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Genetic causes of heterosis in juvenile aspen: a quantitative comparison across intra- and inter-specific hybrids

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Abstract The genetic causes of heterosis in tree growth were investigated by a comparative genetic analysis of intra- and inter-specific crosses derived from *Populus tremuloides* and *P. tremula*. A new analytical method was developed to estimate the effective number of loci affecting a quantitative trait and the magnitudes of their additive and dominant effects across loci. The method combines the assumption of multiple alleles, as frequently found in outcrossing species, and the family structure analysis at different hierarchical levels. During the first 3 years of growth, interspecific hybrids displayed strong heterosis in stem growth, especially volume index, over intraspecific hybrids. By a series of joint analyses on the combining ability and the genetic component, we found that F_1 heterosis might be due to overdominant interaction between two alleles, one from the *P. tremuloides* parent and the other from the *P. tremula* parent, at the same loci. This inference was derived from the finding that heterozygotes, newly formed through species combination, showed much greater growth than the heterozygotes from intraspecifc crosses at a reference locus. Heterosis in aspen growth appeared to be under multigenic control, with a slightly larger number of loci for stem diameter and volume (9–10) than for height (6–8). For traits with non-significant heterosis, such as stem allometry and internode number and length, the number of underlying loci seemed to be much fewer (3–4). While additive effects appeared to influence seedling traits collectively across loci, a few major dominant loci had much larger effects on stem growth.

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Introduction

The importance of interspecific hybridization in the genetic improvement of *Populus* has been demonstrated by significant and stable heterosis, or hybrid vigor, expressed in F_1 hybrids (Heimburger 1936, 1968; Zsuffa 1975; Stettler et al. 1988; Wu et al. 1992; Li et al. 1993). The term, heterosis, first proposed by G.H. Schull (see Hayes 1952), is usually described in terms of the superiority of F_1 hybrids over the mid-parent value (average between the two parents) or over the better parent. Since growth superiority was occasionally observed in F_1 hybrids between *P. deltoides* and *P. nigra* in the 18th century (Muhle-Larsen 1970), efforts to pursue the artificial hybridization of *Populus* have been made (Zsuffa 1975) and have contributed significantly to operational forestry throughout all the world (Zobel and Talbert 1984). In the Pacific Northwest, F_1 hybrids between *P. trichocarpa* and *P. deltoides*, pioneered by R. F. Stettler at the University of Washington (Stettler 1968), showed more than twice the productivity over their native female parent, *P. trichocarpa* (Heilman and Stettler 1985; Abelson 1991). By crossing *P. deltoides* and *P. simonii*, which is native to northern China, scientists were able to select superior F_1 genotypes that have much better rooting capacity than the fast-growing *P. deltoides* parent (Wu et al. 1992). These selected genotypes can typically adapt to the drought environments of northern and northwestern China. Strong heterosis has also been demonstrated in aspen hybrids between *P. tremuloides* and *P. tremula* (Heimburger 1936, 1968; Melchior and Seitz 1966; Zufa 1969; Einspahr 1984; Li et al. 1993). Cross-breeding strategies have been implemented for the long-term improvement of aspens in the Lake States (Li and Wyckoff 1991) and Canada (Li 1995).

Although F_1 heterosis is commonly observed in *Populus*, little information is available on the genetic

basis underlying this phenomenon. Two major hypotheses have been promulgated to explain heterosis (Crow 1952): the dominance hypothesis and the overdominance hypothesis. The dominance hypothesis, proposed by Davenport (1908), Bruce (1910) and Keeble and Pellew (1910), supposes that heterosis is due to the suppression of deleterious recessives from one parent by dominant alleles from the other parent in the heterozygous F_1. The overdominance hypothesis, proposed by Shull (1908) and East (1936), assumes that a heterozygous combination of the alleles at a single locus is superior to either of the homozygous combinations of the alleles at that locus. Evidence for these two hypotheses has been recently observed in many plants through a molecular genetic approach (Stuber et al. 1992; Bradshaw and Stettler 1995; Mitchell-Olds 1995; Xiao et al. 1995). However, heterosis can also be due to non-allelic interactions between different genetic loci. Jinks and Jones (1958) first included epistasis into their quantitative genetic models dealing with heterosis. By means of a two-locus diallelic model, Minvielle (1987) later showed that heterosis might stem from multiplicative epistatic interactions rather than dominance effects. The contribution of multiplicative accumulation to heterosis in a complex trait has been investigated both theoretically and empirically (Wright 1922; Dempster 1942; Richey 1942; Griffing 1990; Schnell and Cockerham 1992).

However, all current quantitative resolutions of the genetic basis for heterosis rely on the use of advanced generations initiated with inbred lines, which are difficult to obtain in outcrossing, long-lived forest trees, such as apsen. In the present study, a novel analytical method is proposed to explore the genetic causes of heterosis through the partitioning of F_1 family structure from a modified mating design. The study includes two parts: the first focuses on the effect of dominance on heterosis and the second on the epistatic components of heterosis. The study materials used here are derived from a factorial mating design between two aspen species, *P. tremuloides* and *P. tremula*, including both intra- and inter-specifc crosses. In the present paper, only the results of the first part are reported.

Materials and methods

Mating design

In 1993, intra- and inter-specific crosses were made among eight *P. tremuloides* (*T*1–*T*8) and eight *P. tremula* (*Ta*1–*Ta*8) parents from the Aspen Genetics Program at the University of Minnesota. Each of these two species had four male parents (denoted by 1–4) and four female parents (denoted by 5–8) (Table 1). The *T* parents originated from the Upper Peninsula of Michigan and northern Wisconsin, and the *Ta* parents from East Prussia and Germany. All these parents were randomly selected from the current aspen breeding population which is assumed to be panmictic. The entire 8 × 8 factorial mating design was intended to include four mating subsets each on a 4 × 4 scheme, two intra- (*T* × *T* and *Ta* × *Ta*) and two inter-specific crosses (*T* × *Ta* and *Ta* × *T*), as described in Table 1. However, because of limited flowers in the *Ta* parents, two families were missing in *T* × *Ta* and seven in *Ta* × *T*. Additionally in *Ta* × *Ta*, only one family was obtained (Table 1).

Nursery and field assessments

In the late spring of 1993, seeds were germinated in mini-plug containers filled with peat and then transplanted into 15-cm^3 styroblock containers in the greenhouse. These transplants were subsequently grown in a nursery in a randomized complete block design, with ten replicates and 11 seedlings per family in each replicate, at Grand Rapids, Minnesota (47°14′ N, 93°31′ W). Seedlings were watered and fertilized as needed during the growing period. Six seedlings per family were randomly chosen from six replicates to measure growth and morphological traits. The number of green leaves on the stem (LVS#) was counted near the end of growing season in September. Seedling height (HT), root collar diameter (DIA), and the number of internods (NOD#) on the stem were measured at the end of the growing season. In May 1994, 60 1-year-old seedlings from each family were transplanted to a test field near Rhinelander, Wisconsin (45°40′ N, 89°25′ W). This experimental plantation was established in a randomized complete block design with ten replicates and six seedlings per family in each replicate at a

Table 1 The factorial mating design used for intra- and inter-specific crosses among *P. tremuloides* (*T*) and *P. tremula* (*Ta*), which is composed of four subsets, i.e., *T* × *T*, *T* × *Ta*, *Ta* × *T*, and *Ta* × *Ta*, each with female (*Fgca*) and male general combining abilities (*Mgca*) as indicated. Missing cells are denoted by dashes

Female parents	Male parents									
	T					*Ta*				
	*T*1	*T*2	*T*3	*T*4	*Fgca*	*Ta*1	*Ta*2	*Ta*3	*Ta*4	*Fgca*
T	*T* × *T*					*T* × *Ta*				
*T*5	×	×	×	×	*t*5	×	×	×	×	*t*5′
*T*6	×	×	×	×	*t*6	×	×	×	×	*t*6′
*T*7	×	×	×	×	*t*7	×	×	×	×	*t*7′
*T*8	×	×	×	×	*t*8	×	–	×	–	*t*8′
Mgca	*t*1	*t*2	*t*3	*t*4		*ta*1	*ta*2	*ta*3	*ta*4	
Ta	*Ta* × *T*					*Ta* × *Ta*				
*Ta*5	×	×	×	×	*ta*5	–	–	–	–	*ta*5′
*Ta*6	–	×	×	–	*ta*6	×	–	–	–	*ta*6′
*Ta*7	×	×	×	–	*ta*7	–	–	–	–	*ta*7′
*Ta*8	–	–	–	–	*ta*8	–	–	–	–	*ta*8′
Mgca	*t*1′	*t*2′	*t*3′	*t*4′		*ta*1′	*ta*2′	*ta*3′	*ta*4′	

2.5 × 2.5 m spacing. At the end of each of the first 2 years in the field, total stem height and diameter were measured for each seedling. The volume index (VOL) was calculated as HT × (DIA)^2. Stem allometry was described by the HT:DIA ratio. In the nursery stage, mean internode length (MIL) on the stem was estimated by dividing height by internode number.

Data analyses

The relative difference between inter- and intra-specific crosses was used to estimate the hybrid vigor for a quantitative trait. It was described as:

$$HV=\frac{\mu_{T\times Ta}(\text{or }\mu_{Ta\times T})-\frac{1}{2}(\mu_{T\times T}+\mu_{Ta\times Ta})}{\frac{1}{2}(\mu_{T\times T}+\mu_{Ta\times Ta})}\times 100,$$

where $\mu_{T\times Ta}(\text{or }\mu_{Ta\times T})$ is the overall mean of progenies from interspecific crosses, $T\times Ta$ (or $Ta\times T$), and $\mu_{T\times T}$ (or $\mu_{Ta\times Ta}$) is the overall mean of progenies from intraspecific crosses, $T\times T$ (or $Ta\times Ta$). Because of a problem in adaptation to the day-length regime, pure *Ta* trees, which originated from high-latitude Europe, grow poorly in the Lake States (Pauley et al. 1963a; Li 1995). Therefore, heterosis is expressed as F_1 superiority relative to the better *T* parent, that is:

$$HV=\frac{\mu_{T\times Ta}\ (\text{or }\mu_{Ta\times T})-\mu_{T\times T}}{\mu_{T\times T}}\times 100.$$

The significance of the hybrid vigor calculated on the better parent *T* was further tested with a *t*-statistic.

Three statistical models were used to analyze the data. The first is a "full" model for the entire 8 × 8 factorial mating design, which detects the effects due to family, replicate, and their interaction:

$$Y_{ijk}=\mu+FAM_i+R_j+(FAM\times R)_{ij}+E_{ijk},$$

where Y_{ijk} is the observed value of the *k*-th seedling grown in the *j*-th replicate from the *i*-th family, μ is the overall mean, FAM_i is the effect of the *i*-th family, R_j is the effect of the *j*-th replicate, $(FAM\times R)_{ij}$ is the effect of interaction between the *i*-th family and the *j*-th replicate, and E_{ijk} is the residual effect. The family effects were partitioned into the effects due to the combining ability of the female and male parents:

$$FAM_i=Fgca_u+Mgca_v+(F\times M)_{uv},$$

where $Fgca_u$ and $Mgca_v$ are the effects due to the general combining ability (GCA) of the *u*-th female and *v*-th male parent, respectively, and $(F\times M)_{uv}$ is the effect due to the specific combining ability (SCA) of the *u*-th female and *v*-th male parent. The effects due to GCA and SCA were further partitioned at both the species and the individual-within-species level:

$$Fgca_u=FS_r+(FI/FS)_{s/r}$$
$$Mgca_v=MS_{r'}+(MI/MS)_{s'/r'}$$
$$(F\times M)_{uv}=(FS\times MS)_{rr'}+(FS\times MI/MS)_{r(s'/r')}+(FI/FS\times MS)_{(s/r)r'}+(FI/FS\times MI/MS)_{(s/r)(s'/r')},$$

where FS_r and $MS_{r'}$ are the effects due to the GCA of the *r*-th female- and *r'*-th male-parent species, respectively, and $(FI/FS)_{s/r}$ and $(MI/MS)_{s'/r'}$ are the effects due to the GCA of the *s*-th and *s'*-th individual within the *r*-th female- and *r'*-th male-parent species, respectively; $(FS\times MS)_{rr'}$ is the effect due to the SCA of the *r*-th female and *r'*-th male parent species, $(FS\times MI/MS)_{r(s'/r')}$ is the effect due to the SCA of the *r*-th female-parent species and the *s'*-th individual within the *r'*-th male-parent species, $(FI/FS\times MS)_{(s/r)r'}$ is the effect due to the SCA of the *s*-th individual within the *r*-th female-parent species and the *r'*-th male-parent species, and $(FI/FS\times MI/MS)_{(s/r)(s'/r')}$ is the effect due to the SCA of the *s*-th individual within the *r*-th female-parent species and the *s'*-th individual within the *r'*-th male-parent species. The corresponding partitioning was also carried out for the interaction effects with replicates:

$$(FAM\times R)_{ij}=(Fgca\times R)_{uj}+(Mgca\times R)_{vj}+(F\times M\times R)_{uvj}$$
$$(Fgca\times R)_{uj}=(FS\times R)_{rj}+(FI/FS\times R)_{(s/r)j},$$
$$(Mgca\times R)_{vj}=(MS\times R)_{r'j}+(MI/MS\times R)_{(s'/r')j}$$
$$(F\times M\times R)_{uvj}=(FS\times MS\times R)_{rr'j}+(FS\times MI/MS\times R)_{r(s'/r')j}+(FI/FS\times MS\times R)_{(s/r)r'j}+(FI/FS\times MI/MS\times R)_{(s/r)(s'/r')j}.$$

The second model is a "reduced" model, which was used to analyze the data from the four mating subsets separately. This model can detect the effects due to the GCA of the female and male parents, the SCA of female × male parents, the replicate, and the interaction between the combining abilities and replicates in each mating subset. In the case of interspecific crosses, the reciprocal effect at the species level was also ruled out, i.e.,

$$Y_{ijkl}=\mu+C_i+(FAM/C)_{j/i}+R_k+(C\times R)_{ik}+(FAM/C\times R)_{(j/i)k}+E_{ijkl}$$
$$(FAM/C)_{j/i}=Fgca_{u/i}+Mgca_{v/i}+(F\times M)_{(uv)/i}$$
$$(FAM/C\times R)_{(j/i)k}=(Fgca\times R)_{(u/i)k}+(Mgca\times R)_{(v/i)k}+(F\times M\times R)_{[(uv)/i]k},$$

where Y_{ijkl} is the observed value of the *l*-th seedling grown in the *k*-th replicate from the *j*-th family of the *i*-th species combination, μ is the overall mean, C_i is the effect of the *i*-th species combination, i.e., the reciprocal effect at the species level, $(FAM/C)_{j/i}$ is the effect of the *j*-th family within the *i*-th species combination, R_k is the effect of the *k*-th replicate, E_{ijkl} is the residual effect; $Fgca_{u/i}$ and $Mgca_{v/i}$ are the effects due to the GCA of the *u*-th female and *v*-th male parent within the *i*-th species combination, respectively, and $(F\times M)_{(uv)/i}$ is the effect due to the SCA of the *u*-th female and *v*-th male parent within the *i*-th species combination; the other terms are the corresponding interaction effects between the combining abilities and replicates. In the present study, the combining ability analysis could not be performed for $Ta\times Ta$ due to only a single family being included in that cross.

All effects in the two models were considered to be random. The approximate *F*-values were calculated from the expected mean squares. Variance components accounted for by all sources were estimated by equating the mean squares to their expected values (SAS PROC GLM, SAS Institute 1988).

The third model is an analysis of covariance model, which was used to calculate four types of covariances, i.e., those in the general combining ability effect of the female parent between $T\times T$ and $T\times Ta$ (t_j vs t'_j, $j=5,6,7,8$), in the general combining ability effect of the male parent between $T\times T$ and $Ta\times T$ (t_j vs t'_j, $j=1,2,3,4$), in the general combining ability effect of the female parent between $Ta\times Ta$ and $Ta\times T$ (ta'_j vs ta_j, $j=5,6,7,8$), and in the general combining ability effect of the male parent between $Ta\times Ta$ and $T\times Ta$ (ta'_j vs ta_j, $j=1,2,3,4$) (Table 1). However, in this study, the last two covariances could not be estimated because most families of the $Ta\times Ta$ cross were missing.

Genetic models

A fundamental assumption for current quantitative genetic analysis is that the same allele system is present between populations of the female and male parents (Falconer 1989). However, this assumption is largely frustrated when the parental populations have different alleles, as commonly observed in outcrossing species (e.g.,Groover et al. 1994; Leonards-Schippers et al. 1994; van Eck et al. 1994). Here, a new analytical method, relaxing this assumption, will be developed to examine the progenies of two outcrossing *Populus* species, *P. tremuloides* and *P. tremula*. If the two species possess a completely different allele system, at least four alleles can be identified at each locus. Given a possible evolutionary relatedness between the two species (both are from the section *Leuce* of the genus), however, we can assume that

there is a recessive at each locus which is identical by descent for both species (see van Eck et al. 1994). Here, a total of three different alleles at each locus are assumed to exist between the populations of the two species. Let us consider a specific locus at which two alleles, A (dominant) and a (recessive), are in the population of *P. tremuloides* (T) and two alleles, A' (dominant) and a (recessive), are in the population of *P. tremula* (Ta). Thus, there are three different genotypes, AA, Aa, and aa, with assumed frequencies of p^2, $2pq$, and q^2, in the T population. The values for these three genotypes are assigned a_1, d, and a_2, respectively. Similarly, three genotypes, $A'A'$, $A'a$, and aa, in the Ta population are assumed to have the values of a'_1, d', and a_2 and frequencies of $p'^2, 2p'q'$, and q'^2. When the individuals from the T population are crossed with those from the Ta population, a new genotype, AA', is formed whose genotypic value is denoted D. Based on mating frequencies and the mean genotypic values of the progenies per family, the analysis of family structure can be carried out at different hierarchical levels. However, owing to the complexity of derivation, it is assumed in this paper that the frequencies of the three genotypes are 1/4, 1/2, and 1/4, respectively, in either population. (The influence of allele frequency on heterosis is analyzed elsewhere.) Given this assumption, the mean of all hybrids between the two species ($T\times Ta$ or $Ta\times T$) can be derived from Table 2 as:

$$\mu_{T\times Ta} \text{ or } \mu_{Ta\times T} = \tfrac{1}{4}(d + d' + a_2 + D).$$

The variance among the mean genotypic values of progenies per family, i.e., the covariance of full-sibs, is obtained for the interspecific crosses as:

$$Cov_{T\times Ta}(FS) \text{ or } Cov_{Ta\times T}(FS) = \tfrac{5}{64}(a_2^2 + d^2 + d'^2 + D^2) - \tfrac{1}{32}(da_2 + d'a_2 + dD + d'D + 3a_2D + 3dd').$$

If alleles are neither linked nor interact among all n loci of interest, the above expression can be written taking into account all of these loci:

$$Cov_{T\times Ta}(FS) \text{ or } Cov_{Ta\times T}(FS) = \sum_{i=1}^{n} [\tfrac{5}{64}(a_{2i}^2 + d_i^2 + d'^2_i + D_i^2) - \tfrac{1}{32}(d_ia_{2i} + d'_ia_{2i} + d_iD_i + d'_iD_i + 3a_{2i}D_i + 3d_id'_i)],$$

where i is denoted the i-th locus. Although additive or dominance effects are generally different among loci, they may follow a particular distribution. Many quantitative genetic studies show that from numerous genes affecting a trait only a few have relatively large effects with many others having smaller effects (Gregory 1965, 1966; Sprickett and Thoday 1966; Thompson 1975; Edwards et al. 1987; Deobely and Stec 1991, 1993; Paterson et al. 1991). This suggests that the distribution of genotypic values of AA, $A'A'$, Aa, $A'a$, aa, and AA' among loci may be approximated by a geometric series,

Locus:	1	2	3	...	i	...	n
A_iA_i	a_1	ua_1	u^2a_1	...	$u^{i-1}a_1$	...	$u^{n-1}a_1$, $u\neq 1$
$A'_iA'_i$	a'_1	$u'a'_1$	$u'^2a'_1$	...	$u'^{i-1}a'_1$	...	$u'^{n-1}a'_1, u'\neq 1$
A_ia_i	d	vd	v^2d	...	$v^{i-1}d$	...	$v^{n-1}d$, $v\neq 1$
A'_ia_i	d	$v'd'$	v'^2d'	...	$v'^{i-1}d'$	...	$v'^{n-1}d'$, $v'\neq 1$
a_ia_i	a_2	ρa_2	ρ^2a_2	...	$\rho^{i-1}a_2$	...	$\rho^{n-1}a_2$, $\rho\neq 1$
$A_iA'_i$	D	wD	w^2D	...	$w^{i-1}D$	...	$w^{n-1}D$, $w\neq 1$

where locus 1 is viewed as the reference locus and u, u', v, v', ρ, and w are the coefficients of common proportion that determine the relative magnitude of the additive or dominant effects at each genetic locus. Since we have no $Ta\times Ta$ in this study, genotype $A'A'$ will not be considered. Also, the recessive homozygote aa, existing in both T and Ta populations, is further assumed to have a constant value across all n loci. Thus, $Cov_{T\times Ta}(FS)$ or $Cov_{Ta\times T}(FS)$ can be expressed in terms of these proportion parameters as:

$$Cov_{T\times Ta}(FS) \text{ or } Cov_{Ta\times T}(FS) = \tfrac{5}{64}(na_2^2 + H_{dd} + H_{d'd'} + H_{DD}) - \tfrac{1}{32}(\Pi_{da_2} + \Pi_{d'a_2} + \Pi_{dD} + \Pi_{d'D} + 3\Pi_{a_2D} + 3\Pi_{dd'}),$$

where

$$H_{dd} = \frac{(1+v^{n-1})(1-v^{n-1})}{(1+v)(1-v)}d^2,\ H_{d'd'} = \frac{(1+v'^{n-1})(1-v'^{n-1})}{(1+v')(1-v')}d'^2,$$

$$H_{DD} = \frac{(1+w^{n-1})(1-w^{n-1})}{(1+w)(1-w)}D^2,\ \Pi_{da_2} = \frac{1-v^{n-1}}{1-v}da_2,$$

$$\Pi_{d'a_2} = \frac{1-v'^{n-1}}{1-v'}d'a_2,\ \Pi_{dD} = \frac{1-(vw)^{n-1}}{1-vw}dD,$$

$$\Pi_{d'D} = \frac{1-(v'w)^{n-1}}{1-v'w}d'D,\ \Pi_{a_2D} = \frac{1-w^{n-1}}{1-w}a_2D,$$

$$\Pi_{dd'} = \frac{1-(vv')^{n-1}}{1-vv'}dd'.$$

The variance due to differences among the GCAs of the T (or Ta) parents, i.e., the covariance of half sibs, can be obtained as the variance of column (or row) means of Table 2 (i.e., the array means)

Table 2 Mating types, mating frequencies, and the mean genotypic values of progenies per mating between *P. tremuloides* (T) and *P. tremula* (Ta)

P. tremula (Ta)	*P. tremuloides* (T)			
	AA 1/4 a_1	Aa 1/2 d	aa 1/4 a_2	Ta-parental array means
$A'A'$ 1/4 (frequency) a'_1 (mean)	1/16 D	1/8 $(d+D)/2$	1/16 d'	1/4 $(d'+D)/2$
$A'a$ 1/2(frequency) d' (mean)	1/8 $(d+D)/2$	1/4 $(d+d'+a_2+D)/4$	1/8 $(a_2+d')/2$	1/2 $(d+d'+a_2+D)/4$
aa 1/4(frequency) a_2(mean)	1/16 d	1/8 $(a_2+d)/2$	1/16 a_2	1/4 $(d+a_2)/2$
T-parental array means	1/4 $(d+D)/2$	1/2 $(d+d'+a_2+D)/4$	1/4 $(d'+a_2)/2$	Overall mean $(d+d'+a_2+D)/4$

around the overall progeny mean:

$$Cov_{T\times Ta}(FS)_T \text{ or} Cov_{Ta\times T}(FS)_T = \tfrac{1}{32}(na_2^2 + H_{dd} + H_{d'd'} + H_{DD}) - \tfrac{1}{16}(\Pi_{da_2} + \Pi_{a_2D} + \Pi_{dd'} + \Pi_{d'D} - \Pi_{d'a_2} - \Pi_{dD}). \quad (1)$$

$$Cov_{T\times Ta}(FS)_{Ta} \text{ or} Cov_{Ta\times T}(FS)_{Ta} = \tfrac{1}{32}(na_2^2 + H_{dd} + H_{d'd'} + H_{DD}) - \tfrac{1}{16}(\Pi_{d'a_2} + \Pi_{a_2D} + \Pi_{dd'} + \Pi_{dD} - \Pi_{da_2} - \Pi_{d'D}). \quad (2)$$

The interaction between the T and Ta parents (SCA) can be obtained as the difference between the total variance among progeny family means and the sum of the variances due to differences among the general combining abilities of the T and Ta parents. This equals:

$$V_{S(T\times Ta)} \text{ or } V_{S(Ta\times T)} = \tfrac{1}{64}(na_2^2 + H_{dd} + H_{d'd'} + H_{DD}) - \tfrac{1}{32}(\Pi_{da_2} + \Pi_{d'a_2} + \Pi_{dD} + \Pi_{d'D} - \Pi_{Da_2} - \Pi_{dd'}). \quad (3)$$

A similar procedure is used to derive the variance due to the difference among the GCAs of the female or male parents and the variance due to the GCAs between the female and male parents in the intraspecific cross ($T\times T$). These variances are:

$$Cov_{T\times T}(FS) = \tfrac{1}{32}(na_2^2 + H_{a_1a_1} - 2\Pi_{a_1a_2}), \quad (4)$$

$$V_{S(T\times T)} = \tfrac{1}{64}(na_2^2 + H_{a_1a_1}) + \tfrac{1}{32}(2H_{dd} - 2\Pi_{a_1d} - 2\Pi_{da_2} + \Pi_{a_1a_2}), \quad (5)$$

where

$$H_{a_1a_1} = \frac{(1+u^{n-1})(1-u^{n-1})}{(1+u)(1-u)}a_1^2,\ \Pi_{a_1a_2} = \frac{1-u^{n-1}}{1-u}a_1a_2,$$

$$\Pi_{a_1d} = \frac{1-(uv)^{n-1}}{1-uv}a_1d.$$

In the mating design described in Table 1, the same T individuals are used as parents for both the intra- and inter-specific crosses, in which case the covariance between the T-parental array means from $T\times T$ and $T\times Ta$ or $Ta\times T$ can be calculated as:

$$Cov(T\times T,\ T\times Ta \text{ or } Ta\times T)_T = \tfrac{1}{32}(na_2^2 + \Pi_{a_1d} + \Pi_{a_1D} + \Pi_{d'a_2} - \Pi_{a_1a_2} - \Pi_{a_1d'} - \Pi_{da_2} - \Pi_{a_2D}), \quad (6)$$

where

$$\Pi_{a_1d'} = \frac{1-(uv')^{n-1}}{1-uv'}a_1d',\ \Pi_{a_1D} = \frac{1-(uw)^{n-1}}{1-uw}a_1D.$$

Also, the parental means from $T\times T$ and $T\times Ta$ or $Ta\times T$ may constitute a family structure in which the variances due to differences of the GCAs at the species level and the variance due to differences of the SCAs between species × individual-within-species or individual-within-species × species can be derived. These two variances are expressed, respectively, as:

$$Cov_{(T\times T,\,T\times Ta \text{or} Ta\times T)}(HS)_s = \tfrac{1}{64}[H_{a_1a_1} + H_{dd} + H_{d'd'} + H_{DD} + 2(\Pi_{a_1d} - \Pi_{a_1d'} - \Pi_{a_1D} - \Pi_{dd'} - \Pi_{dD} + \Pi_{d'D})], \quad (7)$$

$$V_{S(T\times T,\,T\times Ta \text{or} Ta\times T)} = \tfrac{1}{128}[H_{a_1a_1} + H_{dd} + H_{d'd'} + H_{DD} + 2(\Pi_{a_1d} - \Pi_{a_1d'} - \Pi_{dd'} - \Pi_{dD} + \Pi_{d'D})]. \quad (8)$$

The difference in the overall progeny means between inter- ($T\times Ta$ or $Ta\times T$) and intra-specific crosses ($T\times T$) can be expressed as:

$$\Delta = \mu_{T\times Ta} \text{ or } \mu_{Ta\times T} - \mu_{T\times T} = \tfrac{1}{4}(H_{d'} + H_D - H_{a_1} - H_d), \quad (9)$$

where

$$H_{d'} = \frac{1-v'^n}{1-v'}d',\ H_D = \frac{1-w^n}{1-w}D,\ H_{a_1} = \frac{1-u^{n-1}}{1-u}a_1,\ H_d = \frac{1-v^n}{1-v}d.$$

So far, we have constructed nine independent normal equations that include ten unknown parameters, $a_1, d, d', a_2, D, n, u, v, v'$ and w. According to the section on data analyses, the terms of these equations at the left side can be unbiasedly estimated by appropriate statistical models. However, because the number of parameters is greater than that of equations, one cannot solve these unknown parameters. However, if $v = v' = w$ is assumed for the common proportions of three heterozygotes, the number of parameters to be estimated is reduced to eight. Thus, from Eqs. 1–9, we will have nine likelihoods to construct a group consisting of eight equation on which a perfect solution can be computed for each parameter. The computer program package employed to solve non-linear equations is MATHEMATICA (Wolfram Research Inc. 1992). The final solution for each parameter is the mean of its nine estimators.

Results

Hybrid vigor

In the nursery stage (1993), mean survival was higher for $T\times Ta$ (91%) and ($Ta\times T$) (90%) than for $T\times T$ (76%). This pattern was attained in the first year of the field test (1994), despite slight decreases of survival for all the three subsets of mating. In the first 3 years, stem growth displayed significant hybrid vigor in the interspecific crosses relative to the intraspecific cross of the better parent T (Table 3). In year 1, hybrid vigor was 4–17% for stem height and diameter, and 33% for volume index. These values were largely increased when seedlings were planted in the field and further increased with stand development. In year 3, the stem volume index in the two interspecific crosses was 463–603% more than that in the intraspecifc cross of the better parent. The best families in terms of growth were also found in $T\times Ta$ and $Ta\times T$ where family ranges were larger as compared to $T\times T$ (Table 3; Fig. 1A–C).

Non-significant differences were observed in stem allometry between the three mating subsets, $T\times T$, $T\times T$, and $Ta\times T$ (Table 3). However, stems in all the subsets were stouter in the field test than in the nursery stage. Seedlings with the most slender stems were found in $T\times T$. In year 1, two structural components of stem height, internode number and length, generally showed no hybrid vigor, whereas there were more green leaves on the stem (17–22%) for the inter- than for the intra-specific cross when height growth approximated to the end.

Combining ability effects

The mean squares for all traits estimated from the "full" model are given in Table 4, together with the results of the F-tests. The effect due to family differences was

Table 3 Hybrid vigor (*HV*, %) of interspecific crosses ($T \times Ta$ and $Ta \times T$) for growth and morphological traits in the nursery and field test, as compared to the intraspecific cross ($T \times T$) of the better parent in *P. tremuloides* (*T*) and *P. tremula* (*Ta*). In all the three subsets of mating, family ranges of traits are also given. HT = stem height, DIA = root collar diameter, VOL = volume index, ALL = stem allometry, as described by the height:diameter ratio, NOD# = the number of nodes on the stem, MIL = mean internodal length, LVS# = the number of living leaves on the stem. Year 1 (1993) was in the nursery, and years 2 (1994) and 3 (1995) were in the field trial.

Trait (unit)	$T \times T$		$T \times Ta$		$Ta \times T$	
	Mean	Range	*HV*(%)	Range	*HV*(%)	Range
Year 1						
HT (cm)	42.3	19.4–50.3	0*	38.2–55.7	12***	40.5–51.2
DIA (mm)	3.1	1.8–3.7	17***	3.1–3.9	11***	2.7–4.0
VOL (cm^3)	5.08	0.69–8.19	33***	4.80–11.31	33***	4.11–9.37
ALL (cm/mm)	13.43	10.42–15.69	-10^{ns}	10.49–13.59	2^{ns}	12.77–14.72
NOD# (no)	22	13–23	2^{ns}	20–28	1^{ns}	19–25
MIL (cm)	1.9	1.4–2.0	3^{ns}	1.7–2.1	12***	2.0–2.2
LVS# (no)	11	3–15	17***	10–18	22***	9–15
Year 2						
HT (cm)	48.2	17.9–60.2	37***	58.9–78.7	46***	62.2–76.5
DIA (mm)	5.5	4.6–7.4	30***	6.2–8.7	42***	7.2–8.9
VOL (cm^3)	17.29	8.83–31.15	125***	28.85–63.98	180***	39.78–68.71
ALL (cm/mm)	8.83	5.53–10.55	6^{ns}	8.47–10.16	3^{ns}	8.41–9.83
Year 3						
HT (cm)	86.1	62.9–103.3	63***	119.2–178.9	80***	139.6–177.5
DIA (mm)	10.4	8.3–12.5	82***	13.8–26.8	104***	18.1–25.2
VOL (cm^3)	122.71	66.47–240.74	463***	266.32–1428.86	603***	574.57–1296.69
ALL (cm/mm)	8.39	7.60–9.51	-6^{ns}	6.12–9.13	-10^{ns}	7.06–8.09

*** Significant at $P < 0.001$, * significant at $P < 0.05$, ^{ns} non-significant

significant at $P < 0.001$ in all cases. Replicates had a significant effect on stem growth only in the field test, but on stem allometry significant effects occurred in both the nursery and field stages. In 1993, the number of leaves differed significantly among the replicates, whereas there was a non-significant replicate effect for the number and length of internodes. In all traits, family differences varied among replicates, as revealed by significant family × replicate effects.

The effects due to the GCA of the female (*Fgca*) and male parents (*Mgca*) and to the SCA of female × male parents ($F \times M$) were generally significant (Table 4). Some traits showed significant GCA effects at the species level, some at the individual-within-species level, and others at both levels. Although there were non-significant SCA effects due to one species × individuals-within-the-other-species, the SCA effects due to individuals within-one-species × individuals within-the-other-species were consistently significant. The effects due to GCA × replicate interaction were non-significant (Table 4). The partitioning of these effects, however, gave different results between the female and male parents. Interactions between the GCAs of the male species and replicates seemed to be significant for many traits, especifally those measured in 1993. The SCA effects varied significantly among replicates, which is attributed to the influences of the SCA of individuals-within-one-species × individuals-within-the-other-species.

The analyses of combining ability are described separately for intra- and inter-specific crosses in Table 5. The replicate effects showed similar trends between the two types of crosses in all 3 years. In $T \times T$ crosses, the GCA effects due to the female-parental differences were

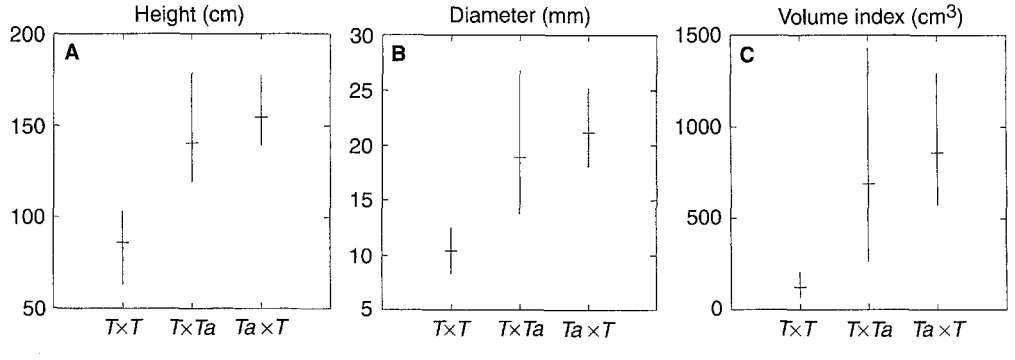

Fig. 1 Mean (+) and family ranges for 3rd-year stem height (**A**), diameter (**B**), and volume index (**C**) in $T \times T$, $T \times Ta$, and $Ta \times T$

Table 4 Mean squares estimated from the "full" model for growth and morphological traits in the nursery and field test in the F_1 progenies between *P. tremuloides* and *P. tremula*.[a] HT $\times 10^{-4}$, DIA $\times 10^{-2}$, VOL $\times 10^{-6}$, and HDR $\times 10^{-2}$. See Table 3 for explanation of traits. *R* = replicates, *FAM* = families from all three mating subsets, *Fgca* (*Mgca*) = general combining abilities of female (male) parents at the mixed species and individual-within-species level, *F* × *M* = female × male specific combining ability at the mixed species and individual-within-species level. The effects due to *R*, *FAM*, *FAM* × *R*, and residual are indicated in boldface. *FS*(*MS*) = female (male) general combining ability at the species level. *FI*/*FS*(*MI*/*MS*) = female (male) general combining ability at the individual-within-species level.

Sources of variation	Year 1							Year 2				Year 3[a]			
	HT	DIA	VOL	HDR	NOD#	LVS#	MIL	HT	DIA	VOL	HDR	HT	DIA	VOL	HDR
R	**389**^{ns}	**1.03**^{ns}	**32.5**^{ns}	**20.1****	**3.5**^{ns}	**0.616*****	**38**^{ns}	**4246*****	**29.7*****	**7228*****	**11.0*****	**2.03****	**2.47*****	**1.98*****	**0.080***
FAM	**1043*****	**4.91*****	**99.6*****	**43.9*****	**171.6*****	**0.388*****	**189*****	**5180*****	**46.7*****	**8923*****	**21.2*****	**3.49*****	**8.38*****	**4.97*****	**0.162*****
Fgca	2845***	9.78***	237.0**	53.2**	265.2^{ns}	1.475***	546**	16 215***	117.2***	21498***	68.3**	8.17***	18.77***	9.49***	0.198***
FS	9239***	42.45***	865.0***	23.5^{ns}	520.4^{ns}	6.559***	1865***	55 287***	403.9***	70548***	46.7^{ns}	34.72***	80.87***	37.33***	0.296**
FI/*FS*	1201^{ns}	3.97***	104.6**	49.7**	170.9^{ns}	0.262^{ns}	291**	2191***	5.1^{ns}	1519^{ns}	54.1***	0.35^{ns}	1.40^{ns}	1.41^{ns}	1.40^{ns}
Mgca	1382^{ns}	15.21***	209.3**	73.4***	262.0^{ns}	0.421*	326*	10 758***	87.7***	16184**	25.7^{ns}	8.58***	19.62***	11.45***	0.226***
MS	2371*	55.93***	630.4***	149.9***	252.1^{ns}	1.103***	998***	60 738***	331.0***	62408***	143.5***	23.47***	50.43**	22.36**	0.003^{ns}
MI/*MS*	1059^{ns}	8.86***	140.6**	43.6**	236.7***	0.300^{ns}	244***	1866^{ns}	25.4***	3827***	10.4^{ns}	1.36^{ns}	4.46^{ns}	3.67^{ns}	0.233**
F × *M*	645***	2.37***	54.8***	13.5**	124.2***	0.142^{ns}	107**	1722***	13.8***	3309***	11.9**	0.67**	1.51***	1.28***	0.067**
FS × *MS*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS × *MI*/*MS*	367^{ns}	0.62^{ns}	32.6^{ns}	14.0^{ns}	47.7^{ns}	0.064^{ns}	28^{ns}	1686^{ns}	1.1^{ns}	409^{ns}	18.5^{ns}	0.45^{ns}	0.75^{ns}	0.60^{ns}	0.100^{ns}
FI/*FS* × *MS*	585^{ns}	0.73^{ns}	39.8^{ns}	16.3^{ns}	122.5^{ns}	0.103^{ns}	93^{ns}	208^{ns}	3.8^{ns}	463^{ns}	7.9^{ns}	0.15^{ns}	1.07^{ns}	0.27^{ns}	0.035^{ns}
FI/*FS* × *MI*/*MS*	681**	2.82**	58.6**	11.6*	130.8***	0.153^{ns}	114**	2036***	17.2***	4232***	11.4***	0.67***	1.54***	1.46***	0.058*
FAM × *R*	**247*****	**0.89*****	**20.5***	**6.9*****	**32.7****	**0.134*****	**34*****	**396*****	**4.1*****	**866*****	**4.0*****	**0.39*****	**0.44*****	**0.40*****	**0.031***
Fgca × *R*	159^{ns}	0.56^{ns}	10.7^{ns}	9.1^{ns}	20.5^{ns}	0.140^{ns}	18^{ns}	535^{ns}	4.1^{ns}	1049^{ns}	4.4^{ns}	0.39^{ns}	0.42^{ns}	0.40^{ns}	0.036^{ns}
FS × *R*	63^{ns}	0.14^{ns}	3.7^{ns}	8.9^{ns}	0.6^{ns}	0.149^{ns}	1^{ns}	348^{ns}	5.6^{ns}	1635^{ns}	3.4^{ns}	0.11^{ns}	0.60^{ns}	0.61*	0.040^{ns}
FI/*FS* × *R*	185^{ns}	0.68^{ns}	12.8^{ns}	9.4^{ns}	24.0^{ns}	0.137^{ns}	23^{ns}	594*	4.7*	1083**	5.3^{ns}	0.42*	0.49*	0.43^{ns}	0.036^{ns}
Mgca × *R*	205^{ns}	0.65^{ns}	16.2^{ns}	5.9^{ns}	26.4^{ns}	0.117^{ns}	28^{ns}	377^{ns}	3.6^{ns}	676^{ns}	3.4^{ns}	0.43^{ns}	0.48^{ns}	0.32^{ns}	0.036^{ns}
MS × *R*	89***	0.42*	6.9**	1.2*	24.8^{ns}	0.082*	29*	278^{ns}	2.4^{ns}	621*	5.5^{ns}	0.60^{ns}	0.24^{ns}	0.28^{ns}	0.032^{ns}
MI/*MS* × *R*	235^{ns}	0.73^{ns}	17.9^{ns}	6.7^{ns}	28.1^{ns}	0.127^{ns}	32^{ns}	401^{ns}	3.8^{ns}	690^{ns}	3.2^{ns}	0.33^{ns}	0.54^{ns}	0.36^{ns}	0.034^{ns}
F × *M* × *R*	233**	0.89**	20.2^{ns}	5.9***	32.3*	0.116***	35***	361***	4.2***	816***	4.3***	0.32***	0.47***	0.41***	0.031*
FS × *MS* × *R*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS × *MI*/*MS* × *R*	89^{ns}	0.44^{ns}	6.6^{ns}	3.6^{ns}	23.4^{ns}	0.048^{ns}	20^{ns}	185^{ns}	3.0^{ns}	512^{ns}	5.0^{ns}	0.17^{ns}	0.35^{ns}	0.26^{ns}	0.036^{ns}
FI/*FS* × *MS* × *R*	226^{ns}	0.59^{ns}	17.5^{ns}	5.8^{ns}	28.8^{ns}	0.088^{ns}	29^{ns}	322^{ns}	2.5^{ns}	343^{ns}	4.2^{ns}	0.21^{ns}	0.31^{ns}	0.15^{ns}	0.037^{ns}
FI/*FS* × *MI*/*MS* × *R*	257***	1.02***	23.0***	5.9***	33.5***	0.128***	37***	361***	4.8***	980***	3.5*	0.36***	0.55***	0.51***	0.027^{ns}
Residual	**165**	**0.63**	**17.5**	**3.2**	**32.3**	**0.076**	**22**	**212**	**2.0**	**451**	**2.7**	**0.13**	**0.28**	**0.26**	**0.026**

*** Significant at $P < 0.001$; ** significant at $P < 0.01$; * significant at $P < 0.05$; ^{ns} non-significant

Table 5 Mean squares estimated from the "reduced" model for growth and morphological traits in the nursery and field test separately for intra- and inter-specific crosses for *P. tremuloides* and *P. tremula*.[a] HT $\times 10^{-4}$, DIA $\times 10^{-2}$, VOL $\times 10^{-6}$, and HDR $\times 10^{-2}$. *REC* = reciprocal effects, *FAM*/interspecific = families within the two interspecific crosses, *Fgca* (*Mgca*) = general combining abilities of female (male) parents for each mating subset, $F \times M$ = female × male specific combining ability for each mating subset. See Tables 3 and 4 for explanation of traits and the other abbreviations, respectively

Sources of variation	Year 1							Year 2				Year 3[a]			
	HT	DIA	VOL	HDR	NOD#	MIL	LVS#	HT	DIA	VOL	HDR	HT	DIA	VOL	HDR
Intraspecific															
R	238^{ns}	0.37^{ns}	14.3^{ns}	12.0^{ns}	9.9^{ns}	0.424^{ns}	10.5^{ns}	1283***	4.66*	472***	11.60*	0.186^{ns}	0.319*	0.027^{ns}	0.060^{ns}
Fgca	59^{ns}	0.18^{ns}	7.7^{ns}	18.9^{ns}	1.0^{ns}	0.203^{ns}	18.7^{ns}	1320^{ns}	3.59**	992^{ns}	25.89^{ns}	0.185*	0.359*	0.068***	0.019^{ns}
Mgca	2094***	7.94***	146.5***	40.3***	371.0***	0.217^{ns}	337.9***	3395***	4.39^{ns}	1048^{ns}	21.55^{ns}	0.074^{ns}	0.216^{ns}	0.025^{ns}	0.058^{ns}
$F \times M$	1071**	3.45**	58.0**	19.7**	215.0***	0.233^{ns}	180.1**	1281*	14.58***	656**	18.67^{ns}	0.134^{ns}	0.174^{ns}	0.016^{ns}	0.071*
Fgca × *R*	217^{ns}	0.66^{ns}	15.0^{ns}	6.4^{ns}	28.1^{ns}	0.105^{ns}	38.0^{ns}	286^{ns}	2.15^{ns}	174^{ns}	4.89^{ns}	0.094^{ns}	0.148^{ns}	0.016^{ns}	0.038^{ns}
Mgca × *R*	198^{ns}	0.61^{ns}	14.4^{ns}	4.6^{ns}	23.1^{ns}	0.105^{ns}	23.9^{ns}	240^{ns}	2.13^{ns}	123^{ns}	5.86^{ns}	0.189^{ns}	0.147^{ns}	0.015^{ns}	0.025^{ns}
$F \times M \times R$	247***	0.79***	15.2^{ns}	5.4**	37.5***	0.111***	39.9**	443***	1.43^{ns}	128^{ns}	7.14***	0.200***	0.263**	0.029**	0.029^{ns}
Residual	147	0.50	12.3	3.0	22.4	0.062	21.5	170	1.21	107	3.29	0.068	0.106	0.013	0.032
Interspecific															
R	285^{ns}	0.93^{ns}	22.6^{ns}	14.5^{ns}	5.5^{ns}	0.410^{ns}	39.7^{ns}	3346**	28.70*	7267*	5.76^{ns}	1.801^{ns}	2.824*	2.966*	0.032^{ns}
REC	1069^{ns}	5.92^{ns}	6.6^{ns}	356.7**	21.5^{ns}	3.499***	4.1^{ns}	1816^{ns}	78.94^{ns}	14 778^{ns}	34.10^{ns}	2.983^{ns}	6.965^{ns}	4.757^{ns}	0.189^{ns}
FAM/interspecific	881***	3.92***	105.3***	29.7***	153.2***	0.169***	273.6***	1692***	16.29***	4195***	14.78***	1.224***	3.394***	3.433***	0.138***
●*T* × *Ta*: *Fgca*	3125***	3.20^{ns}	180.9***	1.2***	483.7***	0.380*	466.4***	1324^{ns}	7.00^{ns}	928^{ns}	0.50***	0.473^{ns}	1.647^{ns}	1.144^{ns}	0.412***
Mgca	48^{ns}	4.13***	60.7^{ns}	0.4***	66.6^{ns}	0.432***	29.8^{ns}	1600^{ns}	30.52^{ns}	5861***	0.11*	2.830***	9.854***	7.839***	0.520***
$F \times M$	325^{ns}	2.10^{ns}	60.8^{ns}	0.0^{ns}	59.5^{ns}	0.057^{ns}	53.9^{ns}	2249***	24.73***	5809**	0.05^{ns}	1.237***	3.048***	2.947***	0.065^{ns}
●*Ta* × *T*: *Fgca*	1165***	7.74***	156.2***	0.0^{ns}	225.1***	0.043^{ns}	458.3***	2654^{ns}	2.79^{ns}	1081^{ns}	0.55***	0.280^{ns}	2.944***	3.263**	0.180***
Mgca	687^{ns}	8.21***	149.2***	0.2**	141.4***	0.050^{ns}	195.9**	521^{ns}	11.66^{ns}	2429^{ns}	0.03^{ns}	0.685^{ns}	0.691^{ns}	1.532^{ns}	0.054^{ns}
$F \times M$	340^{ns}	2.64*	61.6^{ns}	0.1^{ns}	44.9^{ns}	0.138^{ns}	54.4^{ns}	3403***	27.00***	10 089***	0.07^{ns}	0.732**	1.551**	1.783**	0.009^{ns}
REC × *R*	203^{ns}	0.51^{ns}	18.2^{ns}	10.9^{ns}	37.1^{ns}	0.153^{ns}	28.5^{ns}	243^{ns}	3.54^{ns}	894^{ns}	3.04^{ns}	1.248^{ns}	0.729^{ns}	0.771^{ns}	0.027^{ns}
FAM/inter. × *R*	251**	0.98**	23.6^{ns}	8.4***	30.9^{ns}	0.158***	32.6**	623***	6.29***	1461***	3.23*	0.581***	0.682***	0.663***	0.030*
●*T* × *Ta*: *Fgca* × *R*	74^{ns}	0.58^{ns}	14.9^{ns}	0.1^{ns}	25.3^{ns}	0.138^{ns}	16.7^{ns}	468^{ns}	4.08^{ns}	863^{ns}	0.04^{ns}	0.804*	0.487^{ns}	0.418^{ns}	0.028^{ns}
Mgca × *R*	161^{ns}	0.38^{ns}	12.2^{ns}	0.1^{ns}	22.5^{ns}	0.093^{ns}	25.5^{ns}	419^{ns}	4.97^{ns}	887^{ns}	0.03^{ns}	0.417^{ns}	0.627^{ns}	0.384^{ns}	0.038^{ns}
$F \times M \times R$	289***	1.35***	31.4**	0.1***	35.2***	0.148***	37.5***	434***	5.86***	1265***	0.03^{ns}	0.456***	0.694***	0.664***	0.027^{ns}
●*Ta* × *T*: *Fgca* × *R*	214^{ns}	0.85^{ns}	15.2^{ns}	0.1^{ns}	28.9^{ns}	0.103^{ns}	19.2^{ns}	885***	7.19^{ns}	1940^{ns}	0.05^{ns}	0.420**	0.886***	0.839*	0.043**
Mgca × *R*	228^{ns}	0.90^{ns}	17.0^{ns}	0.1^{ns}	36.0^{ns}	0.124^{ns}	35.7^{ns}	334^{ns}	4.54^{ns}	1079^{ns}	0.02^{ns}	0.248^{ns}	0.747***	0.678*	0.048**
$F \times M \times R$	206^{ns}	0.73^{ns}	19.4^{ns}	0.1***	21.5^{ns}	0.117^{ns}	28.9^{ns}	294^{ns}	4.69***	862^{ns}	0.02^{ns}	0.173^{ns}	0.359^{ns}	0.406^{ns}	0.025^{ns}
Residual	173	0.69	19.7	3.3	24.9	0.082	22.4	228	2.24	581	2.59	0.213	0.440	0.435	0.028

*** Significant at $P < 0.001$; ** significant at $P < 0.01$; * significant at $P < 0.05$; ^{ns} non-significant

non-significant in the first 2 years, whereas they became significant in year 3. Significant GCA effects of the male parents were generally observed for traits in the nursery but not in the field. In years 1 and 2, the SCA effects were significant, but were not so in year 3. No traits showed significant GCA × replicate interactions, whereas interaction effects between the SCAs and replicates were important for most traits. Non-significant reciprocal effects at the species level were found between the two interspecific crosses ($T \times Ta$ and $Ta \times T$), except for stem allometry and internode length in 1993 (Table 5). The effects due to family-within-the -interspecific-crosses were partitioned into the GCA and SCA effects within each of the two crosses. In year 1, either the GCA effect of the female or male parents, or both, was significant for all traits, but none was significant in year 2, with exception of stem volume and allometry in $T \times Ta$. In year 3, significant effects due to the GCA were detected only on the female parent side in $T \times Ta$ and the male parent side in $Ta \times T$. In both interspecific crosses, stem growth showed increased SCA effects in the field relative to the nursery stage. Interactions between reciprocals and GCAs vs replicates were not significant in almost all cases, whereas significant interactions were observed between SCAs and replicates in $T \times Ta$.

The number and action of genetic factors

By assuming that the same set of genetic loci are segregating in the intra- and inter-specific crosses of aspens, the number of genetic loci and the relative magnitude of allelic effects across loci can be estimated as shown in Table 6. Growth trait were found to be multigenic, with more loci for stem diameter than for height. Stem volume was controlled by the largest number of loci (9–13), whereas the number for stem allometry was much less (3–5). The locus number affecting stem traits slightly increased from years 1 to 3. There were 3–4 loci governing the number and length of internodes on the stem. Variation in the number of leaves in late autumn was due to six genetic loci.

In the intraspecific cross, $T \times T$, a strong over-dominance effect was observed at the reference locus for all traits in the first 2 years, with the ratio of additivity to dominance being $\lambda = 2.12$–7.49 (Table 6). However, by year 3, stem growth traits appeared to be under the control of additive or partially dominant loci in that cross. The mode of gene action could not be estimated for $Ta \times Ta$ in this study since only the genotypic value of the heterozygote was calculated from this mating subset. However, given the poor performance of $Ta \times Ta$ relative to $T \times Ta$ or $Ta \times T$, as observed in many earlier studies (Pauley et al. 1963b; Melchior and Seitz 1966; Mohrdiek 1979, 1980; Li et al. 1993; Li 1995), one would not expect a high homozygous value ($A'A'$) in $Ta \times Ta$. The heterozygous genotype, AA', not present in the intraspecific crosses, was formed when crosses were carried out between species. This new genotype

Table 6 The estimators for the effective number of genetic factors ($\tilde{n}$) and additive and dominant effects at the reference locus for growth and morphological traits in the nursery and field test in the intra- and inter-specific crosses of *P. tremuloides* and *P. tremula*. See Table 3 for explanation of traits. λ = the degree of dominance in $T \times T$, expressed as $(a_1 - a_2)/2\,[d - (a_1 + a_2)/2]$

Trait (unit)	$\tilde{n}$	Intraspecific ($T \times T$)				Intraspecific ($Ta \times Ta$)			Intraspecific ($T \times Ta$ and $Ta \times T$)				The coefficient of proportions		
		a_2 (aa)	d (Aa)	a_1 (AA)	λ	a_2 (aa)	d' ($A'a$)	a_1' ($A'A'$)	a_2 (aa)	d (Aa)	d' ($A'a$)	D (AA')	u (AA)	u' ($A'A'$)	$v(Aa, A'a, AA')$
Year 1															
HT (cm)	5.9	35.10	47.52	38.89	5.60	35.10	36.03	–	35.10	47.52	36.03	63.62	0.803	–	0.400
DIA (mm)	8.3	2.91	3.21	3.03	4.19	2.91	3.01	–	2.91	3.21	3.01	4.95	0.781	–	0.381
VOL (cm^3)	8.6	4.85	5.23	5.02	3.53	4.85	4.97	–	4.85	5.23	4.97	11.97	0.814	–	0.321
ALL (cm/mm)	2.1	11.26	14.97	12.53	4.86	11.26	12.74	–	11.26	14.97	12.74	12.99	0.622	–	0.532
NOD# (no.)	3.4	18.74	24.57	20.11	7.49	18.74	20.61	–	18.74	24.57	20.61	25.34	0.500	–	0.514
MIL (cm)	4.0	1.53	2.13	1.73	4.86	1.53	1.80	–	1.53	2.13	1.80	2.62	0.421	–	0.429
LVS# (no.)	6.1	8.57	11.84	9.52	5.91	8.57	9.73	–	8.57	11.84	9.73	19.64	0.848	–	0.267
Year 2															
HT (cm)	7.4	42.12	51.33	48.02	2.12	42.12	52.06	–	42.12	51.33	52.06	127.05	0.751	–	0.343
DIA (mm)	9.3	4.32	6.31	4.97	5.12	4.32	6.34	–	4.32	6.31	6.34	12.81	0.713	–	0.255
VOL (cm^3)	9.9	14.76	19.03	16.34	4.42	14.76	21.25	–	14.76	19.03	21.25	119.80	0.898	–	0.267
ALL (cm/mm)	4.2	7.03	10.05	8.18	4.25	7.03	10.21	–	7.03	10.05	10.21	9.65	0.674	–	0.787
Year 3															
HT (cm)	8.1	73.42	88.12	94.73	0.38	73.42	90.39	–	73.42	88.12	90.39	152.79	0.831	–	0.391
DIA (mm)	10.4	8.35	10.64	11.96	0.27	8.35	10.77	–	8.35	10.64	10.77	18.43	0.656	–	0.220
VOL (cm^3)	12.7	109.88	123.61	133.75	0.15	109.88	120.48	–	109.88	123.61	120.48	789.22	0.702	–	0.237
ALL (cm/mm)	4.0	6.47	9.59	7.91	3.34	6.47	8.24	–	6.47	9.59	8.24	10.18	0.677	–	0.653

displayed a strikingly larger dominant value at the reference locus for growth traits than did the heterozygotes *Aa*, and *A'a*, in the intraspecific crosses. (Table 6). The relative importance of *AA'* to *Aa* or *A'a* for these traits increased significantly from the nursery to the field test, with a ratio of about 5.5 for volume. However, for stem allometry, the dominance of *AA'* showed no advantage over that of *Aa* or *A'a* in each year. Internode number and length, and leaf number on the stem, were somewhat intermediate between stem growth and allometry.

The coefficients of proportion for additive effects across loci did not largely differ from 1.0 in most traits, indicating smaller additive differences among genetic loci. However, the coefficients for dominance were significantly less than 1.0 in growth traits. This suggested that only a few dominant loci had major effects on stem growth.

Discussion

The mating scheme used in this study is designed to shed light on the genetic causes of F_1 heterosis in interspecific crosses. While the traditional quantitative genetic method is sufficiently powerful to analyze the heterosis of intraspecific crosses (Jinks and Jones 1958; Falconer 1989), it cannot be appropriately used to handle interspecific hybridization, especially between two outcrossing species. In forest trees, characterized by high heterozygosity, more than two alleles commonly exist at each genetic locus (Groover et al. 1994) and, additionally, these alleles may be different between species or even between populations of a species. In addition, the traditional biometric estimates are derived at the average levels of gene effect over all loci and, therefore, fail to rule out the relative importance of a few major loci with larger effect and polygenes each with a small effect on heterosis. In this paper, we propose a new analytical method to explore the genetic mechanisms of heterosis in interspecific crosses at the individual locus level. The method was illustrated by a quantitative comparison of intra- and inter-specific hybrids derived from the outcrossing of *P. tremuloides* and *P. tremula*. Consider three different alleles at each locus of interest which are segregating between the populations of these two species. Since both species are members of the *Leuce* section, evolved from a common ancestor and having some common orthologous genes, a recessive allele at each locus is assumed to be shared between their populations. By comparing the family structure of intra- and interspecific crosses, it was possible to estimate the difference in the phenotypic means of heterozygotes and homozygotes averaged across all portions of the genome. The results from our quantitative comparisons revealed the following: (1) the interspecific hybrids displayed much more stem growth, especially in volume index, relative to the intraspecific hybrids of the better parent, *P. tremuloides*. (2) in the intraspecific cross of *P. tremuloides* the heterozygote had much higher phenotypic values than their respective homozygotes for all growth and growth-related traits. (3) when the two species were crossed, a new heterozygote at a locus with one allele from *P. tremuloides* and the other from *P. tremula* showed tremendously high phenotypic values over the heterozygotes generated from both intraspecific crosses in all growth traits. Stem allometry influencing stem form had no such advantages for this new heterozygote. These results suggest that overdominant interactions between two alleles at the same loci from the two different species is the major contributor to their F_1 heterosis.

The significant role of non-additivity (possible including overdominance) in stem growth was also observed in intraspecific progenies of *P. deltoides*, where non-additive variance explained almost all of the genetic variance in 4-year volume (Foster and Shaw 1988). Using molecular-marker-based QTL mapping on the F_2 progenies of *P. trichocarpa* and *P. deltoides*, Bradshaw and Stettler (1995) found that heterozygotes at key QTLs showed larger 2-year stem volume growth than the corresponding homozygotes. This phenomenon was explained either by true overdominance at a single locus or by pseudo-overdominance, i.e., the occurrence of dominant and recessive alleles in repulsion at closely linked loci (see also Stuber et al. 1992). Although it is impossible to distinguish between these two mechanisms in this kind of work, this opens the possibility that the overdominance discovered here may be actually pseudo-overdominance, i.e., interactions between a deleterious recessive allele at one locus and a repulsive beneficial dominant allele at a closely linked locus (Crow 1952). The possible impact of pseudo-overdominance on the growth of poplars is not unexpected in terms of the outcrossing property of the species. In outcrossing species, there are notable accumulations of deleterious recessives that are usually masked by their corresponding dominant counterparts (Allard 1960). This can be broadly supported from QTL mapping in that overdominance, likely pseudo-overdominance, is implicated as the prominent factor conditioning heterosis in outcrossing maize (Stuber et al. 1992) but not in self-pollinated species like rice (Xiao et al. 1995).

The most interesting observation in the present study is the superiority of a newly formed heterozygote at a locus of interest, through species combination, over those existing in the original parental populations for growth traits. This phenomenon, which explains both the F_1 heterosis and the generation of larger extremes in the interspecific crosses of *P. tremuloides* and *P. tremula*, should have its underlying molecular or biochemical mechanism with which the strong overdominance occurs between two different alleles at a locus, each from one of the two species. The two alleles of the new heterozygotes at each locus, contributed by the respective parents, could be different functionally, as evidenced by significant general combining ability effects at the species level (Table 4). Apart from unknown mechanisms, the superiority of the new heterozygote may be

quantitatively re-inforced by the multiplicative interaction of genes for composite traits. For a complex quantitative trait like volume growth, larger heterosis is due to the product of smaller heterosis for its individual composite traits, such as height, diameter, and stem taper which was not studied here (see also Wright 1922; Richey 1942; Moll et al. 1962). Provided each component is controlled by a different set of genes, the genetic system of the complex trait will show multiplicative action between genes belonging to different sets (Williams 1959; Schnell and Cockerham 1992). At the molecular level, Kacser and Burns (1981) suspect that enzyme-dependent characters are influenced far above average by those genes which specify enzymes more directly involved in the respective biosynthetic pathways. The effect of multiplicative action on the heterosis of a complex trait can be also seen from the relation of stem height to internode number and length, in that the heterosis of height was approximated by the product of heterosis of the two component traits (Table 3). It should be noted that while the heterosis documented in the interspecific crosses of aspens may also result from epistasis among non-alleles at different loci, it may also result from differences in allele frequency between the two parental populations (see Minvielle 1987) an issue that will be discussed elsewhere.

The method proposed in the present paper has the power to explain the genetic basis underlying heterosis through an estimate of the number of genetic loci contributing to this phenomenon. Gene enumeration from the present method is more accurate, and also more simple in terms of the study material, relative to Wright's estimator for the number of genes (Zeng 1992). This is reflected in the following aspects: (1) the present estimator is based on fewer genetic assumptions in that only linkage and epistasis are assumed to be absent. (2) neither inbred lines nor advanced generations, such as F_2 and backcrosses, are necessary. In forest trees, including *Populus,* these entities are very difficult to generate. According to our estimates, growth appears to be under the control of a multigenic system, with a larger gene number for volume than for height and diameter. The numbers of genes for these growth traits were found to be larger than those for their components, such as internode number and length and leaf number. Relatively speaking, stem allometry is simple in terms of gene number. Stuber et al. (1992) also observed that the number of QTLs for a trait in maize may be a function of the degree of its complexity; for example, grain yield is affected by more QTLs than other traits. In the present study, we further found that for growth traits there was considerable variation among individual loci in dominant effect but not in additive effect. This suggests that only a few major loci exert an important overdominant effect on the growth performance of hybrids, whereas additive effect influences growth more collectively over all loci.

Heterosis, and its underlying genetic basis for growth, supports the genetic improvement strategies in aspen (Li and Wyckoff 1991; Li 1995). In these proposals, selection for full-sib interspecific hybrids was suggested to be the most appropriate approach to capture the genetic gain of aspen in North America. In the present study, we found that the size of heterosis, and therefore the corresponding gene number and overdominance of the new heterozygotes, increased from years 1 to 3. However, there is no reason to expect that all the increases will occur during the entire rotation, even when strong inter-tree competition obtains in the stand (see Wu and Stettler 1996). It would be important to examine the ontogenetic change of heterosis during the course of growth in order to conduct effective early prediction and selection for superior growth in the hybrids between *Populus* species.

The mating design and subsequent quantitative genetic analyses presented in this study provide a high resolution for heterosis genetics. The new biometric approach can be used as an alternative to the molecular approach in that individual loci for heterosis can be mapped on the chromosomes (e.g., Stuber et al. 1992; Bradshaw and Stettler 1995; Mitchell-Olds 1995). However, as pointed out above, our estimator for genetic parameters might be biased by linkage and epistasis among loci. Linkage seems to exist in our aspen material, as evidenced by Fig. 1A–C in which the lowest families in the two interspecific crosses displayed greater growth than the highest family in the intraspecific cross as a result of linkage among loci. Epistasis has been inferred to play a key role in plant breeding and evolution, despite the fact that there is currently a methodological difficulty in detecting it even via QTL mapping (Tanksley 1993; Bradshaw and Stettler 1995; Doebley et al. 1995). In addition to possible violations of these two assumptions, the results from the present material should be interpreted with caution because the number of parents chosen to be crossed within and between species was modest. A small sample size would most likely result in large sampling errors for parameter estimates. Also, the missing *Ta* × *Ta* cross, due to the limited flowers of the *Ta* parents, has reduced the power to estimate genetic parameters. Expanded crosses will be required to better exploit the results in a practical breeding scheme.

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