franco, *Calocedrus decurrens* (Torr.), and *Pinus taeda* L. Theor. Appl. Genet. 81: 83—89.

Asmussen, M. A., Arnold, J. and Avise, J. C. 1987. Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. Genetics 115: 755—768.

Blasko, K., Kaplan, S. A., Higgins, K. G., Wolfson, R. and Sears, B. B. 1988. Variation in copy number of a 24-base pair tandem repeat in the chloroplast DNA of *Oenothera hookeri* strain Johansen. Curr. Genet. 14: 287—292.

Bobola, M. S., Eckert, R. T. and Klein, A. S. 1992a. Restriction fragment variation in the nuclear rDNA repeat unit within and between *Picea rubens* and *Picea mariana*. Can. J. For. Res. 22: 255—363.

Bobola, M. S., Smith, D. E. and Klein, A. S. 1992b. Five major nuclear ribosomal repeats represent a large and variable fraction of the genomic DNA of *Picea rubens* and *P. mariana*. Molec. Biol. Evol. 9: 125—137.

Börner, T. and Sears, B. B. 1986. Plastome mutants. Plant Molec. Biol. Rep. 4: 69—92.

Bousquet, J., Simon, L. and Lalonde, M. 1990. DNA amplification from vegetative and sexual tissues of trees using polymerase chain reaction. Can. J. For. Res. 20: 254—257.

Brown, A. H. D. 1978. Isozymes, plant population genetic structure and genetic conservation. Theor. Appl. Genet. 52: 145—157.

Burley, J. 1965. Karyotype analysis of Sitka spruce, *Picea sitchensis* (Bong.) Carr. Silvae Genet. 14: 127—132.

Bush, R. M. and Smouse, P. E. 1992. Evidence for the adaptive significance of allozymes in forest trees. This issue (pp. 179—196).

Cheliak, W. M., Dancik, B. P., Morgan, K., Yeh, F. C. H. and Strobeck, C. 1985. Temporal variation of the mating system in a natural population of jack pine. Genetics 109: 569—584.

Chesnoy, L. 1987. L'origine des organites du cytoplasme embryonnaire chez les Gymnospermes. Bull. Soc. Bot. Fr., Actual. Bot. 134: 51—56.

Chiu, W.-L. and Sears, B. B. 1985. Recombination between chloroplast DNAs does not occur in sexual crosses of *Oenothera*. Molec. Gen. Genet. 198: 525—528.

Chiu, W.-L., Stubbe, W. and Sears, B. B. 1988. Plastid inheritance in *Oenothera*: organelle genome modifies the extent of biparental plastid transmission. Curr. Genet. 13: 181—189.

Clark, A. G. 1988. Deterministic theory of heteroplasmy. Evolution 42: 621—626.

Clegg, M. T., Learn, G. H. and Golenberg, E. M. 1991. Molecular evolution of chloroplast DNA. pp. 135—149. In: Selander, R. K., Clark, A. G. and Whittam, T. S. (Eds) Evolution at the Molecular Level. Sinauer Assoc., Inc., Sunderland, MA.

Conde, M. F., Pring, D. R. and Levings, C. S. III, 1979. Maternal inheritance of organelle DNAs in *Zea mays — Zea perennis* reciprocal crosses. J. Hered. 70: 2—4.

Cullis, C. A., Creissen, G. P., Gorman, S. W. and Teasdale, R. D. 1988a. The 25S, 18S, and 5S ribosomal RNA genes from *Pinus radiata* D. Don. pp. 34—40. In: Cheliak, W. M. and Yapa, A. C. (Eds) Molecular Genetics of Forest Trees. Petawawa National Forestry Institute Inform. Rep. PI-X-80, Chalk River, Ontario, Canada.

Cullis, C. A. Creissen, G. P., Gorman, S. W. and Teasdale, R. D. 1988b. The 25S and 18S ribosomal DNAs from *Pinus radiata*. p. 463. In: Hanover, J. W. and Keathley, D. E. (Eds) Genetic Manipulation of Woody Plants. Plenum Press, New York, New York.

Dhir, N. K. and Miksche, J. P. 1974. Intraspecific variation of nuclear DNA content in *Pinus resinosa* Ait. Can. J. Genet. Cytol. 16: 77—83.

Dong. J. (pers. comm.) Department of Forestry, University of Kentucky, Lexington, KY.

El-Kassaby, Y. A., Sigurgeirsson, A. and Szmidt, A. E. 1988. The use of restriction analysis of chloroplast DNA in classifying hybrid spruce seedlots, pp. 67—88. In: Hällgren, J.-E. (Ed) Molecular genetics of forest trees: Proceedings of the Frans Kempe symposium in Umeå, June 14—16, 1988. Swedish University of Agricultural Sciences, Umeå, Sweden.

El-Lakany, M. H. and Sziklai, O. 1971. Intraspecific variation in nuclear characteristics of Douglas-fir. Adv. Front. Plant Sci. 28: 363—378.

Ellstrand, N. C. 1992. Gene flow among seed plant populations. This issue (pp. 241—256).

Epperson, B. K. and Allard, R. W. 1984. Allozyme analysis of the mating system in lodgepole pine populations. J. Hered. 75: 212—214.

Epperson, B. K. and Allard, R. W. 1989. Spatial autocorrelation analysis of the distribution of genotypes within populations of lodgepole pine. Genetics 121: 369—377.

Ernst, S. G. (pers. comm.) Department of Forestry, Fisheries and Wildlife, University of Nebraska, Lincoln, NE.

Furnier, G. R. (pers. comm.) Department of Forest Resources, University of Minnesota, St. Paul, MN.

Govindaraju, D. R., Dancik, B. P. and Wagner, D. B. 1989a. Novel chloroplast DNA polymorphism in a sympatric region of two pines. J. Evol. Biol. 2: 49—59.

Govindaraju, D. R., Wagner, D. B., Lidholm, J., Gustafsson, P. and Szmidt, A. E. 1989b. A chloroplast DNA probe identifies unexpectedly high levels of polymorphism in *Pinus banksiana* and *P. contorta*. South. forest tree improvement conf. 20: 147—152.

Govindaraju, D. R., Wagner, D. B., Smith, G. P. and Dancik, B. P. 1988. Chloroplast DNA variation within individual trees of a *Pinus banksiana — P. contorta* sympatric region. Can. J. For. Res. 18: 1347—1350.

Gustafsson, P. and Sitbon, F. 1986. Studies on genetic differences between pine species by modern DNA technology; the nuclear genome, pp. 253—268. In: Lindgren, D. (Ed) Provenances and forest tree breeding for high latitudes: Proceedings of the Frans Kempe symposium in Umeå, June 10—11, 1986. Swedish University of Agricultural Sciences, Umeå, Sweden.

Hartl, D. L. and Clark, A. G. 1989. Principles of Population Genetics. 2nd edition. Sinauer Associates, Inc., Sunderland, Massachusetts.

Keim, P. (pers. comm.) Department of Biology, Northern Arizona State University, Flagstaff, AZ.

Keim, P., Paige, K. N., Whitham, T. G. and Lark, K. G. 1989a. Genetic analysis of an interspecific hybrid swarm of Populus: occurrence of unidirectional introgression. Genetics 123: 557—565.

Keim, P., Whitham, T. G. and Lark, K. G. 1989b. The genetic structure of a *Populus* hybrid swarm and the correlation of insect susceptibility to specific genetic classes. Genetics 122: s30.

Klekowski, E. J., Jr. 1988. Mutation, Developmental Selection, and Plant Evolution. Columbia University Press, New York.

Kreike, J., Burg, K., Zechmeister, M., Haider, T. and Glössl, J. 1991. DNA-fingerprint and RFLP analysis as tools to study genetic diversity in populations of fir, spruce and oak, pp. 95—107. In: Müller-Starck, G. and Ziehe, M. (Eds) Genetic Variation of Forest Tree Populations in Europe. Sauerländer-Verlag, Frankfurt/Main.

Kreitman, M. 1983. Nucleotide polymorphism at the alcohol dehydorogenase locus of *Drosophila melanogaster*. Nature 304: 412—417.

Kremer, A., Petit, R., Zanetto, A., Fougère, V., Ducousso, A., Wagner, D. and Chauvin, C. 1991. Nuclear and organelle gene diversity in *Q. robur* and *Q. petraea*, pp. 141—166. In:

Müller-Starck, G. and Ziehe, M. (Eds) Genetic Variation of Forest Tree Populations in Europe. Sauerländer-Verlag, Frankfurt/Main.

Ledig, F. T. and Conkle, M. T. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Carr.). Evolution 37: 79—85.

Leigh Brown, A. J. 1989. Population genetics at the DNA level: a review of the contribution of restriction enzyme studies. Oxford Surv. Ecol. Biol. 6: 207—242.

Li, T. (pers. comm.) Department of Forestry, University of Kentucky, Lexington, KY.

Libby, W. J., Stettler, R. F. and Seitz, F. N. 1969. Forest genetics and forest tree breeding. Ann. Rev. Genet. 3: 469—494.

Lidholm, J., Szmidt, A. E., Hällgren, J.-E. and Gustafsson, P. 1988. The chloroplast genomes of conifers lack one of the rRNA-encoding inverted repeats. Molec. Gen. Genet. 212: 6—10.

Lidholm, J., Szmidt, A. and Gustafsson, P. 1991. Duplication of the *psbA* gene in the chloroplast genome of two *Pinus* species. Molec. Gen. Genet. 226: 345—352.

Matos, J. A. and Schaal, B. A. (pers. comm.) Department of Biology, Washington University, St. Louis, MO 63130—4899.

McDaniel, R. G. 1984. Plant genetic engineering via organelle transfer. Plant Breeding Rev. 2: 283—302.

Mergen, F. and Thielges, B. A. 1967. Intraspecific variation in nuclear volume in four conifers. Evolution 21: 720—724.

Merkle, S. A., Chou, P. L. and Sommer, H. E. 1988. Stability of highly repeated sequences in the DNA of embryogenic cultures of yellow poplar, pp. 85—88. In: Cheliak, W. M. and Yapa, A. C. (Eds) Molecular Genetics of Forest Trees. Petawawa National Forestry Institute Inform. Rep. PI-X-80, Chalk River, Ontario, Canada.

Miksche, J. P. 1968. Quantitative study of intraspecific variation of DNA per cell in *Picea glauca* and *Pinus banksiana*. Can. J. Genet. Cytol. 10: 590—600.

Miksche, J. P. 1971. Intraspecific variation of DNA per cell between *Picea sitchensis* (Bong.) Carr. provenances. Chromosoma 32: 343—352.

Millar, C. I. and Westfall, R. D. 1992. Allozyme markers in forest genetic conservation. This issue (pp. 347—371).

Neale, D. B., Marshall, K. A. and Harry, D. E. 1991. Inheritance of chloroplast and mitochondrial DNA in incense-cedar (*Calocedrus decurrens*). Can. J. For. Res. 21: 717—720.

Neale, D. B., Marshall, K. A. and Sederoff, R. R. 1989. Chloroplast and mitochondrial DNA are paternally inherited in *Sequoia sempervirens* D. Don Endl. Proc. Natl. Acad. Sci. USA 86: 9347—9349.

Neale, D. B. and Sederoff, R. R. 1988. Inheritance and evolution of conifer organelle genomes, pp. 251—264. In: Hanover, J. W. and Keathley, D. E. (Eds) Genetic Manipulation of Woody Plants. Plenum Press, New York, New York.

Neale, D. B. and Sederoff, R. R. 1989. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor. Appl. Genet. 77: 212—216.

Neale, D. B., Wheeler, N. C. and Allard, R. W. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. Can. J. For. Res. 16: 1152—1154.

Neale, D. B. and Williams, C. G. 1991. Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. Can. J. For. Res. 21: 545—554.

Nybom, H. 1990. DNA fingerprints in sports of 'Red Delicious' apples. Hortscience 25: 1641—1642.

Nybom, H. and Hall, H. K. 1991. Minisatellite DNA "fingerprints" can distinguish *Rubus* cultivars and estimate their degree of relatedness. Euphytica 53: 107—114.

Nybom, H. and Rogstad, S. H. 1990. DNA "fingerprints" detect genetic variation in *Acer negundo* (*Aceraceae*). Plant Syst. Evol. 173: 49—56.

Nybom, H., Rogstad, S. H. and Schaal, B. A. 1990. Genetic variation detected by use of the M13 "DNA fingerprint" probe in *Malus*, *Prunus*, and *Rubus* (Rosaceae). Theor. Appl. Genet. 79: 153—156.

Nybom, H. and Schaal, B. A. 1990a. DNA "fingerprints" reveal genotypic distributions in natural populations of blackberries and raspberries (Rubus, Rosaceae). Amer. J. Bot. 77: 883—888.

Nybom, H. and Schaal, B. A. 1990b. DNA "fingerprints" applied to paternity analysis in apples (*Malus* x *domestica*). Theor. Appl. Genet. 79: 763—768.

Nybom, H., Schaal, B.A. and Rogstad, S. H. 1989. DNA "fingerprints" can distinguish cultivars of blackberries and raspberries. Acta Hort. 262: 305—310.

Ohba, K., Iwakawa, M., Okada, Y. and Murai, M. 1971. Paternal transmission of a plastid anomaly in some reciprocal crosses of Sugi, *Cryptomeria japonica* D. Don. Silvae Genet. 20: 101—107.

Owens, J. N. and Morris, S. J. 1990. Cytological basis for cytoplasmic inheritance in *Pseudotsuga menziesii*. I. Pollen tube and archegonial development. Amer. J. Bot. 77: 433—445.

Owens, J. N. and Morris, S. J. 1992. Cytological basis for cytoplasmic inheritance in *Pseudotsuga menziessii*. II. Fertilization and proembryo development. Amer. J. Bot. 78: 1515—1527.

Painter, R. H. 1966. Lessons to be learned from past experience in breeding plants for insect resistance, pp. 349—355. In: Gerhold, H. D., Schreiner, E. J., McDermott, R. E. and Winieski, J. A. (Eds) Breeding Pest Resistant Trees. Pergamon Press, Oxford, UK.

Palmer, J. D., Jansen, R. K., Michaels, H. J., Chase, M. W. and Manhart, J. R. 1988. Chloroplast DNA variation and plant phylogeny. Ann. Missouri Bot. Gard. 75: 1180—1206.

Parks, C. R. and Wendel, J. F. 1990. Molecular divergence between Asian and North American species of *Liriodendron* (Magnoliaceae) with implications for interpretation of fossil floras. Amer. J. Bot. 77: 1243—1256.

Paterson, A. H., Damon, S., Hewitt, J. D., Zamir, D., Rabinowitch, H. D., Lincoln, S. E., Lander, E. S. and Tanksley, S. D. 1991. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127: 181—197.

Perry, T. O. 1976. Maternal effects on the early performance of tree progenies, pp. 473—481. In: Cannell. M. G. R. and Last, F. T. (Eds) Tree Physiology and Yield Improvement. Academic Press, London, UK.

Pring, D. R. and Lonsdale, D. M. 1985. Molecular biology of higher plant mitochondrial DNA. Internat. Rev. Cytol. 97: 1—46.

Righter, F. I. and Stockwell, P. 1949. The fertile species hybrid, *Pinus murraybanksiana*. Madroño 10: 65—69.

Rogstad, S. H., Nybom, H. and Schaal, B. A. 1991. The tetrapod DNA fingerprinting M13 repeat probe reveals genetic diversity and clonal growth in quaking aspen (*Populus tremuloides*, Salicaceae). Plant Syst. Evol. 175: 115—123.

Rogstad, S. H., Patton, J. C. and Schaal, B. A. 1988a. A human minisatellite probe reveals RFLPs among individuals of two angiosperms. Nucleic Acids Res. 16: 11378.

Rogstad, S. H., Patton, J. C. II and Schaal, B. A. 1988b. M13 repeat probe detects DNA

minisatellite-like sequences in gymnosperms and angiosperms. Proc. Natl. Acad. Sci. USA 85: 9176—9178.

Ryskov, A. P., Jincharadze, A. G., Prosnyak, M. I., Ivanov, P. L. and Limborska, S. A. 1988. M13 phage DNA as a universal marker for DNA fingerprinting of animals, plants and microorganisms. FEBS Lett. 233: 388—392.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. and Erlich, H. A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487—491.

Saylor, L. C. and Smith, B. W. 1966. Meiotic irregularity in species and interspecific hybrids of *Pinus*. Amer. J. Bot. 53: 453—468.

Schnabel, A. and Asmussen, M. A. 1989. Definition and properties of disequilibria within nuclear-mitochondrial-chloroplast and other nuclear-dicytoplasmic systems. Genetics 123: 199—215.

Sears, B. B. 1980. The elimination of plastids during spermatogenesis and fertilization in the plant kingdom. Plasmid 4: 233—255.

Sigurgeirsson, A., Szmidt, A. E. and Karpinska, B. 1991. Alaskan *Picea sitchensis* populations infiltrated with *Picea glauca* genes: a study using DNA markers, pp. 197—207. In: Fineschi, S., Malvolti, M. E., Cannata, F. and Hattemer, H. H. (Eds) Biochemical Markers in the Population Genetics of Forest Trees. SPB Academic Publishing bv, The Hague, The Netherlands.

Sitbon, F. and Gustafsson, P. 1988. Ribosomal DNA polymorphism in *Pinus sylvestris*, pp. 485—486. In: Hanover, J. W. and Keathley, D. E. (Eds) Genetic Manipulation of Woody Plants. Plenum Press, New York, New York.

Smith, R. L. and Sytsma, K. J. 1990. Evolution of *Populus nigra* (Sect. *Aigeiros*): introgressive hybridization and the chloroplast contribution of *Populus alba* (Sect. *Populus*). Amer. J. Bot. 77: 1176—1187.

Soliman, K., Fedak, G. and Allard, R. W. 1987. Inheritance of organelle DNA in barley and *Hordeum* x *Secale* intergeneric hybrids. Genome 29: 867—872.

Stine, M. (pers. comm.) School of Forestry, Wildlife, and Fisheries, Louisiana State University, Baton Rouge, LA.

Stine, M. and Keathley, D. E. 1990. Paternal inheritance of plastids in Engelmann spruce x blue spruce hybrids. J. Hered. 81: 443—446.

Stine, M., Sears, B. B. and Keathley, D. E. 1989. Inheritance of plastids in interspecific hybrids of blue spruce and white spruce. Theor. Appl. Genet. 78: 768—774.

Strauss, S. H., Bousquet, J., Hong, Y.-P. and Hipkins, V. D. 1992. Biochemical and molecular genetic markers in biosystematic studies of forest trees. New Forests. This issue (pp. 125—158).

Strauss, S. H., Hong, Y.-P. and Hipkins, V. (pers. comm.) Department of Forest Science, Oregon State University, Corvallis, OR.

Strauss, S. H. and Howe, G. T. 1990. An investigation of somatic variability for ribosomal RNA gene number in old-growth Sitka spruce. Can. J. For. Res. 20: 853—856.

Strauss, S. H., Neale, D. B. and Wagner, D. B. 1989. Genetics of the chloroplast in conifers: biotechnology research reveals some surprises. J. Forestry 87: 11—17.

Strauss, S. H., Palmer, J. D., Howe, G. T. and Doerksen, A. H. 1988. Chloroplast genomes of two conifers lack a large inverted repeat and are extensively rearranged. Proc. Natl. Acad. Sci. USA 85: 3898—3902.

Strauss, S. H. and Tsai, C.-H. 1988. Ribosomal gene number variability in Douglas-fir. J. Hered. 79: 453—458.

Sutton, B. C. S., Flanagan, D. J. and El-Kassaby, Y. A. 1991a. A simple and rapid method for estimating representation of species in spruce seedlots using chloroplast DNA restriction fragment length polymorphism. Silvae Genet. 40: 119—123.

Sutton, B. C. S., Flanagan, D. J., Gawley, J. R., Newton, C. H., Lester, D. T. and El-Kassaby, Y. A. 1991b. Inheritance of chloroplast and mitochondrial DNA in *Picea* and composition of hybrids from introgression zones. Theor. Appl. Genet. 82: 242—248.

Szmidt, A. E. (pers. comm.) Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå, Sweden.

Szmidt, A. E., Aldèn, T. and Hällgren, J.-E. 1987. Paternal inheritance of chloroplast DNA in *Larix*. Plant Molec. Biol. 9: 59—64.

Szmidt, A. E., El-Kassaby, Y. A., Sigurgeirsson, A., Aldèn, T., Lindgren, D. and Hällgren, J.-E. 1988. Classifying seedlots of *Picea sitchensis* and *P. glauca* in zones of introgression using restriction analysis of chloroplast DNA. Theor. Appl. Genet. 76: 841—845.

Szmidt, A. E. and Wang, X.-R. 1991. DNA markers in forest genetics. In: Müller-Starck, G. and Ziehe, M. (Eds) Genetic Variation of Forest Tree Populations in Europe. Sauerländer-Verlag, Frankfurt/Main (in press).

Tsai, C.-H. and Strauss, S. H. 1989. Dispersed repetitive sequences in the chloroplast genome of Douglas-fir. Curr. Genet. 16: 211—218.

van Damme, J. M. M. 1986. Gynodioecy in *Plantago lanceolata* L. V. Frequencies and spatial distribution of nuclear and cytoplasmic genes. Heredity 56: 355—364.

Wagner, D. B., Dong, J., Carlson, M. R. and Yanchuk, A. D. 1991c. Paternal leakage of mitochondrial DNA in *Pinus*. Theor. Appl. Genet. 82: 510—514.

Wagner, D. B., Furnier, G. R., Saghai-Maroof, M. A., Williams, S. M., Dancik, B. P. and Allard, R. W. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proc. Natl. Acad. Sci. USA 84: 2097—2100.

Wagner, D. B. and Govindaraju, D. R. 1988. Associations of chloroplast DNA and cone morphology in the sympatric region of *Pinus banksiana* and *Pinus contorta*, pp. 492—493. In: Hanover, J. W. and Keathley, D. E. (Eds) Genetic Manipulation of Woody Plants. Plenum Press, New York, New York.

Wagner, D. B., Govindaraju, D. R. and Dancik, B. P. 1989a. Tests of cytonuclear population-genetic structure involving the chloroplast genome in a sympatric region of *Pinus banksiana* and *P. contorta*. Genetics 122: s42.

Wagner, D. B., Govindaraju, D. R., Yeatman, C. W. and Pitel, J. A. 1989b. Paternal chloroplast DNA inheritance in a diallel cross of jack pine (*Pinus banksiana* Lamb.). J. Hered. 80: 483—485.

Wagner, D. B., Nance, W. L., Nelson, C. D., Li, T., Patel, R. N. and Govindaraju, D. R. 1992. Taxonomic patterns and inheritance of chloroplast DNA variation in a survey of *Pinus echinata* Mill., *P. elliottii* Engelm., *P. palustris* Mill., and *P. taeda* L. Can. J. For. Res. 22: 683—689.

Wagner, D. B., Sun, Z.-X., Govindaraju, D. R. and Dancik, B. P. 1991b. Spatial patterns of chloroplast DNA and cone morphology variation within populations of a *Pinus banksiana* — *Pinus contorta* sympatric region. Am. Nat. 138: 156—170.

Wang, C. 1990. A DNA rearrangement found in chloroplast genome from eastern hemlock (*Tsuga canadensis*). M.S. Thesis, University of Louisville, Louisville, KY, 64 pp.

Wang, X.-R. and Szmidt, A. E. 1990. Evolutionary analysis of *Pinus densata* (Masters), a putative Tertiary hybrid. 2. A study using species-specific chloroplast DNA markers. Theor. Appl. Genet. 80: 641—647.

White, E. E. 1990a. Chloroplast DNA in *Pinus monticola*. 1. Physical map. Theor. Appl. Genet. 79: 119—124.

White, E. E. 1990b. Chloroplast DNA in *Pinus monticola*. 2. Survey of within-species variability and detection of heteroplasmic individuals. Theor. Appl. Genet. 79: 251—255.

Whittemore, A. T. and Schaal, B. A. 1991. Interspecific gene flow in sympatric oaks. Proc. Natl. Acad. Sci. USA 88: 2540—2544.

Wilcox, M. D. 1983. Reciprocal cross effects in *Pinus radiata*. N. Z. J. For. Sci. 13: 37—45.
Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531—6535.
Zimmerman, P. A., Lang-Unnasch, N. and Cullis, C. A. 1989. Polymorphic regions in plant genomes detected by an M13 probe. Genome 32: 824—828.