Theor Appl Genet (1994) 89:551–558

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Molecular genetics of growth and development in *Populus*. II. Segregation distortion due to genetic load

Received: 3 January 1994 / Accepted: 2 February 1994

Abstract Distortion of expected Mendelian segregation ratios, commonly observed in many plant taxa, has been detected in an experimental three-generation inbred pedigree of *Populus* founded by interspecific hybridization between *P. trichocarpa* and *P. deltoides*. An RFLP linkage map was constructed around a single locus showing severe skewing of segregation ratio against F_2 trees carrying the *P. trichocarpa* allele in homozygous form. Several hypotheses for the mechanism of segregation distortion at this locus were tested, including directional chromosome loss, segregation of a pollen lethal allele, conflicts between genetic factors that isolate the parental species, and inbreeding depression as a result of genetic load. Breeding experiments to produce inbred and outcrossed progenies were combined with PCR-based detection of RFLPs to follow the fate of the deficient allele throughout embryo and seedling development. A recessive lethal allele, *lth*, inherited from the *P. trichocarpa* parent, was found to be tightly linked to the RFLP marker locus *POP1054* and to cause embryo and seedling mortality. Heterozygotes (*lth*/+) appear to be phenotypically normal as embryos, seedlings, and young trees.

Key words Cottonwood · Inbreeding depression · Lethal equivalent

Abbreviations *RFLP* restriction fragment length polymorphism · *PCR* polymerase chain reaction · *STS* sequence-tagged site · *SDS* sodium dodecyl sulfate

Communicated by P. M. A. Tigerstedt

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Introduction

Deviations from expected Mendelian inheritance are intrinsically interesting because they reveal disturbances in the orderly transmission of genetic information from one generation to the next. Skewed segregation ratios at some loci have been found in most plant pedigrees when large numbers of markers are mapped. Among intraspecific crosses, segregation distortion has been observed at RFLP loci in maize (e.g., Edwards et al. 1987; Beavis and Grant 1991; Reiter et al. 1991), maize-teosinte hybrids (Doebley et al. 1990; Doebley and Stec 1991), lettuce (Landry et al. 1987), rice (McCouch et al. 1988), barley (Graner et al. 1991), loblolly pine (Devey et al. 1991), and common bean (Vallejos et al. 1992). Segregation distortion is generally more prevalent in the backcross or F_2 offspring of interspecific hybrids (Zamir and Tadmor 1986) and has been documented with RFLP markers in *Lycopersicon* (e.g., Bernatzky and Tanksley 1986; Weller et al. 1988; Paterson et al. 1988, 1991), *Solanum* (Bonierbale et al. 1988; Gebhardt et al. 1991), *Glycine* (Keim et al. 1990), *Hordeum* (Graner et al. 1991), and *Citrus* (Durham et al. 1992). Despite the seeming ubiquity of segregation distortion in plant pedigrees, and its implications for the genetic fate of natural and managed populations, very few explanations for the skewing have been given. The differences in life history (annual, perennial) and mating system (selfing, outcrossing) among plant taxa showing segregation distortion make it likely that many different mechanisms are capable of producing skewed inheritance ratios.

We have examined segregation distortion in a three-generation pedigree of *Populus*. Trees in the genus *Populus* include the poplars, aspens, and cottonwoods. Crossability within the genus is good (Zsuffa 1975: Stettler et al. 1980), and many interspecific hybrids display striking heterosis for growth (e.g., Zsuffa, 1975; Heilman and Stettler 1985). In the course of constructing a genetic linkage map for an interspecific *Populus* hybrid, we found a linkage group containing a cluster of several loci

showing severe segregation distortion against one homozygous class of genotypes in the F_2. Several mechanisms are capable of producing such a skewed distribution of genotypes, including: (1) chromosome loss, (2) presence of an allele for pollen lethality, (3) conflict between genetic isolating mechanisms evolved in the parental species, both genic and chromosomal, and (4) expression of genetic load *via* a lethal recessive allele. To distinguish among these possibilities, we examined inheritance patterns in the F_2 and BC_1 generations of our mapping pedigree and followed the fate of the "missing homozygotes" through embryo and seedling development in outcrossed and inbred F_2 offspring.

Materials and methods

Linkage mapping pedigree

We have produced an inbred three-generation *Populus* pedigree suitable for genetic mapping. The founding parents of the pedigree are a female black cottonwood (*Populus trichocarpa*; "T"; clone 93-968) and a male eastern cottonwood (*P. deltoides*; "D"; clone 59-129-17, also known as ILL-129). A controlled cross between the two was carried out in 1981 to produce the F_1 hybrid family 53 (T × D), consisting of 11 clones consecutively numbered from 238 through 248. In 1988, female F_1 clone 53-246 was used as the seed parent in a backcross to ILL-129 (BC_1 family 342; TD × D) and in an F_2 breeding to her male full-sibling 53-242 (F_2 family 331; TD × TD). The F_1, BC_1, and F_2 breedings were repeated in the spring of 1990, and a backcross between *P. trichocarpa* 93-968 and 53-242 (BC_1 family 354; T × TD) was added to make the breeding design symmetrical. A total of about 600 advanced-generation offspring is maintained in the seedling beds and clone banks at Washington State University Farm 5 in Puyallup, Washington.

Growth measurements of BC_1 and F_2 progeny in a replicated clonal trial

A replicated field trial (FT 7.23) was established at Farm 5 in 1991. This trial contains six ramets of each of 107 genotypes from families 331 (F_2) and 342 (BC_1), along with the parental *P. trichocarpa*, *P. deltoides*, and F_1 offspring 53-246 and 53-242. The trial was laid out in a modified randomized block design with 2-tree plots in each of three blocks. To minimize competition, the ramets of a given ortet were assigned to one of four size classes based on the ortet's 2-year height in the seedling bed. The four size classes were arranged in ascending and descending order perpendicularly to the three blocks. Plots were randomized within their block/size-class compartment. Unrooted cuttings 30 cm long were planted at a spacing of 1.5 m. The plantation was kept weed-free and during the first year was irrigated several times during summer. Heights and diameters were measured at the end of each of the two growing seasons.

Controlled crosses to study embryo and seedling development

In the spring of 1992, floral branches were collected from 53-246, 53-242, and two T × D hybrids from an unrelated family: 47-169 (female) and 47-165 (male). Both sib-matings (53-246 × 53-242 = F_2 family 331; 47-169 × 47-165 = F_2 family 359) and outcrosses (53-246 × 47-165 = F_2 family 329; 47-169 × 53-242) were performed in the greenhouse (Stettler and Bawa 1971). Two female branches were used for each cross, with at least three catkins pollinated on each branch. The cross 47-169 × 53-242 failed due to premature catkin abscission, but catkin, capsule, and seed development proceeded normally in the other crosses.

DNA extraction

Twenty-one days after pollination, individual embryos were removed from their ovules under a dissecting microscope, placed in a microfuge tube, and frozen at −70 °C prior to DNA extraction. Following seed maturation (6–8 weeks post-pollination), seeds were germinated on moist filter paper, harvested, and frozen. To extract DNA suitable for PCR, embryos and germinants were ground in microfuge tubes with a motor-driven disposable pestle (Kontes) in 50–200 µl of 10 m*M* Tris-Cl pH 7.9/0.3 *M* sucrose/1 m*M* EDTA. An equal volume of DNA extraction buffer (100 m*M* Tris-Cl pH 7.9/500 m*M* NaCl/20 m*M* EDTA/1% SDS/0.1% 2-mercaptoethanol) was added, followed by phenol-chloroform (1:1) extraction and ethanol precipitation. The resulting nucleic acid pellet was dissolved in 100 µl TE (10 m*M* Tris-Cl pH 7.9/1 m*M* EDTA), treated with 10 µg/ml RNase A (Sigma) for a minimum of 1 h at 37 °C, extracted with phenol/chloroform, and precipitated with 2.5 *M* ammonium acetate and ethanol. About 200 ng of DNA was obtained from each embryo and germinant of normal size. For RFLP mapping by Southern blot hybridization, DNA was extracted from young leaves and purified as previously described (Bradshaw and Stettler 1993).

RFLP probes and linkage mapping

Cloned RFLP probes, primarily random *Pst*I fragments of genomic DNA from *P. trichocarpa* 93-968, were radiolabelled and used in Southern blot hybridizations as previously described (Bradshaw and Stettler 1993). Linkage between nuclear markers was established by statistical tests for independent assortment; χ^2 ($P < 0.001$) was used for the BC_1 population and maximum likelihood (LOD > 3) for the F_2 (Ott 1985). Distance between markers was estimated using the Haldane mapping function (Haldane 1919). Markers were ordered by inspection in the BC_1 and by MAPMAKER (Lander et al. 1987) in the F_2, accepting the order with a likelihood at least 20 times greater than any alternative (95% confidence interval).

STS determination for locus *POP1054*

RFLP probe pPOP1054 is an approximately 600-bp *Pst*I fragment cloned from *P. trichocarpa* 93-968 into pBluescript (Stratagene). The ends of pPOP1054 were sequenced using the chain termination method (Sanger et al. 1977) and Sequenase (U.S. Biochemical) on double-stranded template prepared by alkaline lysis (Birnboim and Doly 1979).

PCR amplification of the *POP1054* locus

Primers suitable for amplification of the *POP1054* locus were chosen from the STS data with the help of OLIGO (Rychlik and Rhoads 1989). Amplifications were performed in 10-µl volumes containing 10–50 ng genomic DNA, 100 ng primer POP1054F (5′-CTACTGCC-GCTTCCGAATTAAG), 100 ng primer POP1054R (5′-TGCAG-TTGGAAACACTTACTCG), 200 µ*M* each of dATP, dGTP, dCTP, and TTP, 10 m*M* Tris-Cl pH 8.3/50 m*M* KCl/2 m*M* $MgCl_2$/0.001% gelatin, and 0.5 µ Amplitaq (Perkin-Elmer Cetus). PCR was done under mineral oil in a COY or MJ Research thermocycler, with 30 cycles of: 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 2 min. Allelic forms of the fragments amplified from the *POP1054* locus were distinguished by separation of *Alu*I digestion products on a 5% polyacrylamide gel in Tris-borate/EDTA (Maniatis et al. 1982).

Results and discussion

Segregation distortion in the F_2 of the *Populus* mapping pedigree

RFLP genotypes were determined at 229 loci in the F_2 generation of the mapping pedigree. All markers were

inherited in a codominant manner. For linkage mapping, genotypes at each RFLP locus were scored as TT (homozygous), TD (heterozygous), or DD (homozygous) so that only "interspecific" recombinations between chromosomes originating from the *P. trichocarpa* and *P. deltoides* parents were counted. The resulting segregation data were treated as if they had come from a cross between inbred parental lines, despite the fact that as many as 4 alleles sometimes could be seen to segregate at a single locus in the F_2. At most loci, 26 F_2 and 27 BC_1 offspring were genotyped by Southern blot analysis of RFLPs; at a few loci the RFLP analysis was extended to larger numbers of progeny following PCR amplification of the desired locus as described for *POP1054*.

In the F_2, deviation ($P < 0.01$) from the expected Mendelian 1:2:1 segregation ratio was observed at 14 of 229 loci (Table 1), far more often than the 2 or 3 loci expected by chance (assuming independent assortment). The probes revealing segregation distortion fall into five linkage groups and 3 unlinked loci. Summed over all loci (1335 TT:3556 TD:1621 DD), there is significant segregation distortion ($\chi^2 = 80.3$; $P < 0.0001$; $df = 2$), with an apparent deficiency of TT homozygotes and a surplus of TD heterozygotes.

In the BC_1 to the *P. deltoides* male parent, 8 of 87 loci showed significant ($P < 0.01$) segregation distortion (Table 1). The distorted loci are found in three different linkage groups, none of which are shared with those showing skewed segregation ratios in the F_2. At locus *POP1018* in linkage group E, there is marginal evidence for distortion in the BC_1 at a locus that is clearly distorted in the F_2. At all of the skewed loci there is an excess of heterozygotes, and summed over all loci (1333 TD:1126 DD) there is a highly significant excess of heterozygotes ($\chi^2 = 17.4$, $P < 0.0001$, $df = 1$).

Given the magnitude of segregation distortion in our *Populus* mapping pedigree and the possibility that different mechanisms of skewing could be operating at each locus, we chose a single locus as the focus of our subsequent efforts. The *POP1054* locus was chosen because of its central location in a large linkage group (see below), the successful development of PCR-based detection of RFLPs at this locus, and severe segregation distortion against the TT homozygous class of F_2 offspring. Of 64 F_2 trees, only 1 individual (vs. 16 expected) is a TT homozygote at *POP1054* (Table 1).

Possible mechanisms of segregation distortion

We considered four mechanisms capable of explaining the segregation distortion observed in the F_2 at the *POP1054* locus: preferential loss of the T chromosome carrying this locus, linkage to a gene lethal to male gametes, conflict between genetic isolating mechanisms in the two parental species, and expression of genetic load as a lethal recessive allele. We will first explain these four (possible) mechanisms and, in the subsequent section, examine their validity in light of our experimental data.

Table 1 Segregation distortion as measured by deviation (χ^2) from Mendelian ratios (1:2:1 for F_2 family 331; 1:1 for BC_1 family 342). The number of offspring in each genotype class is shown. (*nd* not determined)

Locus[a]	Linkage group[b]	F_2 genotypes[c]				BC_1 genotypes[c]		
		TT	TD	DD	χ^2	TD	DD	χ^2
1054	B	1	42	21	****	34	33	ns
1094	B	0	13	12	**	nd	nd	–
1196	B	0	13	11	**	nd	nd	–
1284	B	0	14	12	**	nd	nd	–
69	P	4	14	7	ns	21	6	**
754	P	11	33	18	ns	37	13	***
757	P	17	30	17	ns	44	17	***
834	P	nd	nd	nd	–	22	5	**
214	Y	8	10	6	ns	22	5	**
792	Y	5	14	6	ns	21	5	**
237	H	2	8	15	***	8	17	ns
1059	H	4	8	13	**	nd	nd	–
1077	H	1	8	11	**	nd	nd	–
1083	L	0	20	4	**	nd	nd	–
1093	L	1	19	3	**	nd	nd	–
771	–	3	19	4	ns	21	6	**
1018	E	12	33	3	**	19	7	*
1277	A	2	20	2	**	nd	nd	–
1079.3	–	14	40	3	**	nd	nd	–
1132	–	6	19	0	**	nd	nd	–
BSP	–	1	21	4	**	nd	nd	–

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; *ns* = $P > 0.05$

[a] Loci are designated by RFLP probe numbers

[b] Linkage group designations do not necessarily represent different chromosomes, but are unlinked to each other according to the criteria given in the text

[c] RFLP genotypes at each locus were classified as homozygous for the *P. trichocarpa* allele (TT), heterozygous (TD) or homozygous for the *P. deltoides* allele (DD)

Chromosome loss, such as the directional loss of *Hordeum bulbosum* chromosomes in hybrids with *H. vulgare* (Kasha and Kao 1970), could lead to the underrepresentation of some linkage groups in advanced generations of interspecific *Populus* hybrids. This hypothesis for segregation distortion predicts that similar skewing would be found at all loci in the same linkage group and that heterozygotes as well as one class of homozygotes would be underrepresented in the F_2.

The *P. trichocarpa* allele near *POP1054* could be lethal to male gametes in F_1 hybrid 53-242, either during gametogenesis or in the gametophyte itself. Such pollen lethals have been found in tobacco (Cameron and Moav 1957) and are known to lead to segregation distortion in rice (Lin et al. 1992). Acceptance of this hypothesis leads to the conclusion that the T allele at *POP1054* is never paternally transmitted unless it becomes dissociated from the pollen lethal by recombination (a rare event judging from the severity of segregation distortion in the F_2). Pollen lethality may be verified by observing a near-zero rate of paternal transmission of the *POP1054* T allele to the F_2 (if the paternal allele can be distinguished from the maternal T allele) or by the near-absence of TT homozygotes in the backcross to the female *P. trichocarpa* 93-968 (T × TD family 354). Both the TT homozygotes and TD heterozygotes should be found at half the expected frequency in the F_2.

It has been proposed that segregation distortion in wide crosses can result from the action of genetic isolating mechanisms that have arisen during speciation (Stephens 1949; Zamir and Tadmor 1986). These reproductive barriers may be genic or the result of structural differentiation between homologous chromosomes (Stebbins 1950; Grant 1971). In *Solanum*, linkage to the self-incompatibility locus is responsible for some of the observed segregation distortion (Gebhardt et al. 1991). Barriers to gene flow are known to exist in *Populus* and may be pre- or postzygotic. Prezygotic intersterility between *Populus* species is often the result of pollen-style interactions (Guries and Stettler 1976; Stettler et al. 1980). Postzygotic isolating mechanisms in *Populus* include hybrid breakdown (Johnson 1947) and embryo abortion (Melchior and Seitz 1968). Unknown mechanisms are responsible for asymmetric introgression in hybrid swarms of *Populus* species found in natural ecosystems (Keim et al. 1989; Paige et al. 1991). If the segregation distortion at *POP1054* is the result of genetic isolation between *P. trichocarpa* and *P. deltoides*, we would expect that many or all TD × TD F_2 crosses would show similar distortion at this locus, independent of inbreeding.

The low frequency of TT homozygotes at *POP1054* in the F_2 could be due to expression of genetic load. Under this hypothesis, the female *P. trichocarpa* 93-968 is presumed to be heterozygous (*lth*/+) for a lethal recessive allele near the *POP1054* locus and to have transmitted the lethal allele (*lth*) to the two F_1 offspring used to produce the F_2. Genetic loads tend to be high in outcrossing perennial plants (Klekowski 1988) and have been proposed as a potential cause of segregation distortion in trees (Sorensen 1969). This model for segregation distortion predicts that there should be normal Mendelian segregation at *POP1054* in the backcross to *P. deltoides* (family 342), outcrossed F_2 progeny, and most inbred F_2 pedigrees not having *P. trichocarpa* 93-968 as a parent (assuming the *lth* allele is rare in the *P. trichocarpa* population). The backcross to 93-968 should segregate 1 TT:2 TD at *POP1054*, since half the expected number of TT homozygotes will inherit two *lth* alleles and thus fail to survive. We have also considered the possibility that the lethal phenotype may not be expressed until appreciable embryo or seedling development has taken place. The ovule may be capable of supporting an embryo that cannot survive as a plant. Embryo lethals and defectives are very frequently found in chemical mutagenesis experiments with such model plants as *Arabidopsis* (Patton et al. 1991).

Linkage data show that chromosome loss cannot account for the observed segregation distortion at *POP1054*

A linkage group from the *Populus* genome was established based on the segregation of anonymous RFLPs in the F_2 of our mapping pedigree (Fig. 1). *POP1054*, a locus showing severe segregation distortion against the TT homozygous class of genotypes (Table 1), is near the center of the linkage group. *POP1054* is flanked on one side by markers with no statistically significant segregation distortion: *POP869* (9 TT:34 TD:20 DD; $\chi^2 = 4.2$; $P > 0.1$; $df = 2$) and *POP1123* (12 TT:37 TD:20 DD; $\chi^2 = 2.2$; $P > 0.2$; $df = 2$). Since not all markers on the same chromosome are equally underrepresented in the F_2, we conclude that simple loss of entire chromosomes cannot account for the segregation distortion observed at *POP1054* in the F_2. Further, the number of heterozygotes is larger than expected under Mendelian segregation, rather than smaller as predicted by the "chromosome loss" hypothesis.

The most distal marker on the other side of *POP1054*, *POP1074* (Fig. 1), shows some distortion (1 TT:14 TD:12 DD; $\chi^2 = 9.0$; $P < 0.05$; $df = 2$). We do not know if *POP1074* is telomeric; however, it is possible that there are yet further distal markers in this linkage group but that we have not detected linkage because of segregation distortion. In our mapping we routinely use a panel of 26 F_2 offspring to establish the approximate map position of a marker. In the absence of segregation distortion, linkage can be detected at a distance of about 20 cM (LOD > 3). Segregation distortion reduces the effective "range" of each marker as the observed two-point allele frequencies approach the expectation under the null hypothesis of no linkage. In theory, despite the difficulty of establishing linkage when skewed segregation reduces

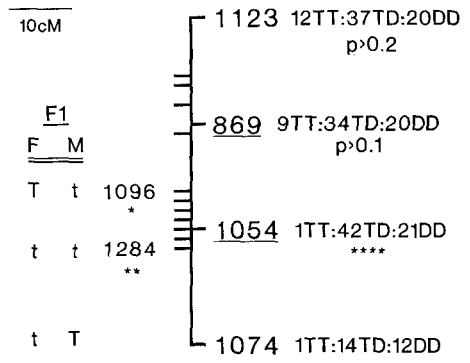

Fig. 1 Linkage group B containing *POP1054*, showing that not all linked loci are equally distorted in F_2 family 331. The *vertical line* represents the linkage group, with a *horizontal bar* for each locus in the linkage group. For clarity, only 6 of the 15 linked loci are *numbered*. Segregation ratios in F_2 family 331 are given for 4 loci, and probability of Mendelian segregation is given for all 6 numbered loci (see Table 1). At 3 loci (*POP1096*, *POP1284*, and *POP1074*) the maternal *P. trichocarpa* parent is heterozygous; the alleles inherited by her F_1 offspring 53-246 (*F*) and 53-242 (*M*) are shown. Though knowledge of linkage phase is implied by the notation, the low number of known F_1 genotypes precludes discrimination between the model shown (one crossover in each of the F_1 offspring) and an alternative model (two crossovers in one of the F_1 offspring). The key point is that both F_1s have inherited the same allele at *POP1284*, which is close to the very distorted *POP1054*. The *underlined loci* have been converted to detection of RFLPs following PCR

the number of fully informative meioses, segregation distortion does not alter the estimate of recombination fraction (although the precision of the estimate is reduced if the number of informative meioses is lowered, a situation which may occur in an F_2 but generally not in a backcross). For example, if all individuals in the pedigree had the DD genotype at 2 linked loci, linkage could not be demonstrated (LOD = 0) but the estimate of recombination fraction ($r = 0$) would be accurate. We have observed this effect experimentally in BC_1 family 342. In this family of the mapping pedigree, there is no segregation distortion at either *POP1054* (34 TD:33 DD; $\chi^2 = 0.01$; $P > 0.9$; $df = 1$) or *POP869* (31 TD:36 DD; $\chi^2 = 0.37$; $P > 0.5$; $df = 1$). In spite of the difference in segregation distortion between the BC_1 and F_2 families (Table 1), the estimated Haldane map distance between *POP1054* and *POP869* in the BC_1 and F_2 families is identical at 35 cM.

The presence of TT homozygotes in the backcross to *P. trichocarpa* 93-968 rules out pollen lethality as the cause of segregation distortion at *POP1054*

We performed two breeding experiments to test the hypothesis that the T allele at *POP1054* is tightly linked to a pollen lethal. One experiment involved the production of an outcrossed F_2 population derived from 53-242, the F_1 hybrid male that must carry the pollen lethal if it exists. Unfortunately, this cross failed (see Materials and methods). The backcross between 53-242 and its female *P. trichocarpa* parent 93-968 was successful, however, and produced 8 TT homozygotes and 23 TD heterozygotes. Since any TT homozygotes in the BC_1 must have received a T allele from each parent, transmission of the T allele via the F_1 pollen parent eliminates the possibility that pollen lethality is the cause of segregation distortion at the *POP1054* locus. A reciprocal backcross using the F_1 as a female parent and *P. trichocarpa* 93-968 as the pollen donor is not possible because most *Populus* are dioecious.

Outcrossed F_2 progeny have undistorted segregation ratios, making genetic isolation unlikely as an explanation for skewed segregation at *POP1054*

To test the hypothesis that genic or chromosome structural differentiation is responsible for the segregation distortion at *POP1054*, we produced outcrossed and inbred F_2 progenies from two unrelated F_1 families (see Materials and methods). If the putative isolating mechanisms are due to alleles fixed in the parental species, segregation distortion should be independent of inbreeding. Both inbred F_2 families showed inbreeding depression as measured by seed germination rate 40 h after sowing: family 331 had a rate of 80% and family 359 had a rate of 71% ($n = 200$ for each family). In contrast, outcrossed F_2 family 329 had a seed germination rate of 99% ($n = 200$). The germinants from family 331 were divided into two classes based on germination speed. The "normal" class germinated overnight, the cotyledons expanded quickly and shed the seed coat, and subsequent root elongation was rapid. The "slow" class of germinants emerged a day later, the seed coat adhered to the cotyledons for several days, and root growth was very slow. Among normal germinants in family 331, the segregation ratio at *POP1054* was distorted against the TT homozygous class (5 TT: 53 TD:35 DD; $\chi^2 = 21.2$; $P < 0.0001$; $df = 2$); among slow germinants the ratio was distorted in favor of TT homozygotes (6 TT:1 TD:0 DD; $\chi^2 = 13.9$; $P < 0.001$; $df = 2$). If all the segregation data are pooled for family 331 germinants, the distortion is still significant (11 TT:53 TD:35 DD; $\chi^2 = 12.1$; $P < 0.01$; $df = 2$). No distinct classes of germinants were noticed in the other inbred F_2 family (359), where the segregation ratio was undistorted at *POP1054* (15 TT:33 TD:20 DD; $\chi^2 = 0.80$; $P > 0.5$; $df = 2$). The F_2 germinants in outcrossed family 329 showed no skewed segregation at *POP1054* with a ratio of 21 TT:38 TD:25 DD ($\chi^2 = 1.1$; $P > 0.5$; $df = 2$). While we cannot formally eliminate the possibility that the segregation distortion in F_2 family 331 is due to a conflict between genetic isolating mechanisms in the parents of the mapping pedigree, the presence of two phenotypic classes of germinants with different distributions of the TT homozygous genotype and the absence of distortion in unrelated inbred and half-sib outcrossed F_2 families suggest a model of distortion due to segregation of a lethal recessive allele.

Segregation distortion at locus *POP1054* in the mapping pedigree is due to a tightly linked recessive allele lethal to TT homozygous offspring

As each of the three previous hypotheses for segregation distortion was tested experimentally, evidence accumulated for the cosegregation of *POP1054* and a lethal recessive allele (*lth*) transmitted from *P. trichocarpa* 93-968 to her F_1 offspring 53-246 and 53-242. Linkage analysis of RFLP data revealed that 93-968 is heterozygous at *POP1284*, which is tightly linked (5.3 cM) to *POP1054*, and that both 53-246 and 53-242 inherited the same *P. trichocarpa* allele at this locus (Fig. 1). Backcrossing 53-242 to 93-968 gave 8 TT homozygotes (presumably all *lth*/+) and 23 TD heterozygotes (a 1:1 mixture of *lth*/+ and +/+) at *POP1054*, significantly skewed from the 1 TT:1 DD segregation expected under normal Mendelian assumptions ($\chi^2 = 7.3$; $P < 0.01$; $df = 1$), but not significantly different from the 1 TT:2 TD segregation ratio expected if 53-242 shares a linked lethal recessive allele with 93-968 ($\chi^2 = 0.79$; $P > 0.2$; $df = 1$). Segregation distortion at *POP1054* is a direct consequence of inbreeding in a specific family, and a subvital class of germinants in the F_2 was almost entirely (6/7) made up of presumptive *lth*/*lth* homozygotes.

We sought to explore further the developmental fate of the putative *lth*/*lth* homozygous class of offspring in F_2 family 331 by taking a census of embryo genotypes at *POP1054*. Embryos were dissected from their ovules 21 days after pollination. It was immediately apparent that the embryos fell into two classes of size and stage of maturity. The largest frequency class consisted of normal cotyledon-stage embryos. Among these embryos, the now-familiar distorted segregation ratio at *POP1054* was 3 TT:40 TD:28 DD. The second class of embryos was made up of very small embryos, delayed in development at the heart stage and devoid of cotyledons. Among the heart-stage embryos, the segregation ratio was 10 TT:3 TD:1 DD. Clearly, the defective embryos are overwhelmingly TT, implying that *POP1054* is linked to a gene with a T allele (*lth*) having an adverse effect on embryo development.

Even if both classes of embryos are taken together, there is a slight deficiency in the TT homozygous class of F_2 offspring at *POP1054* (13 TT:43 TD:29 DD; $\chi^2 = 6.0$; $P < 0.05$; $df = 2$). It would seem that the *lth*/*lth* genotype weakens the zygote and that all subsequent development is affected. The ability of the ovules to support a relatively high proportion of defective embryos is remarkable, given that there must be competition for nutrients among embryos in the same capsule. The few *lth*/*lth* homozygotes that survive seed development and germinate succumb quickly even without competition from neighboring normal seedlings. The single TT offspring (of 64) at *POP1054* that has survived as a tree is very likely to be a recombinant between *POP1054* and *lth*.

The *lth* allele appears to be completely recessive to the normal allele inherited from the *P. deltoides* parent since there is no deficiency in the number of *lth*/+ progeny (heterozygotes at *POP1054*) in the BC_1 family 342 (Table 1). There is no negative effect of the *lth* genotype on growth as a tree in a replicated clonal field trial. Among replicated F_2 family 331 offspring heterozygous at *POP1054* (presumably *lth*/+), the mean height growth after 2 years is 381 ± 85 cm ($n = 40$), while for DD homozygotes (presumably +/+) the corresponding value is 343 ± 90 cm ($n = 20$). Likewise, there is no difference in 2-year height growth performance between BC_1 family 342 trees heterozygous (322 ± 89 cm; $n = 33$) or homozygous (315 ± 73 cm; $n = 33$) at the *POP1054* locus. The *lth* allele near *POP1054* thus appears to be a deleterious recessive component of the genetic load carried by *P. trichocarpa* 93-968.

It is possible that the seedling lethal (*lth*) allele linked to *POP1054* can account for all of the inbreeding depression observed in seed set, embryo size, and germination speed in F_2 family 331. The overall expected mortality in the F_2 from homozygosity at the *lth* locus is 25%. The failure of 20% of the seeds to germinate accounts for the bulk of the skewing, and this figure is augmented by the near-complete loss of the 11% (11/99) of germinants with a TT genotype at *POP1054*. Most (10/14) of the underdeveloped embryos we tested have the TT genotype at *POP1054*. The number of lethal equivalents per zygote (Morton et al. 1956) segregating in F_2 family 331 may be estimated based on seed germination rate (Sorensen 1969) as:

$$2B = -8 \ln R,$$

where B is the number of lethal equivalents per gamete (hence $2B$ is the number of lethal equivalents per zygote) and R is the proportion of seeds germinating from a full-sib mating ($F = 0.25$) versus those germinating in an outcross ($F = 0$). Substituting the seed germination rate for family 331 relative to the outcrossed F_2 family 329 ($0.81 = 0.80/0.99$), we calculate the number of lethal equivalents per zygote as 1.7. This value is near the center of the range estimated for *Salix viminalis* (Kang et al. 1992), which, along with *Populus*, is in the family Salicaceae. Though our estimate of lethal equivalents per zygote is approximately two, there are more than two chromosomal regions showing segregation distortion against homozygous trees in F_2 family 331 (Table 1). Whether distortion at these unlinked loci is due to genetic load acting at developmental stages earlier or later than we found for *lth*, or is the result of other mechanisms such as those we have tested by analysis of the *POP1054* locus, remains to be discovered.

Concluding remarks

The excess of heterozygotes in both the F_2 and BC_1 generations of our mapping pedigree parallels the situation commonly observed in natural populations of forest trees (reviewed in Ledig 1986). Whether this excess is due to heterozygote advantage or inbreeding depres-

sion among homozygotes is not known in either case. Most forest tree species, however, are neither highly inbred (Hamrick et al. 1979) nor the product of interspecific hybridization. Results derived from our experimental populations of *Populus* should be tested for validity in natural populations of trees before being applied generally. In spite of this caveat, the widespread occurrence of segregation distortion in plants calls into question some of the assumptions (Mendelian segregation, Hardy-Weinberg equilibrium) that are made to facilitate the statistical analysis of tree populations in genetic improvement programs and in natural stands.

Among forest trees, segregation distortion has been observed in many gymnosperms with the aid of allozyme data derived from haploid megagametophyte tissue (e.g., Strauss and Conkle 1986; Cheliak et al. 1987; Adams et al. 1990). Skewed segregation among angiosperm trees is known from several studies using allozymes as markers (e.g., Gillet and Gregorius 1992 and references therein). Although mechanisms capable of producing such distortion have been proposed (Sorensen 1967; Gillet and Gregorius 1992), no conclusive evidence for any model has been put forward until now.

Acknowledgements We wish to thank the following members of the UW/WSU Poplar Research Group: Milt Gordon for providing laboratory space and facilities to carry out the RFLP mapping; Paul Heilman for supplying the pedigreed tree cuttings; Brian Watson and Kevin Otto for technical expertise in determining RFLP genotypes, Rongling Wu, Paula Glackin, and Diane Fogle for tree growth measurements, Doug Ewing for his help in maintaining pedigreed trees in the greenhouse, and the Howard Hughes Medical Institute Chemical Synthesis Facility at the University of Washington for oligonucleotide synthesis. This work was supported by grants from the Washington Technology Centers (09-1022), the USDA NRICGP (91-37300-6458), the US Department of Energy (19X-43382C), Boise Cascade Corporation, and James River Corporation.

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