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Genetic control of macro- and micro-environmental sensitivities in *Populus*

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Abstract Understanding the genetic mechanisms for the phenotypic plasticity and developmental instability of a quantitative trait has important implications for breeding and evolution. Two clonally replicated plantations of two 3-generation inbred pedigrees derived from the highly divergent species *Populus trichocarpa* and *P. deltoides* were used to examine the genetic control of macro- and micro-environmental sensitivities and their genetic relationships with the trait mean across two contrasting environments. For all stem-growth traits studied, the trait mean had a higher broad-sense heritability (H^2) level than macroenvironmental sensitivity, both with much higher H^2 values than microenvironmental sensitivity. Genetic correlation analyses indicated that the trait mean was more or less independent of macro- or micro-environmental sensitivity in stem height. Thus, for this trait, the genetic difference in response to the two environments might be mainly due to epistasis between some regulatory loci for plasticity and loci for trait mean. However, for basal area and volume index, pleiotropic loci might be more important for their genetic differences between the two environments. No evidence was found to support Lerner's (1954) homeostasis theory in which macro- or micro-environmental sensitivity is the inverse function of heterozygosity.

Key words Developmental instability · Growth · Phenotypic plasticity · Poplar (*Populus*) · Quantitative genetics

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Introduction

The phenotypic change of a given genotype is determined by two different, but related, processes; one, the predictable environment, and the other, the unpredictable environment (Allard and Bradshaw 1964; Bull 1987; Falconer 1989). The predictable environment, including different climates or soil types, interacts with the developmental "blueprint" of the organism and gives rise to phenotypic plasticity (Schmalhausen 1949; Bradshaw 1965; Schlichting 1986). The unpredictable environment, such as external random errors or internal "accidents", entails changes in developmental pathways of the organism (Palmer and Strobeck 1986), which result in developmental instability that describes different expressions of genetically identical individuals in the same environment (Lerner 1954; Waddington and Robertson 1966). These two environments are typically called "macro" and "micro", respectively, by quantitative geneticists (Jinks and Pooni 1988; Gavrilets and Hastings 1994).

Sensitivities to both macro- and micro-environments are the subject of an increasing number of studies in breeding and evolution (Ford and Seigel 1989; Schlichting 1989; Scheiner and Lyman 1991; Via 1993; Bazzaz et al. 1995; Dutilleul and Potvin 1995). Genotypic responses to heterogeneous environments are ubiquitous in breeding practice (Comstock and Moll 1963; Wescott 1986; Namkoong et al. 1992). Only by a full understanding of the genetic foundations for this phenomenon are breeders able to select genotypes that have a potential capacity to buffer environmental changes or fluctuations (Eberhart and Russell 1966). In evolution, variation in response among genotypes is considered advantageous, since it can maintain latent genetic variation in a population (Dobzhansky et al. 1955; Gupta and Lewontin 1982; Gillespie and Turelli 1989).

Macro- and micro-environmental sensitivities are believed to be under genetic control, but there is no consensus on the underlying genetic mechanisms (Via

et al. 1995). Three genetic hypotheses have been proposed to explain variation in macroenvironmental sensitivity: overdominance, pleiotropy, and epistasis. The overdominance hypothesis suggests that the macroenvironmental sensitivity of a genotype decreases with the number of heterozygous loci it contains (Lerner 1954; Gillespie and Turelli 1989). The pleiotropy hypothesis assumes that the same loci that affect the mean value of a character over all environments also determine its macroenvironmental sensitivity (Via and Lande 1985, 1987; Gomulkiewicz and Kirkpatrick 1992). The epistasis hypothesis assumes that macroenvironmental sensitivity is a character in its own right, separate from the mean phenotype and under its own genetic control (Bradshaw 1965; Jinks and Pooni 1988; Schlichting 1989; Scheiner and Lyman 1991). Although evidence for each of these hypotheses has been obtained in different organisms, geneticists have found it difficult to resolve experimentally the debate about which hypothesis is most appropriate (Scheiner 1993; Schlichting and Pigliucci 1993; Via 1993).

Microenvironmental sensitivity is generally considered to be associated with the level of heterozygosity (Palmer and Strobeck 1986; Jinks and Pooni 1988). Owing to their particular biochemical pathways, heterozygous genotypes tend to display decreased microenvironmental sensitivity compared with homozygous genotypes (Lerner 1954). However, after an extensive review of the literature, Clarke (1993) suggested that genomic coadaptation played a more important role in influencing developmental instability than did heterozygosity. In the past, the study of developmental instability has been largely conducted using animals as material, with little emphasis on plants. In animals, the difference between homological left and right parts on the same individual (fluctuating asymmetry) provides an accurate measurement of developmental instability. A similar measure was developed in plants to use variation within clonal replicates of a genotype planted in the same environment. Inconsistent results have arisen about the genetic control of developmental instability from earlier studies in plants (mostly annuals).

In the present paper, genetic analyses of macro- and micro-environmental sensitivities are performed and the genetic hypotheses for these two variables are tested using stem-growth traits in two 3-generation hybrid pedigrees derived from two divergent *Populus* species. *Populus* provides an ideal model system for the study of macro- and micro-environmental sensitivities in woody plants. First, wide variation and easy crossability among species and populations offer a good opportunity to create types that can be grown in a broad context of environments. Second, easy clonal propagation of many species facilitates the partitioning of the phenotypic variance into genetic, environmental and their interaction components. Clonal replicates also make it possible to accurately measure macro- or micro-environmental sensitivity for a given genotype. Third, rapid progression to advanced generations, such as F_2 or backcrosses (B_1), can be attained due to early flowering, which is crucial for examining the genetic mechanisms underlying macro- or micro-environmental sensitivity at the molecular level (Paterson et al. 1991; Stuber et al. 1992).

Materials and methods

Plant pedigrees and plantations

In 1981, two half-sib F_1 families, 50 and 53, were generated from crosses between a female *Populus trichocarpa* clone (93–968), native to western Washington, and two different male *P. deltoides* clones. The *P. deltoides* parent of Family 50 was from northern Illinois (clone ILL-129) and that of Family 53 from southern Illinois (clone ILL-005). Two siblings of F_1 Family 53, 53–246, and 53–242, were further crossed in 1988 to produce an F_2 family (331). In 1990, the same F_2 cross was repeated to obtain a larger sample size ($n = 325$). At the same time, an additional F_2 family (355) was generated by crossing two siblings of F_1 Family 50, 50–181 and 50–188. Both inbred hybrid pedigrees were maintained in a nursery at Farm 5 of the Washington State University Research and Extension Center in Puyallup, Washington.

In spring 1993, rooted cuttings of this entire material (including the 1988 subset of Family 331) were planted in two environments, one east of the Cascades in Boardman, Oregon, the other west of the Cascades in the lower Columbia River Valley near Clatskanie, Oregon. The two plantation environments differ markedly, Boardman (117°6′W, 45°42′N) being in a continental climate, whereas Clatskanie (123°40′W, 46°6′N) being in the coastal zone with a strong maritime influence. At Boardman, the soil is a sandy loam, whereas the soil at Clatskanie is an alluvial silt loam. Both plantations contain 375 and 225 genotypes of Families 331 and 355, respectively, as well as their corresponding F_1 parents and original parents, laid out in a randomized complete block design with two-tree plots at a spacing of 1.5 × 3.0 m and surrounded by two border rows. Three replicates were used in the plantation at Clatskanie, but four at Boardman, which is irrigated, to allow for a reduced (50%) watering regime in the fourth replicate in year 2.

Trait measurements

Stem height and diameter were measured for each tree at the end of each of the first 2 years. Basal diameter was measured 3 cm above the ground in year 1 and at the mid-point of the first-year stem-height increment in year 2. Stem basal area and volume index were estimated by $(\text{diameter})^2/4*\pi$ and $(\text{height})*(\text{diameter})^2$, respectively. The second-year stem-height increment was calculated based on the difference in height between the first 2 years. Stem allometry was described using the height:diameter ratio. In all trait analyses, a triploid clone identified in Family 331 was excluded.

Measuring macro- and micro-environmental sensitivities

Several methods have been suggested to measure the macroenvironmetal sensitivity of a quantitative trait. First, a general two-way analysis of variance: the proportion of the total phenotypic variance accounted for by genotype × macroenvironment interactions is used to quantify the variation in phenotypic response to macroenvironments. Scheiner and Goodnight (1984) suggested that this might be replaced by the sum of two variance components due to the macroenvironment and genotype × macroenvironment interaction effects, although the two variances are from two different sources, namely the mean and variance of macroenvironmental sensitivity (Schlichting 1986). Second, the coefficient of variation (expressed as the standard deviation over the mean) across all macroenvironments for individual genotypes: this parameter reflects the amount of sensitivity of sepcific genotype (Schlichting 1986). Third, the difference in performance of a

given genotype between a pair of macroenvironments: this measures both the amount and direction of macroenvironmental sensitivity for this genotype (Scheiner and Lyman 1991; Via 1993).

Whereas the first method reflects a global measure of macroenvironmental sensitivity in a given population, the coefficient of variation and difference are typically capable of comparing sensitivities between different genotypes and of estimating the relationship of the mean value of a trait to its sensitivity across environments (Scheiner 1993). In the present study, the third method was used to calculate macroenvironmental sensitivity, which was based on the difference of plot means in each replicate between the two plantations. All three replicates were included at Clatskanie, but only the first three were included at Boardman (since its replicate IV received a different watering regime from the others in year 2). For each genotype, therefore, plot means in the two plantations can be paired with a total of six possibilities. However, a potential pairing was made using the bootstrap resampling statistical technique (Efron 1982). Analyses of trait means across the two environments were calculated based on the same pairing as trait sensitivities.

Microenvironmental sensitivity was measured using the nongenetic (i.e., environmental) variance of a genotype in a more-or-less uniform environment. Since the replicate effect may be due to predictable site conditions and physiographic features, the environmental variance was estimated based only on two trees per genotype within each replicate. The two trees of a genotype were always planted side by side in all replicates and, therefore, their differences in growth and form are considered to stem from some unpredictable factors experienced by the trees, such as external fluctuating errors or internal developmental "accidents".

Genetic analysis

The traditional analysis of variance is less powerful for performing the genetic analysis of macro- and micro-environmental sensitivity as measured in this study, since the variance due to genotype × environment interactions cannot be ruled out based on plot means. Ignoring this variance component, however, would lead to an inflated estimate of the heritability of these two developmental aspects. Here, a more robust alternative, derived from Gimelfarb's (1994) additive-multiplicative model, was applied to estimate the components of variance due to the genetic, environmental, and their interaction effects.

According to the principles of quantitative genetic analysis (Mather and Jinks 1982), the accuracy for estimating genetic variance based on a single F_2 family relies on whether all loci affecting a quantitative trait (QTLs) are fixed for alternative alleles in the two parents. When this condition is met, the estimated genetic variance is unbiased; that is, the variance due to the genotype effect in the F_2 family can exactly represent the total genetic variance of the trait in a randomly mating population. However, any deviation from this condition would lead to an underestimate for the total genetic variance. Fortunately, this may not be serious since most traits studied are so discriminated between the original parents that the parents can be assumed to be fixed for alternative alleles at each locus of interest. Through the component analyses, broad-sense heritabilities and genetic correlations were estimated for both F_2 hybrid progenies.

The effective numbers of QTLs governing variation in the macro- or micro-environmental sensitivity of a quantitative trait were estimated following the equations described in Lande (1981). This method has relaxed the assumption of no dominance, but involves four other simplifying assumptions, i.e., all increasing alleles come from one parent and all decreasing alleles from the other parent, all QTLs have equal effect, and alleles are neither linked nor interact among loci. The sampling variances of the QTL numbers were estimated using the Tayor expansion (e.g., Lande 1981). It should be pointed out that QTL enumeration based on the biometric method may be underestimated when any of the genetic assumptions is violated (Zeng et al. 1990).

Results

Variation and distribution

The means of all stem-growth traits and their macroenvironmental sensitivities across Boardman and Clatskanie differed among generations (Table 1). In both years, the *P. trichocarpa* clone displayed greater height and radial growth (and therefore a greater volume index) than the *P. deltoides* clones. In year 2, clone ILL-005 of *P. deltoides* from southern Illinois had more volume growth than clone ILL-129 from northern Illinois. For both F_1 families, heterosis above the better parent was strong in basal area and volume index; for example, the F_1 superiority was more than 15% for the 2-year volume index. Owing to inbreeding depression

Table 1 Mean values and macroenvironmental sensitivities of stem growth and allometry between Boardman and Claskanie in *P. trichocarpa* (T), *P. deltoides* (D), and their F_1 hybrids and F_2 hybrids

Generation	Mean				Sensitivity			
	Height	Basal area	Volume index	Stem allometry	Height	Basal area	Volume	Stem allometry
Year 1 ILL-129 (D)	1.34	3.01	0.67	0.784	0.76	3.26	0.99	−0.011
F_1 53	1.82	5.97	1.52	0.775	0.28	6.34	1.81	−0.345
$F_2$331	1.08	1.87	0.33	0.828	0.37	1.99	0.41	−0.175
T[a]	**1.70**	**4.84**	**1.14**	**0.752**	**0.35**	**4.37**	**1.15**	**−0.231**
$F_2$355	1.14	2.52	0.47	0.781	0.40	3.05	0.65	−0.251
$F_1$50	1.74	5.87	1.42	0.739	0.32	6.61	1.78	−0.314
ILL-005 (D)	1.44	3.46	0.73	0.774	0.53	2.41	0.76	−0.063
Year 2 ILL-129 (D)	3.82	16.76	8.78	0.888	1.09	6.93	5.06	0.052
$F_1$53	4.88	25.29	16.27	0.870	1.33	10.71	11.07	0.055
$F_2$331	2.87	8.99	4.14	0.956	1.49	8.27	5.03	−0.026
T[a]	**5.10**	**20.72**	**13.95**	**1.008**	**1.04**	**4.50**	**6.32**	**0.103**
$F_2$355	3.10	10.20	5.10	0.965	1.84	10.95	7.04	−0.037
$F_1$50	4.95	24.52	16.38	0.907	1.80	14.58	14.88	0.056
ILL-005 (D)	4.45	17.88	11.71	1.006	2.59	13.72	12.91	0.047

[a] The common female parent (T) is placed in the middle of the two pedigrees in boldface

(R. F. Stettler and R. Wu, unpublished data), the means of the two F_2 families were below the low-value original parent in all growth traits (see Figs. 1A, C, E).

In year 1, the *P. trichocarpa* clone was less sensitive in stem height but more sensitive in radial and volume growth than both *P. deltoides* clones. However, in year 2, the *P. trichocarpa* clone and clone ILL-129 had more-or-less similar sensitivities, which were clearly exceeded by the sensitivity values of ILL-005. For basal area and volume index, the F_1 hybrids were more plastic to the

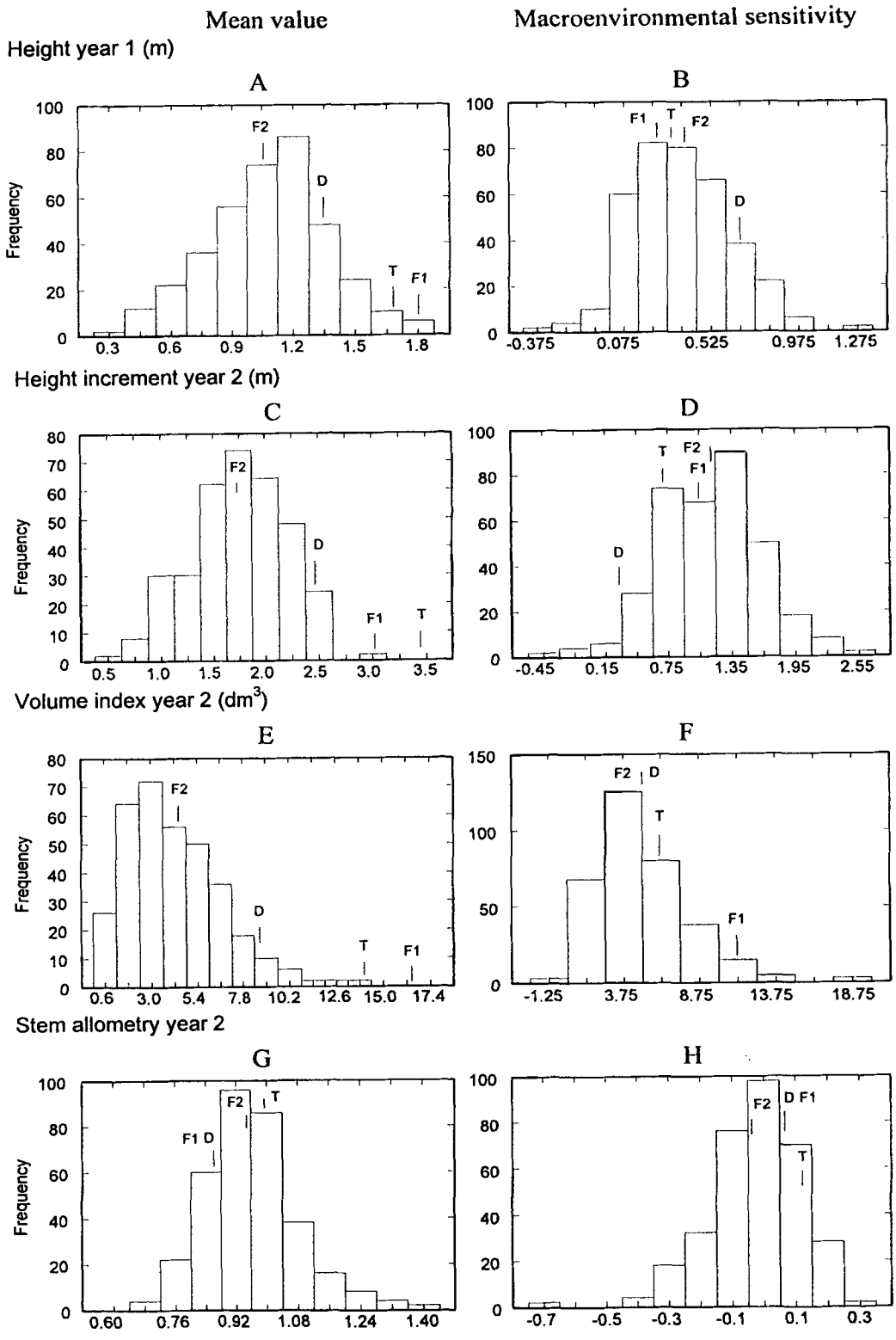

Fig. 1 Distribution of trait means (*A*, *C*, *E*, and *G*) and macroenvironmental sensitivities (*B*, *D*, *F*, and *H*) for stem growth traits across Boardman and Clatskanie in the F_2 family 331. Means for the two original parentals (*P. trichocarpa*, *T* and *P. deltoides*, *D*: ILL-129), the F_1 parents (53) and the F_2 family are indicated

growth environments than their respective parents in both years. On average, the F_2 hybrids had a varying macroenvironmental sensitivity across traits, years, and families.

Although stem allometry over the two plantations did not vary much among generations, its macroenvironmental sensitivity was considerably larger for the *P. trichocarpa* and hybrid clones than for the *P. deltoides* clones in year 1, and for the *P. trichocarpa* clone than for the *P. deltoides* and hybrid clones in year 2 (Table 1; Fig. 1H).

As expected, mean values of stem height and allometry exhibited a perfect normal distribution in both F_2 families; but the normality in stem volume index was approximate, and more skewed toward the small-value side. Macroenvironmental sensitivity had a normal distribution with continuous variation in all traits (Fig. 1).

Microenvironmental sensitivity, expressed as the variance between two clonal replicates of a genotype in the same plot, showed significantly different magnitudes between plantations as well as generations (Fig. 2A–F). The norms of reaction of the microenvironmental sensitivity of stem height crossed strongly between the two *P. deltoides* clones, but all other generations had a small sensitivity and nonsignificant variation in both sites. Basal area and volume index were more sensitive to microenvironments at Boardman than Clatskanie in most generations, especially in F_1 Family 53 and ILL-129. In these two traits, the two F_2 families showed a low microenvironmental sensitivity. For stem allometry, ILL-005, *P. trichocarpa*, and the two F_1 families were more stable than ILL-129 and the F_2 families in both plantations. However, significant interactions were observed between generations and environments.

Broad-sense heritability and QTL number

Mean values and macroenvironmental sensitivities of the traits studied displayed similar broad-sense heritability levels between the F_2 families (Table 2). In all cases, trait means were under stronger genetic control

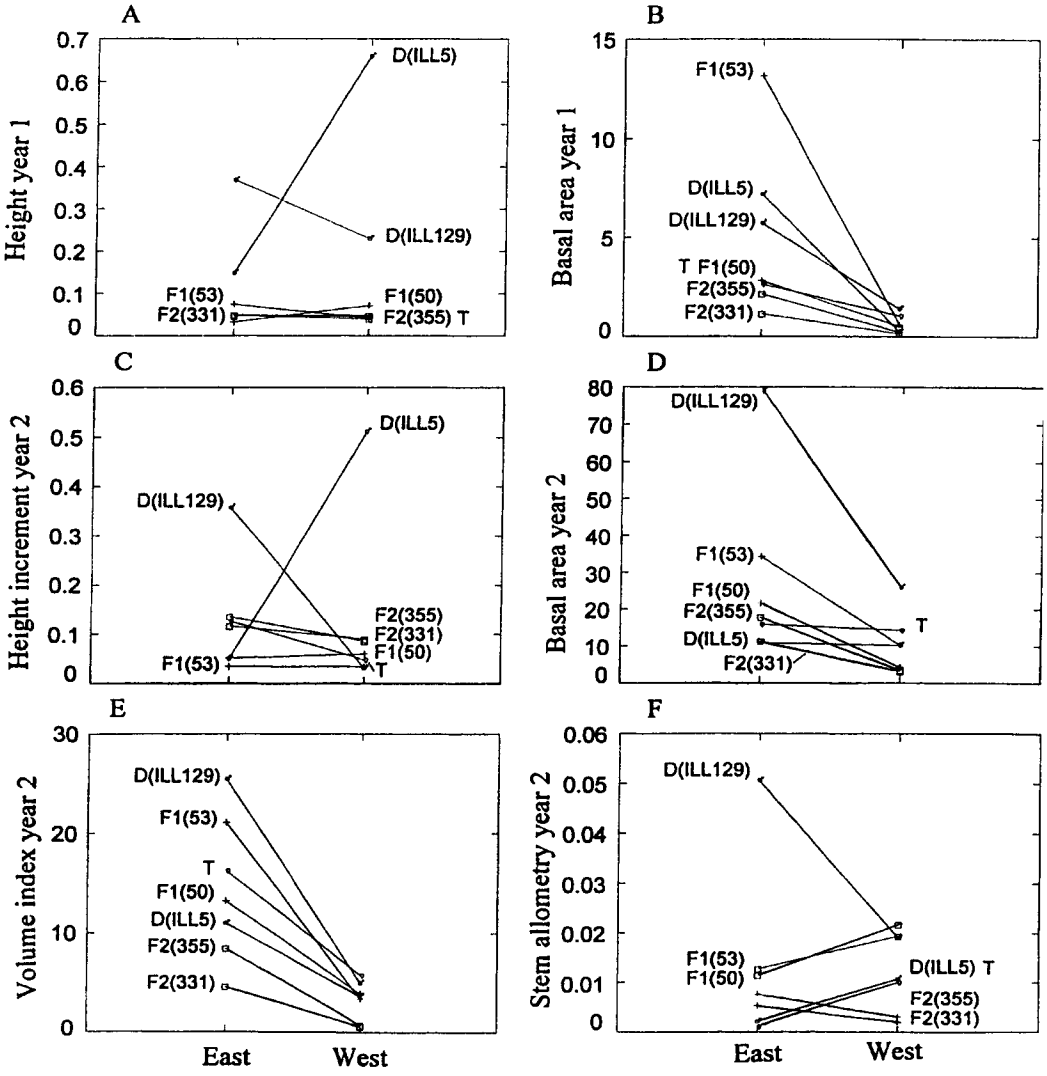

Fig. 2 The norm of reaction of microenvironmental sensitivities for stem growth traits across Boardman (east) and Clatskanie (west) in *P. trichocarpa* (*T*), *P. deltoides* (*D*: ILL-005 and ILL-129), their F_1 hybrids (50 and 53) and F_2 families (331 and 355). The microenvironmental sensitivity was expressed as the environmental variance between two trees per genotype in each replicate

($H^2 = 0.74–0.90$) than macroenvironmental sensitivities ($H^2 = 0.38–0.74$). In Family 331, the heritabilities of trait means increased with age; this was also true for the macroenvironmental sensitivities of growth traits. As compared to trait means and macroenvironmental sensitivities, microenvironmental sensitivities appeared to have very low heritabilities whose values were a function of trait, plantation, and year. In addition, obvious differences were found in these relationships between the two F_2 families (Table 2). In Family 331, the microenvironmental sensitivity of stem height was consistently under low genetic determination in the two plantations. For basal area and volume index, however, microenvironmental sensitivity showed higher heritabilities, and more so at Boardman than Claskanie. The heritability of microenvironmental sensitivity of stem allometry increased at Boardman but decreased at Clatskanie from year 1 to 2 (Table 2).

The numbers of QTLs with large effects on trait means tended to be similar to those for the corresponding macroenvironmental sensitivities (Table 3). For these two developmental variables, basal area and volume index seemed to have more QTLs than height and stem allometry. The QTL numbers underlying trait means and macroenvironmental sensitivities were much lower than those for microenvironmental sensitivities for all traits. However, there appeared to be more QTLs for microenvironmental sensitivities at Boardman than Clatskanie and in year 1 compared with year 2 (Table 3).

Genetic correlation

The relationships between trait mean and macroenvironmental sensitivity varied largely among traits in both F_2 families (Table 4). The mean value of stem height had low, or nonsignificant, correlations with macroenvironmental sensitivity across Boardman and Clatskanie. In Family 331, there was a negative association between height mean and macroenvironmental sensitivity in year 1. Basal area and volume index showed very tight correlations between trait means and macroenvironmental sensitivities, although the values were slightly larger in year 1 than in year 2. For stem allometry, trait mean was significantly negatively correlated with macroenvironmental sensitivity.

The correlations between trait mean and microenvironmental sensitivity were a function of trait and plantation (Table 4). The correlations were very low for stem height, especially at Boardman in Family 331. Basal area and volume index generally had moderate correlations that tended to be larger at Boardman than Clatskanie and in year 1 compared to year 2. There were low or nonsignificant relationships between the trait mean and the microenvironmental sensitivity of stem allometry.

Macro- and micro-environmental sensitivities were nonsignificantly associated with each other for stem height in both plantations (Table 4). The correlations between these two developmental aspects were more

Table 2 Broad-sense heritabilities (± SE) of trait means (M), and macro- (E_e) and micro-environmental sensitivity (E_ε) at Boardman and Clatskanie for stem growth traits in Families 331 and 355. >, < or ≈: Heritability is significantly greater, lower, or close for the left than right variables (pairwise tests for heritability differences between the two variables were based on the bootstrap method, see Efron 1982). Values not significantly different between two years for the same traits are denoted by the same letters

Trait	Family 331					Family 355				
	M		E_e	E_ε		M		E_e	E_ε	
				Boardman	Clatskanie				Boardman	Clatskanie
Height										
year 1	0.897 ± 0.035b	>	0.513 ± 0.079a	0.230 ± 0.115b	≈ 0.247 ± 0.099b	0.898 ± 0.049b	>	0.610 ± 0.092a	0.120 ± 0.122a	< 0.250 ± 0.120a
year 2	0.826 ± 0.041a	>	0.629 ± 0.083b	0.103 ± 0.121a	≈ 0.123 ± 0.117a	0.813 ± 0.046a	>	0.650 ± 0.098a	0.179 ± 0.123a	≈ 0.189 ± 0.129a
Basal area										
year 1	0.810 ± 0.037a	>	0.530 ± 0.080a	0.347 ± 0.114a	> 0.226 ± 0.120b	0.825 ± 0.054a	>	0.681 ± 0.114a	0.069 ± 0.141a	≈ 0.092 ± 0.140a
year 2	0.855 ± 0.033b	>	0.655 ± 0.074b	0.267 ± 0.107a	> 0.088 ± 0.136a	0.822 ± 0.060a	>	0.668 ± 0.101a	0.542 ± 0.099b	> 0.222 ± 0.123a
Volume index										
year 1	0.794 ± 0.050a	>	0.517 ± 0.080a	0.526 ± 0.120a	> 0.361 ± 0.113b	0.818 ± 0.052a	>	0.693 ± 0.103a	0.106 ± 0.124a	≈ 0.060 ± 0.137a
year 2	0.843 ± 0.035b	>	0.716 ± 0.055b	0.484 ± 0.109a	> 0.102 ± 0.129a	0.818 ± 0.048a	>	0.736 ± 0.087b	0.555 ± 0.108b	> 0.236 ± 0.124b
Stem allometry										
year 1	0.775 ± 0.058a	>	0.674 ± 0.076b	0.120 ± 0.118a	< 0.558 ± 0.110b	0.737 ± 0.051a	>	0.573 ± 0.112b	0.203 ± 0.130b	> 0.051 ± 0.147b
year 2	0.821 ± 0.037b	>	0.430 ± 0.105a	0.244 ± 0.120b	> 0.101 ± 0.140a	0.770 ± 0.064a	>	0.378 ± 0.161a	0.087 ± 0.192a	< 0.509 ± 0.118a

Table 3 The effective number of additive-dominant loci (± SE) underlying variation in trait means (M), and macro- (E_e) and micro-environmental sensitivity (E_ε) at Boardman and Clatskanie for stem growth traits in F_2 Families 331 and 355

Trait	Family 331				Family 355			
	M	E_e	E_ε		M	E_e	E_ε	
			Boardman	Clatskanie			Boardman	Clatskanie
Height								
year 1	1 ± 0.3	2 ± 0.3	30 ± 3.3	20 ± 1.9	1 ± 0.4	2 ± 0.4	30 ± 3.4	21 ± 2.2
year 2	1 ± 0.4	1 ± 0.2	12 ± 1.4	5 ± 0.4	1 ± 0.5	1 ± 0.3	12 ± 1.9	7 ± 0.9
Basal area								
year 1	3 ± 0.6	3 ± 0.6	31 ± 3.0	13 ± 1.2	3 ± 0.6	3 ± 0.6	31 ± 3.3	12 ± 1.3
year 2	2 ± 0.4	2 ± 0.3	10 ± 1.1	9 ± 0.8	2 ± 0.5	2 ± 0.4	11 ± 1.9	9 ± 0.9
Volume index								
year 1	4 ± 0.6	4 ± 0.7	30 ± 3.4	5 ± 0.5	4 ± 0.7	4 ± 0.8	29 ± 3.9	5 ± 0.6
year 2	2 ± 0.5	2 ± 0.5	12 ± 1.0	5 ± 0.6	2 ± 0.6	2 ± 0.5	11 ± 1.1	5 ± 0.7
Stem allometry								
year 1	1 ± 0.3	1 ± 0.2	6 ± 0.7	2 ± 0.4	1 ± 0.3	1 ± 0.3	8 ± 0.7	2 ± 0.6
year 2	1 ± 0.4	1 ± 0.3	4 ± 0.5	7 ± 1.0	1 ± 0.5	1 ± 0.3	6 ± 0.5	6 ± 1.1

tight for basal area and volume index, especially in year 2, at Boardman compared with Clatskanie. Stem allometry tended to display nonsignificant relationships between macro- and micro-environmental sensitivities at Boardman, whereas negative increased correlations were found at Clatskanie.

As expected, mean values of two functionally related traits, stem height and basal area, had very tight genetic correlations, which were larger than those between the macro- or micro-environmental sensitivities of these two traits (Table 5). For all these three development variables, stem height showed increased correlations with basal area from years 1 to 2. There were negative genetic correlations between two functionally unrelated traits, stem volume and allometry, in terms of both means and macroenvironmental sensitivities (Table 5). These two traits were not correlated with one another for microenvironmental sensitivity at both plantations.

While genetic correlations of mean values between the first and second year were large for all stem traits, close genetic associations of macroenvironmental sensitivities between the 2 years were limited to basal area and volume index (Table 6). This was also true for microenvironmental sensitivity, but its age-age correlations were smaller than those of macroenvironmental sensitivity. For these two traits, larger age-age correlations were found for microenvironmental sensitivity at Boardman than Clatskanie.

Discussion

Macro- and micro-environmental sensitivities, as synonymous terms of phenotypic plasticity and developmental instability, respectively, have long been considered to have an underlying genetic basis (Gupta and Lewontin 1982; Scheiner and Lyman 1991; Scheiner et al. 1991; Clarke 1993; Via 1993). However, because of limitations of study materials, most investigations on the genetic variation of these two components of development have focused only on comparisons at the species, population, and family level (Bradshaw 1965; Schlichting 1986). Thus, it has been impossible in these studies to estimate the genetic parameters of plastic response of environments. Although the importance of genetically identical materials, such as clonal replicates, in examining this issue has been recognized by some authors (e.g., Sultan and Bazzaz 1993), none of their studies have offered heritability estimates of either phenotypic plasticity or developmental instability.

The two large-cloned pedigrees allowed for the genetic dissection of macro- and micro-environmental sensitivity. In both F_2 families, macroenvironmental sensitivities had significantly different genetic determination from trait means across Boardman and Clatskanie. The values of broad-sense heritabilities for macroenvironmental sensitivities ranged from 0.45 to 0.75, consistently lower than those for trait means. Despite moderate or high genetic control, there were quite few QTLs underlying phenotypic plasticities and trait means, as estimated by a biometric method. Unlike macroenvironmental sensitivity, microenvironmental sensitivity, occuring within the same genotype in a similar environment, is under low genetic control (see also Clarke 1993), but is affected by numerous QTLs. The heritabilities and the underlying QTL numbers for microenvironmental sensitivity were a function of environment, with higher values at continental Boardman than maritime Clatskanie.

Given the high homozygosity of the two original parents for Family 331 [the estimate of homozygosity is around 80% in either parent (Bradshaw et al. 1994)], this pedigree is suitable to test the hypothesis that phenotypic plasticity or developmental instability is inversely related to genic heterozygosity (Lerner 1954). This relationship has been attributed to the greater capacity of heterozygotes to buffer development against perturbation due to some innate biochemical and

Table 4 Genetic correlations (± SE) among trait means (M), and macro-(E_e) and micro-environmental sensitivity (E_ε) at Boardman and Clatskanie for stem growth traits in F_2 Families 331 and 355. >, < or ≈: Genetic correlation is significantly greater, lower, or close for the left than right variables

Trait	Family 331						Family 355					
	M vs E_e	M vs E_ε			E_e vs E_ε		M vs E_e	M vs E_ε			E_e vs E_ε	
		Boardman		Clatskanie	Boardman		Clatskanie		Boardman		Clatskanie	Boardman		Clatskanie
Height														
Year 1	−0.269 ± 0.090	0.083 ± 0.103	<	0.121 ± 0.111	0.036 ± 0.123	<	−0.151 ± 0.107	−0.000 ± 0.141	0.170 ± 0.110	≈	0.175 ± 0.121	0.090 ± 0.129	≈	0.033 ± 0.130
Year 2	0.086 ± 0.102	0.053 ± 0.121	<	0.153 ± 0.114	0.059 ± 0.120	≈	0.059 ± 0.123	0.175 ± 0.120	0.034 ± 0.137	≈	0.104 ± 0.123	0.027 ± 0.136	≈	−0.075 ± 0.132
Incr. year 2	0.212 ± 0.097	0.059 ± 0.110	<	0.120 ± 0.120	0.026 ± 0.120	≈	−0.044 ± 0.124	0.213 ± 0.103	0.039 ± 0.128	<	0.150 ± 0.119	0.013 ± 0.140	<	−0.173 ± 0.111
Basal area														
Year 1	0.848 ± 0.034	0.533 ± 0.050	>	0.454 ± 0.053	0.521 ± 0.057	>	0.228 ± 0.105	0.880 ± 0.048	0.480 ± 0.098	>	0.396 ± 0.102	0.455 ± 0.072	>	0.179 ± 0.110
Year 2	0.603 ± 0.041	0.413 ± 0.061	≈	0.420 ± 0.058	0.432 ± 0.064	>	0.030 ± 0.121	0.716 ± 0.062	0.318 ± 0.105	>	0.381 ± 0.103	0.399 ± 0.086	>	0.074 ± 0.135
Volume ind.														
Year 1	0.895 ± 0.040	0.600 ± 0.049	>	0.452 ± 0.056	0.595 ± 0.050	>	0.222 ± 0.111	0.930 ± 0.037	0.575 ± 0.064	>	0.439 ± 0.085	0.590 ± 0.074	<	0.275 ± 0.109
Year 2	0.819 ± 0.039	0.486 ± 0.064	≈	0.501 ± 0.050	0.534 ± 0.051	>	0.161 ± 0.119	0.880 ± 0.041	0.369 ± 0.073	≈	0.369 ± 0.095	0.421 ± 0.080	>	0.133 ± 0.114
Stem allom.														
Year 1	−0.469 ± 0.054	0.192 ± 0.118	<	0.280 ± 0.104	0.089 ± 0.125	<	−0.342 ± 0.098	−0.467 ± 0.077	0.097 ± 0.126	<	0.016 ± 0.136	0.171 ± 0.110	≈	−0.143 ± 0.110
Year 2	−0.307 ± 0.067	0.191 ± 0.117	>	0.115 ± 0.125	0.075 ± 0.124	<	−0.226 ± 0.108	−0.266 ± 0.104	0.320 ± 0.110	>	0.172 ± 0.121	0.006 ± 0.143	≈	−0.025 ± 0.150

physiological superiority. It has been supported by many empirical studies (Dobzhansky and Levene 1955; Mitton and Grant 1984; Palmer and Strobeck 1986) and also considered as a fundamental assumption of some quantitative genetic models (Gillespie and Turelli 1989; Zhivotovsky and Gavrilets 1992). More recently, the relationship was theoreticallty derived by Gavrilets and Hastings (1994). However, our results tend to contradict this hypothesis since the highly heterozygous F_1 hybrids have a lower developmental homeostasis (Lerner 1954) than the highly homozygous *P. trichocarpa* and *P. deltoides* parents, especially for radial and volume growth. In their QTL analysis of a subset of Family 331, Bradshaw and Stettler (1995) found no significant correlation between stem growth and marker heterozygosity. Thus, from a high positive correlation between the mean values and macroenvironmental sensitivities of basal area and volume index, one cannot predict that the heterozygotes are necessarily less plastic in those traits. Furthermore, if phenotypic plasticity or developmental instability was a function of the number of heterozygous loci, then it should be positively genetically correlated between any pair of traits. However, the macro- or micro-environmental sensitivity of a trait was strongly positively associated with that of another trait only if the two traits were positively correlated. For example, both macro- and micro-environmental sensitivities of stem height were largely correlated with those of basal area, whereas stem volume was only slightly correlated with stem allometry in terms of these two developmental variables.

There was no consistent relationship between phenotypic plasticity and developmental instability. These two variables were more tightly correlated with each other for basal area and volume index at Boardman than at Clatskanie. Yet, they displayed a nonsignificant correlation for stem height at both Boardman and Clatskanie. For stem allometry, there is a change in the sign of their correlations over the two environments. Hence, phenotypic plasticity and developmental instability may be two different variables that are controlled by different genetic systems, not as predicted by the overdominance model. Similar conclusions were also obtained in earlier studies (Waddington 1960; Perkins and Jinks 1973; Santiago et al. 1989; Scheiner et al. 1991).

Developmental instability most likely results from the breakdown of genomic coadaptation that involves both a relational balance of alleles between chromosomes and an internal balance of loci within chromosomes (Thoday 1955). This breakdown may happen when an organism is moved to a novel environment or when a cross is made between two species that have diverged for a long time (Clarke 1993). In the present study, the *P. trichocarpa* parent shows a high developmental stability for stem height at Clatskanie. This is not surprising since this species is native to this environment in which many coadapted genes are harmoniously affecting height growth (see Bradshaw and Stettler 1995). For the *P. deltoides* parents from Illinois and the F_1

Table 5 Genetic correlations (± SE) among stem growth traits over means (M), and macro- (E_e) and micro-environmental sensitivity (E_ε) at Boardman and Clatskanie in F_2 Families 331 and 355

Trait relationship	Family 331				Family 355			
	M	E_e	E_ε		M	E_e	E_ε	
			Boardman	Clatskanie			Boardman	Clatskanie
Height-Area								
year 1	0.797 ± 0.051	0.349 ± 0.089	0.329 ± 0.092	0.148 ± 0.105	0.846 ± 0.055	0.512 ± 0.092	0.202 ± 0.102	0.351 ± 0.098
year 2	0.827 ± 0.043	0.668 ± 0.054	0.447 ± 0.084	0.449 ± 0.087	0.804 ± 0.064	0.693 ± 0.087	0.458 ± 0.087	0.411 ± 0.084
Volume-Allometry								
year 1	−0.112 ± 0.120	−0.274 ± 0.098	−0.012 ± 0.136	0.001 ± 0.125	−0.166 ± 0.130	−0.296 ± 0.118	0.052 ± 0.130	−0.017 ± 0.123
year 2	−0.274 ± 0.100	−0.138 ± 0.101	0.073 ± 0.135	0.128 ± 0.106	−0.222 ± 0.121	−0.187 ± 0.134	0.075 ± 0.131	0.022 ± 0.013

Table 6 Genetic correlations (± SE) between stem growth traits at years 1 and 2 over means (M), and macro- (E_e) and micro-environmental sensitivity (E_ε) at Boardman and Clatskanie in F_2 Families 331 and 355

Trait relationship	Family 331				Family 355			
	M	E_e	E_ε		M	E_e	E_ε	
			Boardman	Clatskanie			Boardman	Clatskanie
Height increment	0.750 ± 0.050	0.113 ± 0.113	0.155 ± 0.106	0.238 ± 0.094	0.640 ± 0.072	0.306 ± 0.103	0.085 ± 0.120	0.240 ± 0.113
Basal area	0.854 ± 0.042	0.705 ± 0.054	0.539 ± 0.056	0.309 ± 0.083	0.761 ± 0.081	0.621 ± 0.058	0.402 ± 0.064	0.348 ± 0.102
Volume index	0.857 ± 0.041	0.751 ± 0.051	0.677 ± 0.042	0.556 ± 0.054	0.779 ± 0.083	0.696 ± 0.062	0.396 ± 0.070	0.356 ± 0.097
Stem allometry	0.650 ± 0.064	0.171 ± 0.102	0.265 ± 0.097	0.051 ± 0.120	0.674 ± 0.075	0.217 ± 0.119	0.058 ± 0.131	0.022 ± 0.140

hybrids, low developmental stability may result from reactions to the new environments and from a breakdown of coadapted gene complexes, respectively. However, in order to validate these interpretations, a large sample size is required.

The other two hypotheses about phenotypic plasticity are the pleiotropy and epistasis model. The pleiotropy model assumes that plasticity is a function of the differential expression of the same gene in different environments (Via and Lande 1985, 1987; Gomulkiewicz and Kirkpatrick 1992; Via 1993). Some degree of genetic independence between traits expressed in different environments results from two genetic mechanisms: (1) different sensitivities of the same alleles to the environments, and (2) environment-specific gene expression (Schmalhausen 1949). The epistasis model predicts that (1) plasticity is a trait independent of the trait mean, and (2) their are genes for plasticity which are separate from those for the trait mean (Jinks and Pooni 1988; Scheiner and Lyman 1991; Scheiner et al. 1991). In this model, plasticity is due to genes that determine the magnitude of response to environmental effects which interact with genes that determine the average expression of the trait.

The genetic correlation between trait mean and macroenvironmental sensitivity can be used as a criterion to test which hypothesis is more important in affecting the phenotypic plasticity of growth traits. If this correlation equals one, these two developmental aspects have a completely identical genetic basis and cannot be regarded as two different variables. Thus, the pleiotropy model determines phenotypic plasticity. However, if this correlation equals zero, then trait mean and macroenvironmental sensitivity should be viewed as two independent variables under the control of different genetic systems. In this case, the epistasis model can effectively interpret plasticity. In the present study, stem height showed nonsignificant genetic correlations between trait mean and macroenvironmental sensitivity. Therefore, for this trait, phenotypic plasticity is independent of trait mean and there may be some genetic loci underlying phenotypic plasticity which are different from those for trait mean (the epistasis model). Schlichting and Pigliucci (1993) suggested that the loci for plasticity may be regulatory loci, through the action of which the expression of multiple structural genes is mediated. Such regulatory loci have been observed in all five biological kingdoms (Goransson et al. 1989; Dixon and Harrison 1990; Liu and Ambros 1991; Song et al. 1991). Significant genetic correlations between trait mean and sensitivity were detected for stem basal area and volume index, which may demonstrate the importance of the pleiotropy model for their phenotypic plasticity. It should be noted that neither of these two models can exclusively explain phenotypic plasticity because no genetic correlation was found to exactly equal one or zero.

From a statistical point of view, the correlation between trait mean and macroenvironmental sensitivity is virtually a function of the difference of genetic variances between the two environments. [this is because

$\mathrm{Cov}_g(m, e) = V_{g1} - V_{g2}$ where $\mathrm{Cov}_g(m, e)$ is the genetic covariance between trait mean and macroenvironmental sensitivity of a trait, and V_{g1} and V_{g2} are the genetic variance of the trait in two different environments, respectively.] Thus, the two models for phenotypic plasticity, pleiotropy and epistasis, can be related to Cockerham's (1963) theory that the variance of genotype × environment interactions is partitioned into two subcomponents due to the heterogeneity of genetic variance and to the lack of genetic correlation across two environments. Whereas the first subcomponent is affected by the pleiotropy model, the second subcomponent is associated with the epistasis model.

In this study, age is detected to influence the variation of macro- and micro-environmental sensitivities, and their genetic relationships with trait means. As an important type of plasticity, ontogenetic reaction norm and its interactions with environmental reaction norm should receive more attention in the future (e.g., Pigliucci and Schlichting 1995).

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