Theor Appl Genet (1993) 86:301–307

THEORETICAL AND APPLIED GENETICS

Molecular genetics of growth and development in *Populus*. I. Triploidy in hybrid poplars

H. D. Bradshaw, Jr.[1] **and R. F. Stettler**[2]

[1] Department of Biochemistry SJ-70 and [2] College of Forest Resources AR-10, University of Washington, Seattle, WA 98195, USA

Received July 25, 1992; Accepted September 19, 1992
Communicated by P. M. A. Tigerstedt

Summary. While constructing a genetic linkage map of a hybrid poplar genome (*Populus trichocarpa* × *P. deltoides*), we identified several restriction fragment length polymorphismus (RFLPs) for which the parental trees are heterozygous. Although 8 of the 11 F_1 hybrid offspring inherited, as expected, single RFLP alleles from each parent, 3 F_1 trees in the mapping pedigree inherited both maternal alleles along with a single paternal allele at some loci. Aneuploidy or polyploidy in these 3 F_1 trees due to partial or complete nondisjunction during female gametogenesis is the simplest explanation for this finding. Of the 3 F_1 offspring with supernumerary RFLP alleles 2 have triploid nuclear DNA contents as measured by fluorescence flow cytometry; the 3rd F_1 with supernumerary alleles has a sub-triploid nuclear DNA content and is probably aneuploid. Among the tri/aneuploid hybrids, leaf quantitative traits either are skewed toward those values characteristic of the *P. trichocarpa* female parent (adaxial stomate density, petiole length: blade length ratio; abaxial color) or show transgressive variation (epidermal cell size). Abaxial leaf color was used to screen a large population of *P. trichocarpa* × *P. deltoides* hybrids for further evidence of tri/aneuploidy. In each case where a "white" abaxial leaf surface was observed and the nuclear DNA content measured, the hybrid proved to be tri/aneuploid. All sexually mature female triploids examined were sterile, although the inflorescences completed their development in the absence of embryo formation. The (probably) aneuploid F_1 hybrid is a fertile female. Of 15 female *P. trichocarpa* parents used in crosses to *P. deltoides*, 10 produced one or more tri/aneuploid hybrid offspring. In an intraspecific cross using a *P. trichocarpa* female that had produced triploid hybrids with five different *P. deltoides* males, no tri/aneuploid offpsring were found.

Key words: RFLP – Polyploid – Nondisjunction – Interspecific hybrid – Cottonwood

Correspondence to: H. D. Bradshaw

Introduction

During the course of experiments designed to construct a genetic linkage map for the poplar genome, unexpected segregation of some restriction fragment length polymorphisms (RFLPs) in the F_1 generation led us to examine transmission genetics and quantitative trait inheritance in an interspecific cross of *Populus*. The prevalence of unusual ploidy levels in some of the hybrid offspring has potential implications for practical genetic improvement of poplars through traditional breeding and biotechnology and for gene flow in natural populations.

Material and methods

The pedigree

Controllled pollinations were performed on detached floral branches in the greenhouse (Stettler and Bawa 1971). In 1981, *Populus trichocarpa* (T) clone 93-968 and *P. deltoides* (D) clone 59-129-17 (also known as ILL129) were crossed to produce F_1 hybrid family 53 (T × D). Eleven clones from family 53, numbered from 238 through 248, have been maintained in the 1984 stoolbed at Washington State University's Farm 5 in Puyallup, Wash. Additional hybrid offspring from other T × D crosses made in 1979–1981 were surveyed for aberrant ploidy levels. In 1990, 93-968 was used as the female parent in a repeat cross to ILL129 and in an intraspecific cross to 147-993, a male *P. trichocarpa* (Dunlap 1991), to produce family 352.

DNA extraction

Leaves were either lyophilized or frozen in liquid nitrogen, pulverized in a small coffee grinder, suspended in nuclei buffer

(10 m*M* *TRIS*-HCl, pH 8, 1 m*M* EDTA, 0.3 *M* sucrose), and homogenized with a Brinkmann Polytron 10-TS tip for 1 min at a power setting of seven to dissociate nuclei from cell-wall debris. Crude nuclei were sedimented by a 5-min centrifugation at 1000 *g*. DNA was extracted and purified according to Parsons et al. (1989).

Southern blot hybridization

Poplar DNA (3 μg/lane) was used for Southern blotting essentially as described by Maniatis et al. (1982). The blots were hybridized in roller bottles (Robbins Scientific) with approximately 5×10^6 cpm/ml [^{32}P]-labeled probe in 0.5 *M* Na_2HPO_4, pH 7.2, 7% SDS, 10 m*M* EDTA, and 0.5% nonfat dry milk at 65 °C for 24 h and washed according to Church and Gilbert (1984). Autoradiography was done at −70 °C with a single intensifying screen. A *win*8 cDNA clone (Parsons et al. 1989) and random genomic *Pst*I fragments cloned from poplar nuclear DNA were used as probes in Southern blot experiments.

Preparation of nuclei and flow cytometry

Crude nuclei were prepared from fully expanded field-grown leaves as for DNA extraction except that the nuclear homogenate was filtered through two layers of cheesecloth and a layer of Miracloth (Calbiochem). The pellet from 10 g of leaves was resuspended in 1 ml of nuclei buffer, diluted into 0.15 *M* NaCl containing 10 μg/ml DAPI (Sigma), and the clumped nuclei dispersed with ten passes through a 26 gauge syringe needle. Flow cytometry was performed on an ICP-22 (Ortho Diagnostic Systems, Westwood, Mass.) with chicken erythrocytes (2.34 pg/nucleus; Mirsky and Ris 1949) used as an internal standard. Approximately 1000 nuclei were assayed for each measurement of DNA content.

Leaf quantitative traits

Leaves from T × D F_1 hybrids taken for metric trait analysis were from actively growing (mid-July) first-year resprouts of 6th-year stools coppiced the previous winter. Parental leaves were obtained from outdoor container-grown trees similar to the hybrids in height and age since sprouting. Fully mature leaves (leaf plastochron index 14 and 15; Larson and Isebrands 1971) were harvested and either used immediately or refrigerated up to 18 h prior to processing. Epidermal leaf peels were made by spraying the leaves with a thin coat of clear Krylon acrylic and lifting the dried plastic film with a piece of clear Scotch tape. The peels were affixed to microscope slides for examination. Three microscope fields (field diameter = 446 μm) were counted and averaged to determine the mean number of stomates in a field and the mean number of epidermal cells along the diameter of a field.

Assessment of fertility

All three tri/aneuploid members of family 53 are female. Two of them (53-240 and 53-244) are growing, together with other T × D hybrids, in an 8-year-old plantation at the University of Washington's Pack Forest. In June 1991, mature open-pollinated female catkins were collected, three from each of five tri/aneuploid clones (53-240, 53-244, 51-198, 15-21, 19-61) and five diploid clones (53-246, 53-248, 51-205, 51-201, 15-29). Lengths and diameters of the ten largest capsules per catkin were measured. Ten capsules per clone were then dissected and examined for filled seed.

Fiber length analysis

Stem sections (1.5 m in length approximately at breast height) were cut from the clonal performance trial at Pack Forest, Wash. A single stem from a triploid (53-240) and a diploid (53-242) clone was analyzed. Each of the two stem sections was chipped and kraft pulped at the University of Washington College of Forest Resources Pulp and Paper Laboratory. The distribution of fiber lengths in this pulp was determined with a Kajaani FS-200 fiber length analyzer at the Boise Cascade Research Laboratory in Portland, Ore.

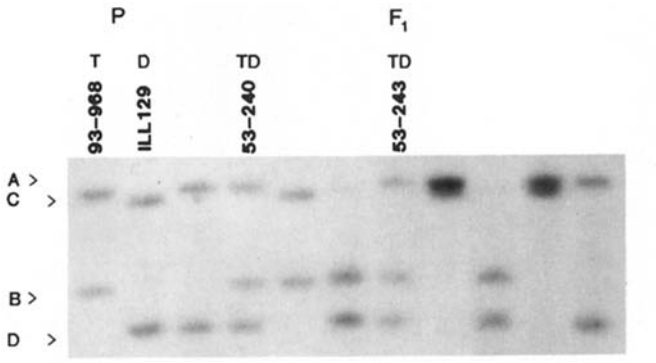

Fig. 1. Southern blot evidence for inheritance of supernumerary RFLP alleles in *Populus trichocarpa* (T) × *P. deltoides* (D) hybrid family 53. The female *P. trichocarpa* parent 93-968 is heterozygous for the A and B alleles at locus pPOP46, while the male *P. deltoides* parent is heterozygous for the C and D alleles. Among the TD hybrids, most inherit a single allele from each parent. Clones 53-240 and 53-243 inherit both maternal alleles (A and B) along with a single paternal allele

Results and discussion

Triploidy and aneuploidy can be detected using RFLPs

In our search for RFLPs informative in our linkage mapping pedigree we frequently encountered probes that reveal apparent heterozygosity in one or both parental trees. Heterozygosity was verified by examining the pattern of RFLP inheritance in the F_1 family 53. Of the 11 F_1 offspring 8 always inherit a single allele from each parent according to Mendelian expectation; however, 3 individuals (53-240, 53-243, and 53-244) occasionally receive two alleles from their maternal parent along with a paternal allele (Fig. 1; Table 1). None of these 3 clones inherits both female alleles at all 12 loci where the seed parent is known to be heterozygous (Table 1). No supernumerary alleles in the F_1 have been found to be derived from the pollen parent at the 7 loci at which the male is known to be heterozygous (Table 1).

The simplest explanation for this unexpected mode of inheritance is that 53-240, 53-243, and 53-244 are aneuploid or polyploid due to partial or complete nondisjunction during female gametogenesis. If so, their nuclear DNA contents should be greater than those of their diploid siblings. Nuclear DNA content in the parental trees and the F_1 family 53 progeny was measured by fluorescence flow cytometry. Two clones, 53-240 and 53-

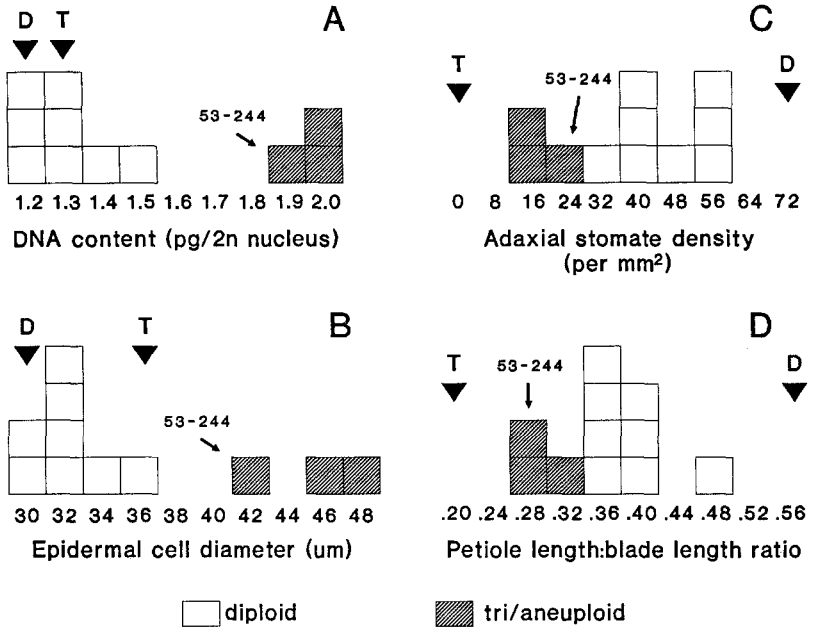

Fig. 2 A–D. Quantitative trait inheritance among tri/aneuploid and diploid siblings in *Populus trichocarpa* (T) × *P. deltoides* (D) hybrid family 53. Nuclear DNA content (*panel A*) and three leaf traits (*panels B–D*) were measured. Clone 53-244 is probably aneuploid. D and T indicate parental values

Table 1. Segregation of alleles at loci where the female *Populus trichocarpa* parent 93-968 is heterozygous

RFLP locus number/enzyme	93-968	ILL129	**53-240**	53-242	**53-243**	**53-244**	53-246[b]
46/*Hin*dIII[a]	AB	CD	**ABD**	BD	**ABD**	AC	AC
65/*Xba*I	AB	C	**ABC**	BC	AC	BC	BC
185/*Hin*dIII	AB	C	**ABC**	BC	AC	**ABC**	BC
191/*Xba*I[a]	AB	CD	BC	AD	BD	AD	AC
192/*Hin*dIII	ABC	D	ACD	ABD	**ABCD**	ACD	ABD
214/*Hin*dIII[a]	AB	CDE	ADE	ADE	–	BDE	BCE
221/*Hin*dIII	AB	C	BC	AC	AC	AC	BC
237/*Eco*RV	ABC	CD	**ABCD**	ACD	ACD	–	ACD
242/*Xba*I	AB	C	**ABC**	AC	**ABC**	**ABC**	AC
761/*Eco*RV	ABC	D	**ABCD**	BCD	–	**ABCD**	ACD
767/*Eco*RV	ABC	AD	**ABCD**	ACD	–	**ABCD**	ABD
win8/*Hin*dIII	AB	–	**AB**	B	A	B	A

Aneuploid or triploid clones and the loci at which this is demonstrated are in **boldface**. Each band on the Southern blot is given a letter; these letters do not necessarily correspond to discrete alleles (e.g., the "A" band for probe 192)

[a] Four additional loci unambiguously heterozygous in the male *P. deltoides* ILL129 have been omitted, but show no aberrant segregation

[b] The six remaining diploid offspring from family 53 have been omitted for clarity, but confirm Mendelian expectations at these loci

243, have approximately 1.5 times the nuclear DNA content (2.0–2.1 pg) of their parents and diploid siblings (Fig. 2A). Clone 53-244 has a nuclear DNA content (1.9 pg) intermediate between the diploid and triploid values (Fig. 2A). Alternative explanations for finding two maternal alleles in individual preparations of F_1 DNA have been considered. The trivial case of DNA sample mixing has been ruled out by using only DNA extracted from the leaves of a single ramet of each clone. Flow cytometric measurements convincingly demonstrate that the nuclei of the tri/aneuploid offspring contain more DNA than those of their diploid siblings. The excess nuclear DNA content, narrowly distributed about a single value, eliminates the possibility that the tri/aneuploid hybrids are chimeras of diploid or mixoploid cells with alternate maternal genotypes. The absolute value of diploid nuclear DNA content we obtained for the *P. deltoides* clone ILL129 (1.2 pg, Fig. 2A) agrees well with estimates made by Dhillon (1987) using cytophotometry (1.08 pg) and flow cytometry (1.20 pg). We find that *P. trichocarpa* 93-968 has a nuclear DNA content of 1.4 pg.

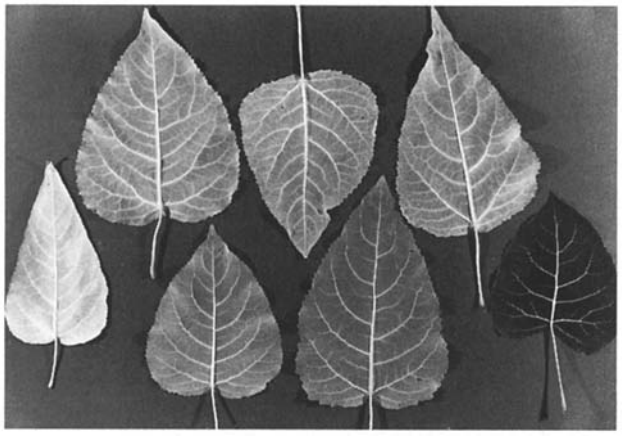

Fig. 3. Variation in abaxial leaf color in *Populus trichocarpa* × *P. deltoides* hybrid family 53. The contrast between the white *P. trichocarpa* 93-968 (*left*) and the dark green *P. deltoides* ILL129 (right) is interpolated by the two diploid F_1 hybrids (53-242 and 53-246) in the *lower row*. Their tri/aneuploid siblings in the *upper row* (left to right: 53-240, 53-243, and 53-244) are lighter in color

Tri/aneuploidy has phenotypic effects on leaf morphology, wood fiber length, and fertility

Reasoning that the additional *P. trichocarpa* genetic contribution to the three tri/aneuploid hybrids might have some quantitative effects on leaf morphology, we examined leaf epidermal cell size, adaxial stomate density, leaf shape, and leaf color parameters in family 53 and its parents. Leaf quantitative traits at the cellular level are quite dissimilar in *P. trichocarpa* and *P. deltoides* (Ridge et al. 1986). *P. trichocarpa* leaves have large epidermal cells and few adaxial stomates when compared with those from *P. deltoides*. In F_1 family 53, epidermal cell size shows a pattern of transgressive variation not seen in the other morphological or biochemical traits (Fig. 2B), with the tri/aneuploid hybrids having larger values than either parents or diploid siblings. Transgressive variation for cell size is not unexpected, as increased cell size frequently is correlated with higher ploidy levels (Swanson 1957). The adaxial stomate density among tri/aneuploids is skewed toward the low values typical of their female *P. trichocarpa* parent (Fig. 2C). Leaf quantitative traits at the macroscopic level corroborate the observations made at the cellular level. Leaf shape traits, such as the ratio of leaf-petiole length to blade length (petiole length ratio; PLR), discriminate well between *P. trichocarpa* 93-968 (PLR = 0.20) and *P. deltoides* ILL129 (PLR = 0.55). Tri/aneuploid hybrids have a PLR more *trichocarpa*-like (smaller) than their diploid siblings (Fig. 2D). A conspicuous difference in abaxial leaf color between *P. trichocarpa* (white) and *P. deltoides* (green) serves as a useful visible marker. The anatomical basis for this contrast in color has been described by Figliola (1986) and Hinckley et al. (1989). Diploid members of family 53 are intermediate in color between the parental species, but the three tri/aneuploid clones are noticeably whiter (Fig. 3).

Wood fiber length was measured in a triploid (53-240) and its diploid full-sib (53-242) to gather preliminary data on potential wood quality differences attributable to ploidy level. The length-weighted average fiber length was greater in the triploid clone ($x = 0.71$ mm, $n = 44{,}108$) than that from the diploid ($x = 0.57$ mm; $n = 41{,}146$), which is in agreement with similar comparisons between naturally occurring triploid and diploid aspen (*P. tremuloides*; van Buijtenen et al. 1958).

Open-pollinated floral catkins from 1 triploid (53-240) were compared to those of 2 diploid siblings (53-246 and 53-248). No filled seeds were found in the triploid. In contrast, filled seed set was consistent in the diploids, if somewhat low ($x = 3.1$ per capsule, $n = 20$), as often is found in T × D hybrids. Capsule morphology in the triploid was skewed towards the pubescent, globose appearance characteristic of *P. trichocarpa* (Harlow et al. 1979). These differences were further explored in a comparison of 3 "white", i.e., putative triploid, versus 3 "green", putative diploid, hybrid clones in three additional families (15, 19, and 51). Capsules from "white" clones were consistently more pubescent and more globose, with larger diameters ($x = 7.57 \pm 0.04$ mm, $n = 90$) than those from "green" clones ($x = 5.87 \pm 0.05$ mm, $n = 90$). No filled seeds were found in the former ($n = 30$), whereas an average of 5.1 per capsule was found in the latter ($n = 30$). Catkin maturation seemed to proceed normally in the "white" clones despite the lack of viable embryos; however, the capsule walls were noticeably thickened, perhaps due to altered allocation of nutrients normally destined to support seed development. Catkins from the triploids abscised just prior to capsule dehiscence, thereby preventing "cotton" shed. The putative aneuploid female clone 53-244 produced capsules with an average of 3.9 filled seed ($n = 10$). Based on nuclear DNA content, it seems likely that 53-244 is aneuploid, and perhaps for this reason is more fertile than the triploids. Alternatively, the seed in 53-244 may have arisen by apomixis; we have not yet directly tested this possibility. Although none of the 3 tri/aneuploids in family 53 is male, in other families of *P. trichocarpa* × *P. deltoides* hybrids we have identified triploid males based on nuclear DNA content and abaxial leaf color. No conclusive pollen fertility tests have been carried out, but previous breeding attempts have failed when pollen was used from male clones now known to be triploid.

Tri/aneuploidy is common in T × D hybrids

Using abaxial leaf color as an indicator of ploidy level in T × D hybrids, we surveyed our stoolbeds for "white" and "green" clones. A total of 92 "white" hybrids and

168 "green" hybrids were found, representing 15 *P. trichocarpa* female parents. All 10 female *P. trichocarpa* with more than four offspring in our stoolbed produced at least 1 "white" hybrid. In every case where nuclear DNA content has been determined [18 clones from 5 families (19, 20, 50, 52, 53) representing 2 *P. trichocarpa* females and 8 *P. deltoides* males] the "white" hybrids have aneuploid or triploid values ($x = 2.0 \pm 0.1$ pg, $n = 8$) as compared with their "green" siblings ($x = 1.4 \pm 0.1$ pg, $n = 10$). Leaf morphological traits were assessed in hybrid families 19, 20, 50, 51, and 52. All "white" hybrids showed the same transgressive variation in epidermal cell size and skewing of adaxial stomate density and PLR as the tri/aneuploids in family 53 (data not shown).

From this we conclude that either the frequency of nondisjunction in *P. trichocarpa* females is generally quite high or the tri/aneuploid condition confers some selective advantage that causes it to be overrepresented in hybrids with *P. deltoides*. To distinguish between these hypotheses, in the spring of 1990 we repeated the 1981 cross between *P. trichocarpa* 93-968 and *P. deltoides* ILL129 and performed an additional intraspecific controlled cross with pollen collected from male *P. trichocarpa* 147-993. The 1990 T × D progeny had 2 "white" hybrids assumed to be tri/aneuploid (versus 10 "green"), which is consistent with the proportion of tri/aneuploid hybrids produced by this cross in 1981. Tri/aneuploids among the T × T family 352 were expected to lack distinguishing gross morphological traits, so 92 seedlings were examined for abnormally large leaf epidermal cells as an indication of increased ploidy. Nuclei were prepared from the T × T seedlings with the largest epidermal cells (352-2312 and 352-2329) and their DNA content measured along with nuclei of two seedlings from the middle of the epidermal cell size distribution (352-2303 and 352-2347). By flow cytometric analysis, all four seedlings appear to be diploid, with nuclear DNA contents of 1.5 pg, 1.6 pg, 1.4 pg, and 1.5 pg, respectively. It appears that tri/aneuploidy may be more common in offspring from interspecific hybridizations than in those from intraspecific matings. *P. trichocarpa* 93-968 has produced a total of 12 "white" hybrids (versus 89 "green") when crossed with 5 different *P. deltoides* males, yet failed to produce any triploids among 92 T × T offspring. Even comparatively rare unreduced female gametes may produce seeds at noticeable frequencies if tri/aneuploidy confers some selective advantage on hybrid zygotes. Alternatively, tri/aneuploidy may have deleterious effects on intraspecific embryos and not on hybrid embryos. It is possible, of course, that our choice of epidermal cell size as an indicator of higher ploidy in the T × T cross was inappropriate and failed to detect triploids. This explanation seems unlikely given the rather extreme transgressive variation for this trait observed in T × D crosses and other polyploid plants.

Aberrant female gametogenesis is not confined to *P. trichocarpa*, since we find that the female T × D hybrid 53-246 has produced at least two F_2 offspring (of the 26 routinely tested as part of the genome mapping effort) with supernumerary RFLP alleles derived from her gametes; transmission of extra alleles from the 53-242 male parent has never been observed (data not shown).

The absence of pollen-derived supernumerary alleles in all of the F_1 and F_2 tri/aneuploids examined argues that either aberrant gametogenesis is more frequent in *P. trichocarpa* and hybrids than in *P. deltoides*, or that aneuploid or unreduced pollen is less likely to produce a zygote than aneuploid or 2n ova. A reciprocal hybridization scheme could be designed to test these alternatives, although identical parents could not be used reciprocally since poplars are dioecious. It would not be surprising if aneuploidy has more severe negative consequences for the male gametophyte whose success depends upon pollen-tube growth, which in the *P. trichocarpa* × *P. deltoides* cross is suboptimal even in normal gametes (Guries and Stettler 1976).

Tri/aneuploidy has implications for applied tree breeding and the genetics of natural populations

P. trichocarpa and *P. deltoides* represent two different sections within the genus *Populus* (Tacamahaca and Aigeros, respectively; Eckenwalder 1977). If aneuploidy/triploidy is a frequent outcome of intersectional crosses in *Populus,* there are many potentially useful ramifications. For example, some aneuploids may be single chromosome addition lines, with attendant utility for RFLP and quantitative trait mapping; or, a collection of triploids produced by first or second meiotic division restitution might be used to map the distance between RFLPs and the centromeres. These applications await a detailed understanding of the events surrounding unreduced female gamete formation in *Populus*.

Sterility of triploid hybrids has several interesting possibilities and some pitfalls. In production plantations, where hybrid poplars begin to flower in the 4th year of a 6- to 10-year rotation, the lack of viable pollen and/or filled seed would prevent hybrid gene flow into adjacent natural populations and reduce or eliminate nuisance pollen allergens and "cotton". Sterile hybrids would be logical candidates for manipulation by genetic engineering since transgenes would not be released into the environment via the gametes. On the other hand, in a tree improvement strategy that requires advanced-generation breeding it will be convenient to eliminate sterile offspring from any performance testing design.

In hybrids between *P. trichocarpa* and *P. deltoides* (and probably other intersectional hybrids, as well), tri/aneuploidy can be detected at the seedling stage by inspection of abaxial leaf color, offering the possibility of

rapid early selection in a poplar improvement program. If triploids outperform their diploid siblings, as is the case in aspen (van Buijtenen et al. 1958), extensive clonal trials could be confined to this subset of the progeny. In the spring of 1991 we planted a replicated clonal trial containing 51 tri/aneuploid hybrids and a matching set of their nearest diploid relatives. This trial will be used to evaluate the growth and yield consequences of increased ploidy in hybrid *Populus*. Since some triploids will be more heterozygous than others due to randomness in the distance between the centromere and successive chiasma for each gamete, the effect of varying heterozygosity (as determined with RFLP markers) can be evaluated simultaneously. Triploids, with a two-thirds contribution from one parent via the unreduced gamete, may capture in a single generation the majority of improvement that could be normally achieved with a backcross having three-quarters of the recurrent parent's genome.

In natural populations of *Populus,* sterility or reduced fecundity are obviously selectively disadvantageous if sexual reproduction is the only mode of propagation. Most *Populus* species are capable of efficient asexual reproduction by suckering from the roots, stump sprouting, or sprouting of abscised short shoots and of broken-off stem and branch fragments. In a long-lived plant capable of propagating vegetatively, faster-growing sterile genotypes may persist for long periods in competition with fertile diploids (Stebbins 1950). Many intersectional F_1 hybrids show heterosis for growth (Zsuffa 1975; Dickmann and Stuart 1983; Heilman and Stettler 1985), and might be effective competitors against their parental species. The occurrence and prevalence of higher ploidy in natural populations of *Populus* deserve further study, especially in hybrid contact zones where intersectional crosses occur (Brayshaw 1965; Keim et al. 1989).

While tri/aneuploidy in *P. trichocarpa* × *P. deltoides* hybrids may be ascertained by simple inspection of abaxial leaf color, prior to RFLP analysis of pedigreed material "white" clones were assumed to be representative of normal variation within a population of diploid hybrids. Molecular techniques have introduced a higher level of resolution to traditional genetic methods and, in this instance, have revealed additional unsuspected sources of variation.

Acknowledgements. We wish to thank the following members of the UW/WSU Poplar Research Group: Milt Gordon and Liz van Volkenburgh for providing laboratory space and facilities to carry out the RFLP mapping and leaf metric trait analyses, respectively; Rong-ling Wu for help with the fertility assessment; Paul Heilman and Diane Fogel for supplying the pedigreed tree cutings; Rick Gustafson for fiber analysis; Alyssa Prinz, Brian Watson, Paula Glackin, and Kevin Otto for contributing expert technical support; and John Davis for helpful discussions and critical reading of the manuscript. Flow cytometry was performed by Anthony Dayton in Peter Rabinovitch's laboratory in the Department of Pathology, University of Washington. Doug Ewing's expertise in the greenhouse growth of poplars was much appreciated. Pollen from ILL129 was kindly provided by Joe Jokela and Dave Harry (Unversity of Illinois). This work was supported by grants from the Washington Technology Centers (09-1022), the USDA NRICGP (91-37300-6458), and the U.S. Department of Energy (19X-43382C).

References

Brayshaw TC (1965) Native poplars of southern Alberta and their hybrids. Can Dep For Publ No. 1109, Ottawa

Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81:1991–1995

Dhillon SS (1987) DNA analysis during growth and development. In: Hanover JW, Keathley DE (eds). Genetic manipulation of woody plants. Plenum Press, New York, pp 265–274

Dickmann DI, Stuart KW (1983) The culture of poplars in eastern North America. Michigan State University Press, East Lansing, Mich.

Dunlap JM (1991) Genetic variation in natural populations of *Populus trichocarpa* T.&G. from four river valleys in Washington. PhD thesis, University of Washington, Seattle, Wash.

Eckenwalder JE (1977) Systematics of *Populus* L. (Salicaceae) in southwestern North America with special reference to Sect. Aigeros Duby. PhD thesis, University of California, Berkeley, Calif.

Figliola AL (1986) Studies in the physiology, morphology, and anatomy of *Populus trichocarpa, Populus deltoides*, and their hybrids. MSc Thesis, University of Washington, Seattle, Wash.

Guries RP, Stettler RF (1976) Pre-fertilization barriers to hybridization in the poplars. Silvae Genet 25:37–44

Harlow WM, Harrar ES, White FM (1979) Textbook of dendrology. McGraw-Hill, New York

Heilman PE, Stettler RF (1985) Genetic variation and productivity of *Populus trichocarpa* T.&G. and its hybrids. II. Biomass production in a 4-year plantation. Can J For Res 15:384–388

Hinckley TM, Ceulemans R, Dunlap JM, Figliola A, Heilman PE, Isebrands JG, Scarascia-Mugnozza G, Schulte PJ, Smit B, Stettler RF, Van Volkenburgh E, Wiard BM (1989) Physiological, morphological, and anatomical components of hybrid vigor in *Populus*. In: Kreeb KH, Richter H, Hinckley TM (eds) Structural and functional responses to environmental stresses. SPB Academic Publ, The Hague, The Netherlands, pp 199–217

Keim P, Paige KN, Whitham TG, Lark KG (1989) Genetic analysis of an interspecific hybrid swarm of *Populus:* occurrence of unidirectional introgression. Genetics 123:557–565

Larsŏn PR, Isebrands JG (1971) The plastochron index as applied to developmental studies of cottonwood. Can J For Res 1:1–11

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Mirsky AE, Ris H (1949) Variable and constant components of chromosomes. Nature 163:666–669

Parsons TJ, Bradshaw HD, Gordon MP (1989) Systemic accumulation of specific mRNAs in response to wounding in poplar trees. Proc Natl Acad Sci USA 86:7895–7899

Ridge CR, Hinckley TM, Stettler RF, Van Volkenburgh E (1986) Leaf growth characteristics of fast growing poplar

hybrids *Populus trichocarpa* × *P. deltoides*. Tree Physiol 1: 209–216

Stebbins GL (1950) Variation and evolution in plants. Columbia University Press, New York

Stettler RF, Bawa KS (1971) Experimental induction of haploid parthenogenesis in black cottonwood (*Populus trichocarpa* T.&G. ex Hook). Silvae Genet 20: 15–25

Swanson CP (1957) Cytology and cytogenetics. Prentice-Hall, Englewood Cliffs, N.J.

van Buijtenen JP, Joranson PN, Einspahr DW (1958) Diploid versus triploid aspen as pulpwood sources with reference to growth, chemical, physical, and pulping differences. TAPPI 41:170–175

Zsuffa L (1975) A summary review of interspecific breeding in the genus *Populus*. In: Fowler DP, Yeatman CW (eds) Proc 14th Meeting Can Tree Improv Assoc, part 2. Fredericton, New Brunswick, pp 107–123