Quantitative genetic variation of leaf size and shape in a mixed diploid and triploid population of *Populus*

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Summary

In the interspecific cross of *Populus trichocarpa* × *P. deltoides*, unexpected simultaneous occurrence of diploid hybrids and triploid hybrids (with two alleles from the female parent and one from the male parent at each locus) led us to examine the evolutionary genetic significance of this phenomenon. As expected, leaf size and shape of the triploid progeny are closer to the female *P. trichocarpa* than male *P. deltoides* parent. Although the pure triploid progeny population did not have higher genetic variance in leaf traits than the pure diploid population, the former appears to hide much non-additive genetic variance and display strong genetic control over the phenotypic plasticity of leaf traits. It is suggested that the cryptic non-additive variance, especially epistasis, can be released when a population is disturbed by changes in the environment. A mixed diploid and triploid progeny population combines phenotypic and genetic characteristics of both pure hybrids and is considered to be of adaptive significance for poplars to survive and evolve in a fluctuating environment. The significant effect due to general and specific combining ability differences at the population level suggests that the population divergence of these two species is under additive and non-additive genetic control.

1. Introduction

Numerous studies have demonstrated that leaf variation is of adaptive significance for growth and competitive survival in a wide range of plants (Raschke, 1960; Parkhurst & Loucks, 1972; Givnish, 1979; Hinckley *et al.*, 1989; Gurevitch, 1992). Remarkable differences have been observed in leaf morphology and physiology between and within plant species. For example, as a riparian species in the Pacific Northwest, black cottonwood (*Populus trichocarpa* T. & G.) has thick and ovate leaves, held by short and stout petioles (Stettler *et al.*, 1988). By contrast, throughout its distribution range of the eastern United States, leaves of eastern cottonwood (*Populus deltoides* Bartr.) are thin and deltoid, hanging from long and laminar petioles (Dickmann *et al.*, 1990). Further studies on *P. trichocarpa* indicate that leaves of this species are larger and have longer petioles at its northeastern region, and are smaller with short petioles as latitude decreases and longitude increases (Weber *et al.*, 1985; Rogers *et al.*, 1989). A similar clinal variation pattern from north and west to south and east was also found for leaf size and petiole length of *P. deltoides*, over much of its natural range (Ying & Bagley, 1976). These leaf size and shape differences are generally suggested to be the result of adaptation to a specific environment (reviewed in Parkhurst & Loucks, 1972; Givnish, 1979), although the genetic mechanisms underlying this environmental adaptation have been poorly understood.

Using trees from different geographic origins of *P. trichocarpa* and *P. deltoides* as parents, the interspecific hybridization between these two species was conducted to exploit heterosis for short-rotation productivity (Heilman & Stettler, 1985) and to explore the genetic structure and variation pattern of morphological traits, such as leaf size and shape, branch structure and crown form (Stettler *et al.*, 1988; Wu & Stettler, 1994, 1996). Unexpectedly, cytogenetic and molecular genetic analyses have indicated that almost all F1 families between these two *Populus* species

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produced approximately half triploid individuals, each with two doses of genes from the female *P. trichocarpa* parent and one dose from the male *P. deltoides* parent, in addition to half normal diploid siblings (Bradshaw & Stettler, 1993). The shift in population composition from diploidy and polyploidy (e.g. triploidy) has often been linked to evolutionary and ecological change in plants (Darlington, 1939; Stebbins, 1950, 1971; Levin, 1983). Polyploids possess more alleles than diploid relatives at each locus, and have higher levels of genetic diversity than the diploid progeny (Hancock & Bringhurst, 1981; Song *et al.*, 1995). As a result, polyploids have frequently been suggested to have an exceptional ability to colonize a wide range of habitats and to survive better in a changing environment (Levin, 1983; Novak *et al.*, 1991). Plants that are grown in different environments may display variation in phenotypic response to these environments (Bradshaw, 1965; Via & Lande, 1985; Gurevitch, 1992). The response of a genotype to heterogeneous environments, termed phenotypic plasticity, shows potential adaptation of the organism to changing environments and is thought to be modelled by selection (Bradshaw, 1965; Scheiner & Lyman, 1989; Wu, 1997, 1998). However, no studies have shown quantitative differentiation in genetic structure and phenotypic plasticity between diploid and triploid progeny populations.

The objectives of this study are to (1) examine the physiological, genetic and evolutionary basis of ploidy differentiation in leaf size and shape in the *P. trichocarpa × P. deltoides*. F1 hybrid population, and (2) estimate the genetic variation of phenotypic plasticity for leaf traits by planting clonal replicates in three different microenvironments.

2. Materials and methods

The *P. trichocarpa* female parents used for the cross were randomly selected from six geographically isolated natural populations along latitude and longitude gradients, as described by Stettler *et al.* (1988). These populations are located in Chilliwack, British Columbia, Canada (49° 05′ N, 121° 56′ W), Rockport, Washington (48° 29′ N, 121° 36′ W), Monroe, Washington (47° 26′ N, 122° 06′ W), IFA, Washington (47° 09′ N, 123° 42′ W), Orting, Washington (47° 03′ N, 122° 12′ W) and Longview, Oregon (46° 06′ N, 122° 58′ W), respectively. The *P. deltoides* pollen for the crosses came from a wide range of distribution, representing five different populations, i.e. Minnesota (44° 35′ N, 92° 40′ W), southern Illinois (37° 46′ N, 89° 09′ W), Oklahoma (36° 30′ N, 99° 30′ W), Mississippi (32° 46′ N, 91° 00′ W) and southern Texas (30° 38′ N, 96° 21′ W). One to four trees with normal growth, stem form and branch pattern selected from each geographic origin

of the two species were mated in an unbalanced factorial mating design, which finally resulted in 28 full-sib families. Almost all families (27) were found to have both diploid and triploid hybrids (Bradshaw & Stettler 1993), whereas only one family produced pure diploid progeny. In spring 1991, a clonal field trial of the mixed diploid and triploid progeny was established at farm 5 of the Washington State University Research and Extension Center near Puyallup, Washington, using 20 cm long, unrooted dormant cuttings. The trial was laid out in a randomized complete block design with three blocks and two-tree plots at a spacing of 3.0×1.5 m. In order to make a visual comparison between the two kinds of progeny, each triploid hybrid was matched by its nearest diploid relatives within each block. A small-scale environmental heterogeneity was observed in soil structure, moisture and fertility among the three blocks (Braatne *et al.*, 1992). Thus, the difference in tree growth and morphology among the blocks, as also observed in other studies by Wu & Stettler (1994, 1996), can be viewed as affected by these environmental factors. The degree to which the same genotype varies in phenotype among the three blocks represents the genotype's capacity to respond to different moisture conditions, fertility levels and soil structures. A border of two rows of trees was planted at the periphery of the experimental area.

Leaf morphometric measurements were made for each tree based on two mature leaves on the terminal at an approximate leaf plastochron index of 10 to 12 during the first growing season. Four traits chosen for measurement were those that best discriminate between the two parental species (Stettler *et al.*, 1988). They were single leaf area (SLA), petiole length (PL), leaf width:leafblade length ratio (WLR) and the distance from leaf base to maximum width:leafblade length ratio (DLR) (Reyment *et al.*, 1984). WLR describes the overall dimensions of the leaf outline, for example the contrast between short, wide leaves (higher WLR) and long narrow ones (lower WLR). DLR illustrates leaf base shape in detail. Higher or lower DLR is related to ovate or deltoid shape of a leaf, respectively.

In this study, two linear regression models were used to examine quantitative genetic variation in leaf morphological traits. The first was employed to detect the effects of female and male general combining ability and female × male specific combining ability at the population level and their interactions with blocks, which is expressed as:

$$Y_{ijkl} = \mu + T_i + D_j + S_{ij} + B_k + (T \times B)_{ik} + (D \times B)_{jk} + (S \times B)_{ijk} + E_{ijkl}, \quad (1)$$

where Y_{ijkl} is the phenotypic value of the *l*th individual in block *k* derived from the cross of population *i* of *P*.

trichocarpa and population j of *P. deltoides*, μ is the overall mean, T_i is the effect of population i of *P. trichocarpa*, D_j is the effect of population j of *P. deltoides*, S_{ij} is the effect of population i of *P. trichocarpa* × population j of *P. deltoides*, B_k is the effect of block k, $(T \times B)_{ik}$, $(D \times B)_{jk}$ and $(S \times B)_{ijk}$ are the corresponding interaction effects, and E_{ijkl} is the residual effect. Statistical analyses using this model were performed for diploid, triploid, and mixed diploid and triploid progeny populations, respectively.

The second model ignoring the parental-population effect was used to estimate the family and clone-within-family effects, as well as the ploidy effect in the mixed diploid and triploid population. The model for the mixed diploid and triploid population is expressed as

$$
\begin{aligned}
Y_{ijklm} = \mu &+ F_i + P_j + (F \times P)_{ij} + [C/(F \times P)]_{k/ij} + B_l \\
&+ (F \times B)_{il} + (P \times B)_{jl} + (F \times P \times B)_{ijl} \\
&+ \{[C/(F \times P)] \times B\}_{(k/ij)l} + E_{ijklm},
\end{aligned}
\tag{2}
$$

where Y_{ijklm} is the phenotypic value of the mth individual of clone k within family i and ploidy level j in block l, μ is the overall mean, F_i is the effect of family i, P_j is the effect of ploidy level j, $(F \times P)_{ij}$ is the effect of interaction between family i and ploidy level j, $[C/(F \times P)]_{k/ij}$ is the effect of clone k within family i and ploidy level j, B_l is the effect of block l, $(F \times B)_{il}$, $(P \times B)_{jl}$, $(F \times P \times B)_{ijl}$ and $\{[C/(F \times P)] \times B\}_{(k/ij)l}$ are the corresponding interaction effects, and E_{ijklm} is the residual effect. The model for the pure diploid or triploid population is the same as (2), except that the ploidy effect and its associated interaction terms are omitted.

By assuming that all these effects are random, the variance partitioning for all the sources described in (1) and (2) was performed by restricted maximum likelihood (REML; SAS Institute, 1988). Maximum likelihood can provide valid estimates for (co)variance components regardless of imbalance or other complexities of data structure like the one in this study (Meyer, 1991). Furthermore, these estimates are unbiased when REML is used (Patterson & Thompson, 1971). All null hypotheses were tested by approximate F-ratios from analysis of variance based on PROC GLM type III sum of squares (SAS Institute, 1988).

The experimental variances estimated by REML were used to estimate the additive, dominant and epistatic components of genetic variance following quantitative genetic models suggested by Wu (1995). The interspecific mating design at the population level offers a prerequisite for estimating these three genetic components in a triploid progeny population (Wu, 1995). The three kinds of genetic variance components were estimated for diploid, triploid and mixed progeny populations, respectively. The contribution of each of these three components to broad-sense heritability was estimated for each population. The same procedures were used to estimate the variance components due to additive × block, dominant × block and epistatic × block interactions. Scheiner & Lyman (1989) defined the heritability of phenotypic plasticity as the proportion of the total phenotypic variance accounted for by genotype × environment interaction effect. According to this definition, the broad-sense heritability of phenotypic plasticity of leaf traits to small-scale block differences was estimated and its additive, dominant and epistatic components separated using the same procedures as for leaf traits themselves. It is noted that the accuracy of partitioning the genetic variance into its causal components from the clonal design is dependent on the assumption about non-allelic epistasis. If epistasis is mainly derived from interactions among many loci (> 3), the method described in Wu (1995) should give high accuracy. On the other hand, if it is due to interactions between few loci (two or three), estimates of genetic variance components will be biased (Wu, 1996).

3. Results

Ploidy was found to have an important effect on leaf size and shape in the mixed progeny population (Fig. 1). On average, triploid leaves had more *P. trichocarpa*-like characteristics, such as larger size (12%), shorter petiole length (9%) and more ovate base shape (DLR was larger by 14%), than leaves from diploid siblings. These findings were not unexpected because triploids have two-thirds of their genes contributed from the *P. trichocarpa* parent.

Components of variance due to various sources are presented in Table 1, with a similar trend in the explained proportions of the total phenotypic variance between the diploid and triploid populations. The small-scale environmental effect of block was detected to be significant for single leaf area and petiole length, accounting for 5–8% of the phenotypic variance. These two leaf size traits differed considerably among families and among clones within families. Family and clone effects together explained 35–45% of the observed variance. For the mixed progeny population, the ploidy effect was found to account for a small but consistently significant proportion of the phenotypic variance (2–5%). Ploidy displayed markedly different effects on the two leaf size traits in different families. The ploidy and ploidy × family effects together explained about 13% of the phenotypic variance.

Leaf shape traits showed a small or non-significant block effect in all three population types. The genotypic effect was significant on leaf shape in the diploid or triploid progeny, but the family or clone effect was generally larger on leaf overall shape than on leaf base shape. In the mixed progeny population,
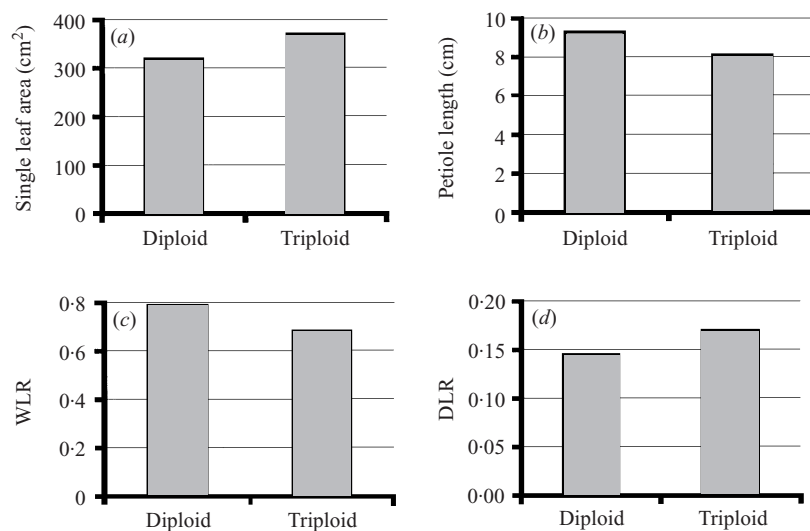
Fig. 1. Phenotypic values of four leaf traits: comparison across the diploid and triploid progeny populations of *P. trichocarpa* × *P. deltoides*. WLR, leaf width:leafblade length ratio; DRL, the distance from leaf base to maximum width:leafblade length ratio.

Table 1. *Variance components and significance tests for four leaf traits in diploid, triploid and mixed progeny populations of* P. trichocarpa × P. deltoides. *The percentages of variance components are given in parentheses*

Progeny/source	d.f.	$SLA \times 10^{-3}$	PL	$WLR \times 10^3$	$DLR \times 10^3$
Mixed					
Block	2	0·75 (6)***	0·17 (5)**	0·01 (0)ns	0·03 (1)*
Family	27	0·33 (2)**	0·15 (5)***	−0·08 (0)ns	0·02 (1)*
Ploidy	1	1·91 (15)**	0·29 (10)*	2·05 (29)**	0·23 (7)*
Family × Ploidy	26	1·40 (11)***	0·26 (8)**	0·45 (6)*	0·09 (3)ns
Clone/(Family × Ploidy)	142	1·87 (14)**	0·66 (22)***	0·75 (11)**	0·25 (8)*
Ploidy × Block	2	0·76 (6)*	0·11 (4)*	0·14 (2)ns	0·12 (4)ns
Family × Block	54	0·05 (0)ns	0·02 (1)ns	0·09 (1)ns	0·04 (1)ns
Family × Ploidy × Block	48	−1·22 (0)ns	−0·27 (0)ns	−0·65 (0)ns	−0·34 (0)ns
Clone/(Family × Ploidy) × Block	183	2·78 (21)***	0·64 (21)***	0·96 (14)***	0·83 (26)***
Residual	352	3·33 (25)	1·03 (34)	2·57 (37)	1·60 (50)
Pure diploid					
Block	2	0·76 (8)***	0·19 (7)***	0·00 (0)ns	0·03 (1)ns
Family	27	2·53 (28)***	0·61 (21)**	1·91 (38)***	0·19 (8)*
Clone/Family	85	1·52 (17)**	0·76 (25)***	0·55 (11)*	0·44 (17)**
Family × Block	54	0·25 (3)ns	0·01 (0)ns	−0·44 (0)ns	−0·28 (0)ns
Clone/Family × Block	99	0·93 (10)*	0·24 (8)*	0·78 (15)*	1·01 (39)***
Residual	192	3·15 (34)	1·17 (39)	1·85 (36)	0·89 (35)
Pure triploid					
Block	2	0·69 (5)***	0·16 (5)***	0·01 (0)ns	0·06 (2)*
Family	25	3·91 (27)***	0·46 (15)**	3·10 (37)***	0·07 (2)ns
Clone/Family	57	2·17 (15)*	0·58 (19)**	0·94 (11)*	0·11 (3)ns
Family × Block	50	−0·85 (0)ns	−0·21 (0)ns	−0·45 (0)ns	−0·15 (0)ns
Clone/Family × Block	84	4·37 (30)***	1·03 (34)***	1·94 (23)**	1·51 (47)***
Residual	160	3·54 (24)	0·85 (27)	2·44 (29)	1·45 (45)

Negative estimates for variance resulting from an unbalanced design were viewed as zero.
SLA, single leaf area; PL, petiole length; WLR, leaf width:leafblade length ratio; DLR, the distance from leaf base to maximum width:leafblade length ratio.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns non-significant.

the ploidy and ploidy × family effects were small or non-significant for these two leaf shape traits.

The interaction between families and blocks was non-significant for all four traits in both diploid and triploid populations (Table 1). Yet, families responded differently to the small-scale environment for two leaf size traits in the mixed population. All leaf traits displayed significant clone-within-family × block in-

Table 2. *Broad-sense heritabilities (H^2) and coefficients of genetic variance (CGV, %) for four leaf traits and their phenotypic plasticity to different blocks in diploid, triploid and mixed progeny populations of* P. trichocarpa $\times$ P. deltoides

Trait		H^2			CGV (%)		
		Diploid	Triploid	Mixed	Diploid	Triploid	Mixed
SLA	Trait	0·38 = 0·38+0·00+0·00	0·43 = 0·11+0·20+0·12	0·44 = 0·15+0·19+0·20	19·9	21·6	21·8
	Phenotypic plasticity	0·13 = 0·00+0·03+0·10	0·31 = 0·00+0·11+0·20	0·28 = 0·06+0·10+0·12	10·7	18·4	17·6
PL	Trait	0·49 = 0·39+0·00+0·00	0·36 = 0·08+0·17+0·11	0·46 = 0·11+0·20+0·15	12·7	12·5	13·2
	Phenotypic plasticity	0·08 = 0·00+0·04+0·04	0·35 = 0·00+0·12+0·13	0·25 = 0·04+0·08+0·13	5·4	12·0	9·8
WLR	Trait	0·49 = 0·49+0·00+0·00	0·46 = 0·22+0·13+0·11	0·46 = 0·29+0·07+0·10	6·4	8·2	7·4
	Phenotypic plasticity	0·15 = 0·00+0·06+0·09	0·23 = 0·00+0·09+0·14	0·16 = 0·02+0·05+0·09	3·6	5·7	4·5
DLR	Trait	0·25 = 0·07+0·18+0·00	0·18 = 0·10+0·06+0·02	0·19 = 0·07+0·04+0·08	14·9	10·7	14·5
	Phenotypic plasticity	0·39 = 0·00+0·09+0·30	0·47 = 0·00+0·12+0·35	0·30 = 0·04+0·20+0·06	18·9	22·3	18·8

H^2 is divided into three components: additive+dominant+epistatic, in order.
SLA, single leaf area; PL, petiole length; WLR, leaf width:leafblade length ratio; DRL, the distance from leaf base to maximum width:leafblade length ratio.

teraction in each of the three population types. No significant interaction effect existed between ploidy levels and blocks for leaf shape and among families, ploidy level, and blocks in the mixed population.

Estimates for broad-sense heritability were generally similar for the leaf traits studied among the diploid, triploid and mixed populations, ranging from 0·20 to 0·50 (Table 2). In the diploid progeny population, additive genetic variance showed a larger contribution to the heritability than non-additive genetic variance and, in some cases, the heritabilities were due to pure additive variance. However, in the triploid and mixed population, the contribution of non-additive genetic variance (including both dominant and epistatic) was remarkably increased. Phenotypic plasticity of all four traits to blocks was much more heritable in the triploid than diploid population (Table 2). For both diploid and triploid populations, the heritabilities of phenotypic plasticity were contributed by non-additive genetic variance, whereas they were contributed by both additive and non-additive genetic variance in the mixed population. Broad-sense genetic variabilities for the leaf traits had similar trends among the three populations, with leaf overall shape displaying a lower coefficient of genetic variance than the other three traits (Table 2). However, for the phenotypic plasticity of these leaf traits, genetic variabilities were consistently larger in the triploid than diploid progeny population.

At the population level, the female- and male-parental general combining ability and their interaction effects were significant for all four leaf traits studied in each of the three *P. trichocarpa* $\times$ *P. deltoides* progeny populations: diploid, triploid and mixed (data not given).

4. Discussion

Interspecific hybridization of *P. trichocarpa* and *P. deltoides* produced a mixed-ploidy hybrid progeny including normal diploid hybrids and triploid hybrids with two alleles from the female *P. trichocarpa* parent and one from the male *P. deltoides* parent at each locus (Bradshaw & Stettler, 1993). The formation of mixed diploid and triploid hybrids in the interspecific crosses probably is the consequence of adaptation of the *Populus* species to some evolutionary force (Stebbins, 1950). In this study, the triploid is found to have a larger leaf size than its diploid counterpart, which may be due to its larger cell size rather than larger cell number because there is a correlation between cell size and ploidy level (Swanson, 1957) and because the *P. trichocarpa* parent that contributes more genes to the triploid has larger size but fewer number of epidermal cells than the *P. deltoides* parent (Hinckley *et al.*, 1989). Earlier comparative physio-

logical studies have demonstrated that polyploids have higher photosynthetic capacity and other metabolic levels than diploid siblings (Levin, 1983). It is thus inferred that, by producing higher leaf area, the triploid can intercept higher solar radiation and, in turn, make more photosynthate than the diploid. However, with its deltoid-shaped leaves, hanging from long petioles, the diploid could be sensitive to wind and sunlight through its particular biomechanic leaf structure and, thereby, diffuse heat loads effectively in the canopy (Dickmann *et al.*, 1990; Vogel *et al.*, 1995). The combination of different characteristics of diploid and triploid hybrids confers a capacity for a mixed-ploidy population to adapt well to environmental conditions. Such a capacity is absent for any single-ploidy population (Stebbins, 1950, 1971; Levin, 1983).

Evolutionary genetic theory suggests that genetic variation is the fuel for the organism to evolve in the wild (Houle, 1992). A population of higher heritability and variability is considered to have an evolutionary advantage over a population of lower heritability and variability. As fitness-related traits, leaf size and morphology can be used to assess such advantage for a population. The present study showed that the triploid progeny population had little more genetic variation in leaf traits than its diploid counterpart. However, the triploid progeny displayed more diverse genetic structures, with more contributions to the genetic variance by dominance and epistasis, than the diploid progeny. A traditional tenet of polyploid evolution suggests that polyploids cannot effectively construct their new adaptive complexes due to the 'buffering' effect of multiple genomes (Stebbins, 1971). Also, because polyploidization events were considered rare, polyploids may have a single origin and, thus, high genetic uniformity is maintained across different individuals. Considerable non-additive genetic variation observed in the triploid poplar population seems to disagree with these traditional views. Although triploids are sterile in the natural condition, their genetic variation may be maintained and 'inherited' through vegetative reproduction. Thus, the formation of triploids in vegetatively propagated plant species may provide a possibility of breaking the evolutionary dead-end for the polyploids (Wagner, 1970). In the current literature, the non-additive genetic variation has frequently been suggested to play a critical but unrecognized role in evolution and speciation (Whitlock *et al.*, 1995). Epistasis is often hidden in a population and can be released when the population is disturbed by external environment. By asexual reproduction of triploidy, *Populus* can maintain and fix high non-additive genetic variation for better adaptation to unpredictable environmental fluctuations.

Another interesting finding in this study is that the heritabilities of phenotypic plasticity of leaf traits are consistently larger in the triploid than diploid population. Phenotypic plasticity is the object of many recent studies and recognized to be an important force for evolution (Scheiner & Lyman, 1989; Via, 1993; Wu, 1997, 1998). Although there is a debate about the genetic mechanisms underlying phenotypic plasticity (Via *et al.*, 1995), it is broadly accepted that a population with larger genetic variation in phenotypic plasticity can better regulate itself to a changing environment than a population with small genetic variation. Therefore, it is possible that the triploid population has a greater capacity to adapt more efficiently to environmental changes than the diploid population. The mixed diploid and triploid progeny population appears to maintain the genetic variation structure typical of triploids, such as higher non-additive variance and stronger genetic control over phenotypic plasticity, and, thus, has an evolutionary advantage over a pure diploid progeny population.

As shown by Wu (1995), the partitioning of genetic variance in a triploid progeny population requires a mating design at the population level. As a by-product of this study, therefore, population combining ability analysis provides new insights into population differentiation and its genetic underpinnings. It was found that the general and specific combining ability effects at the population level were significant for all four leaf traits, which suggests that some kind of evolutionary force is shaping the population differentiation of *P. trichocarpa* and *P. deltoides* over their sampling ranges. In the same sampling region of the mesic west side of the Cascade Ranges, the genetic differentiation of *P. trichocarpa* in growth, morphology and polymorphic traits somewhat parallels certain geographic trends among populations (Weber & Stettler, 1981; Weber *et al.*, 1985; Rogers *et al.*, 1989). A more extensive study in the xeric region has revealed that climatic dichotomy is an important evolutionary force inducing genetic differentiation among *P. trichocarpa* natural populations in the east slope of the Cascade Ranges (Dunlap, 1991). Although the limited sampling ranges of both species used for parents in this study cannot enable inferences about the association between population combining abilities and geographic trends, we anticipate that this kind of experimental design can help to provide a better understanding of the genetic mechanisms (e.g. additive versus non-additive genetic variance) underlying population evolution over environmental gradients.

This study is based on an unbalanced mating and experimental design. It was shown that imbalance of the data structure could result in biased estimates for genetic parameters (Namkoong & Roberds, 1974). Furthermore, the quantitative genetic analysis of non-additive variance relies on the assumption about the relative importance of low- versus high-order epistatic

interactions to the total epistasis (Wu, 1996). The model used to estimate genetic variances in this study is based on the assumption that epistasis results from high-order interactions among many loci affecting a quantitative trait (Wu, 1995). However, the results from a recent molecular experiment using the hybrids between *P. trichocarpa* and *P. deltoides* suggested that as few as one to five loci of large effect were responsible for a large proportion of the genetic variance in many traits (Bradshaw & Stettler, 1995). Thus, a model considering low-order epistatic interaction between a few loci ($\leqslant 3$) should be developed to provide estimates for genetic variance in such a particular population as used in this study. Nonetheless, several preliminary results detected may provide an encouraging line of thinking to design a more powerful population genetic experiment in multiple environments which can shed light on the role of polyploidy in population evolution.

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