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Using clonal replicates to explore genetic variation in a perennial plant species

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Summary. An experimental design is presented for estimating genetic parameters using a family structure with clonally replicated individuals. This experimental design provides a technique to quantify genetic variation in a population, with partial separation of additive, dominance and epistatic gene action. Our method is offered as an alternative to techniques for estimating epistatic gene action that require several generations and/or inbreeding. Such methods are not particularly useful for long-lived perennials with long generation cycles. An example of the analysis is given with a forest tree species, *Populus deltoides* Bartr., and parameter estimates are presented for traits measured over 8 years.

Key words: Additive genetic variance – Dominance genetic variance – Epistatic genetic variance – Eastern cottonwood

Introduction

Traditionally, genetic improvement of metric traits for plant species has relied mainly, if not exclusively, on changes in frequencies for genes with independent and additive effects. Selection response for traits conditioned by genes with additive effect is predictable, and cumulative gains over generations are easily obtained through a variety of selection and breeding programs (Falconer 1960; Hallauer and Miranda 1981). Residual genetic effects, including intra-locus (dominance) and inter-locus (epistasis) interactions, are often pooled under a single classification, termed non-additive effects. Selection response for metric traits based on non-additive effects is not predictable from parental performance and is largely non-cumulative over generations. The presence of non-additive genetic effects and their value in achieving genetic gain are well documented for annual crop species such as maize (*Zea mays* L.), although disagreement remains as to the relative contribution of dominance and epistatic effects (Hallauer and Miranda 1981). Inference about non-additive genetic effects for perennial plants is usually limited to dominance effects, due to difficulties in applying experimental methods that require several generations of mating or inbreeding (Namkoong 1979); application of such methods is especially difficult for species with long generation intervals. Further resolution of non-additive genetic variance into dominance and epistatic components is valuable both for design of efficient methods for their utilization in plant improvement and for estimating their potential bias for programs that utilize additive genetic effects.

Variance among clones provides an estimate of total genetic variance and has been widely studied (e.g., Burton and Devane 1953; Keller and Likens 1955; Libby 1962; Libby and Jund 1962; Mohn and Randall 1973). However, only a few reseachers have recognized its full potential to more completely explore additive and non-additive gene action (Comstock et al. 1958; Cooper and Randall 1973; Burdon and Shelbourne 1974; Stonecypher and McCullough 1986).

In this paper, we present an experimental design that uses information from both clonal replicates and family structure to estimate genetic parameters. Our design allows estimation of additive and non-additive genetic variances, with partial resolution of the latter into dominance and epistatic components. We demonstrate the application of this model using experimental data for a perennial tree species (*Populus deltoides* Bartr.). A second objective of our paper is to examine changes in genetic parameters associated with ontogeny (Atchley 1984).

Table 1. Analysis of variance and expected mean squares for a factorial mating design with clonally replicated individuals within full-sib families

Source	Degrees of freedom	Expected mean squares
Location (L)	l−1	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nmc\sigma^2_{FB(L)}+nfc\sigma^2_{MB(L)}+nb\sigma^2_{LC(MF)}+nbc\sigma^2_{LMF}$ $+nmbc\sigma^2_{LF}+nfbc\sigma^2_{LM}+nfmc\sigma^2_{B(L)}+nfmcb\sigma^2_{L}$
Blocks (B)/L	b−1	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nmc\sigma^2_{FB(L)}+nfc\sigma^2_{MB(L)}+nfmc\sigma^2_{B(L)}$
Males (M)	m−1	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nfc\sigma^2_{MB(L)}+nb\sigma^2_{LC(MF)}+nbc\sigma^2_{LMF}+nfbc\sigma^2_{LM}+nlb\sigma^2_{C(MF)}$ $+nlbc\sigma^2_{MF}+nlbcf\sigma^2_{M}$
Females (F)	f−1	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nmc\sigma^2_{FB(L)}+nb\sigma^2_{LC(MF)}+nbc\sigma^2_{LMF}+nmbc\sigma^2_{LF}+$ $nlb\sigma^2_{C(MF)}+nlbc\sigma^2_{MF}+nlbcm\sigma^2_{F}$
M × F	(m−1)(f−1)	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nb\sigma^2_{LC(MF)}+nbc\sigma^2_{LMF}+nlb\sigma^2_{C(MF)}+nlbc\sigma^2_{MF}$
Clones (C)/(MF)	(c−1)mf	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nb\sigma^2_{LC(MF)}+nlb\sigma^2_{C(MF)}$
L × M	(l−1)(m−1)	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nfc\sigma^2_{MB(L)}+nb\sigma^2_{LC(MF)}+nbc\sigma^2_{LMF}+nfbc\sigma^2_{LM}$
L × F	(l−1)(f−1)	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nmc\sigma^2_{FB(L)}+nb\sigma^2_{LC(MF)}+nbc\sigma^2_{LMF}+nmbc\sigma^2_{LF}$
L × M × F	(l−1)(m−1)(f−1)	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nb\sigma^2_{LC(MF)}+nbc\sigma^2_{LMF}$
L × C/(MF)	(l−1)(c−1)mf	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nb\sigma^2_{LC(MF)}$
M × B/L	(m−1)(b−1)l	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nfc\sigma^2_{MB(L)}$
F × B/L	(f−1)(b−1)l	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}+nmc\sigma^2_{FB(L)}$
M × F × B/L	(m−1)(f−1)(b−1)l	$\sigma^2+n\sigma^2_{B(L)C(MF)}+nc\sigma^2_{MFB(L)}$
(B/L)(C/(MF))	(b−1)l(c−1)mf	$\sigma^2+n\sigma^2_{B(L)C(MF)}$
among ramets/plot	$\sum$(n−1)	σ^2

Experimental design and analysis of variance

The function of a mating design is to provide the required genetic structure among individuals in the population for estimating genetic parameters. Several mating designs are available that could fulfill the above requirement (Hallauer and Miranda 1981). We used a factorial mating design (NC State Design II; Cockerham 1961) where m males are mated to f females to produce c individuals per full sib family. Each individual is then cloned to produce r ramets or clonal copies of each individual (Shaw and Hood 1985).

The field design provides the method for partition of components of genetic and environmental variance. Several experimental locations are chosen at random to represent the set of possible environments. The replicated study is then established at each location with each block containing n ramets of each cloned individual in each of the full sib families. Analysis using a randomized complete block design with l locations and b complete blocks at each location results in the following model:

$$\begin{aligned} X_{ijkpqt} = {} & u+L_i+B_{j(i)}+M_k+F_p+(MF)_{kp}+C_{q(kp)} \\ & +(LM)_{ik}\,(LF)_{ip}+(LMF)_{ikp}+(LC)_{iq(kp)} \\ & +(MB)_{kj(i)}+(FB)_{pj(i)}+(MFB)_{kpj(i)} \\ & +(CB)_{q(kp)j(i)}+E_{ijkpqt} \end{aligned} \quad (1)$$

where $i=1\ldots l$, $j=1\ldots b$, $k=1\ldots m$, $p=1\ldots f$, $q=1\ldots c$, $t=1\ldots n$.

In the above model, X_{ijkpqt} is the value for ramet t of clone q from a cross between female p and male k, tested in block j at location i. A complete description of the elements of this model, together with the form of the analysis of variance and expected mean squares, is given in Table 1. All effects are considered to be random in this model.

Genetic model

The genetic model can now be specified and equated to variance components in the analysis of variance. Transla-

tion of the experimental components of variance given in Table 1 to causal components requires the following assumptions (Comstock et al. 1958):

(1) Regular diploid behavior at meiosis.
(2) No cytoplasmic or maternal effects.
(3) No correlation of genotypes at separate loci. This implies no linkage among genes affecting any single character or that, where linkage existed, the distribution of genotypes was as expected in the absence of linkage.
(4) That the distribution of genotypes in the parents was of the nature to be expected in a random sample from a random breeding population.

One departure from the assumption given by Comstock et al. (1958) is that our model allows some inference in the presence of epistasis. We add one additional assumption usually necessary when testing vegetatively propagated materials:

(5) No C effects (Libby and Jund 1962). C effects arise as common environmental effects associated with specific clones. This results in a positive, non-genetic covariance within clones and causes estimates of between-clone variance to be inflated (Libby and Jund 1962).

When these assumptions are fulfilled, the variance components for our factorial experiment have the following genetic expectations (Becker 1984):

$$\hat{\sigma}^2_M = \hat{\sigma}^2_F = 1/4\,\hat{V}_A + 1/16\,\hat{V}_{AA} + \ldots \quad (2)$$

$$\hat{\sigma}^2_{MF} = 1/4\,\hat{V}_D + 1/8\,\hat{V}_{AA} + 1/8\,\hat{V}_{AD} + 1/16\,\hat{V}_{DD} + \ldots \quad (3)$$

$$\hat{\sigma}^2_{C(MF)} = 1/2\,\hat{V}_A + 3/4\,\hat{V}_D + 3/4\,\hat{V}_{AA} + 7/8\,\hat{V}_{AD} + 15/16\,\hat{V}_{DD} + \ldots \quad (4)$$

where

$\hat{V}_A$ = additive genetic variance
$\hat{V}_D$ = dominance genetic variance
$\hat{V}_{AA}$, $\hat{V}_{AD}$, $\hat{V}_{DD}$ = epistatic genetic variance due to additive × additive effects, additive × dominance effects, and dominance × dominance effects, with similar notation for higher order interactions

Estimates of genetic parameters are then calculated as follows:

$$\hat{V}'_A = 2(\hat{\sigma}^2_M + \hat{\sigma}^2_F) = \hat{V}_A + 1/4\,\hat{V}_{AA} + \ldots \quad (5)$$

$$\hat{V}'_D = 4(\hat{\sigma}^2_{MF}) = \hat{V}_D + 1/2\,\hat{V}_{AA} + 1/2\,\hat{V}_{AD} + 1/4\,\hat{V}_{DD} + \ldots \quad (6)$$

$$\hat{V}'_I = \hat{\sigma}^2_{C(MF)} - (\hat{\sigma}^2_M + \hat{\sigma}^2_F) - 3\hat{\sigma}^2_{MF} = 1/4\,\hat{V}_{AA} + 1/2\hat{V}_{AD} + 3/4\hat{V}_{DD} + \ldots \quad (7)$$

Total genetic variance ($\hat{V}_G$) and phenotypic variance ($\hat{V}_P$) are then calculated as:

$$\hat{V}_G = \hat{\sigma}^2_M + \hat{\sigma}^2_F + \hat{\sigma}^2_{MF} + \hat{\sigma}^2_{C(MF)} \quad (8)$$

$$\hat{V}_P = \hat{V}_G + \hat{\sigma}^2_{LM} + \hat{\sigma}^2_{LF} + \hat{\sigma}^2_{LMF} + \hat{\sigma}^2_{LC(MF)} + \hat{\sigma}^2_{MB(L)} + \hat{\sigma}^2_{FB(L)} + \hat{\sigma}^2_{MFB(L)} + \hat{\sigma}^2_{B(L)C(MF)} + \hat{\sigma}^2 \quad (9)$$

Equations (5) and (6) provide estimates of $\hat{V}_A$ and $\hat{V}_D$ that are confounded with fractional components of epistatic variances. These estimates are identical to those obtained for factorial analysis without clonal replicates, for which epistatic components are usually ignored (Cockerham 1954). Equation (7) is composed entirely of epistatic components, although estimation of the contribution of epistasis to the total phenotypic variance is not possible without additional information about gene action. Note that multiplying Eq. (7) by 4 will provide an upper limit estimate of the contribution of epistasis, with coefficients for all epistatic components greater than or equal to 1.

Precision of the estimates constitutes a major concern with this technique (Comstock et al. 1958), as with all estimates of genetic parameters. Standard errors of the variance components con be calculated (Becker 1984); and as always, their precision is a function of the experimental design.

Although our model includes the assumption of no C effects, their presence in clonal material is real and can cause significant bias (Burdon and Shelbourne 1974). We used an experimental procedure developed to minimize this problem: several primary ramets (donor plants) were developed for each genotype, which then served as the source for subsequent cloning (Libby and Jund 1962; Wilcox and Farmer 1968; Foster et al. 1984). These primary ramets then serve as a level of blocking in the experimental design.

One caution in interpreting genetic variances is that the variance is related to the mean of the trait. This is a particular problem when examining the changes in genetic parameters associated with growth (ontogeny) for a trait (Atchley 1984). For this reason, we express genetic variances in the example as the genetic coefficient of variation (Comstock et al. 1958), which is simply the square root of the genetic variance divided by the mean of the trait.

An example with poplar clones: materials and methods

Seven parent trees of *P. deltoides*, considered as random samples from the natural population, were chosen from an area within 80 km of Stoneville, Mississippi. [Although there is some evidence of their superiority for growth (Cooper and Randall 1973)]. These parents were mated using a factorial design with 4 male and 3 female parents. Seeds were germinated and resultant seedlings planted in a nursery where they were vegetatively propagated. No selection was invoked at the seedling stage.

In February 1976, a clonal field trail was established near Fitler, Mississippi, using dormant, 46 cm long, unrooted cuttings. The cuttings were planted at a spacing of 3.7 × 3.7 m in a randomized complete block design with 4 replications and a split-plot configuration. Families served as the main plots while cloned individuals were randomized within their respective family. Approximately 20 cloned individuals were planted per family

with a single ramet per clone in each replication. Cultural treatments closely followed those described by McKnight (1970).

The trees were measured several times during the first 8 years of growth. The traits scored included total height at ages 1 (HT1), 2 (HT2), 4 (HT4) and 8 (HT8) years; diameter at 1.4 m height at ages 3 (DBH3), 4 (DBH4) and 8 (DBH8) years; and merchantible volume to a 7.6 cm top (Mohn and Krinard 1971) at ages 4 (VOL4) and (VOL8) years.

The analysis of variance for our example differed from the full design given in Table 1; data were available for only a single location and only a single tree per plot within each replication. The appropriate form of the analysis of variance for our experiment is given in Table 2. Coefficients of the variance components were adjusted to compensate for some missing plots (Searle 1971). Components of variance were calculated by equating observed mean squares to expected mean squares and solving the resulting equations. The alteration of the field test design changes the procedure for calculating model components and has some effect on the translation of model components into causal components as described in Eq. (5), (6) and (7). the estimates of genetic components $\hat{V}'_A$, $\hat{V}'_D$ and $\hat{V}'_I$ in this example are inextricably confounded with their interactions with location effects. Standard errors of the variance components were calculated following Becker (1984).

Table 2. Analysis of variance and expected mean squares for a clonally replicated genetic test of *Populus deltoides*

Source	Expected mean squares
Blocks (B)	$\sigma^2 + c\sigma^2_{MFB} + cm\sigma^2_{FB} + cf\sigma^2_{MB} + cfm\sigma^2_B$
Males (M)	$\sigma^2 + c\sigma^2_{MFB} + cf\sigma^2_{MB} + b\sigma^2_{C(MF)} + bc\sigma^2_{MF} + bcf\sigma^2_M$
Females (F)	$\sigma^2 + c\sigma^2_{MFB} + cm\sigma^2_{FB} + b\sigma^2_{C(MF)} + bc\sigma^2_{MF} + bcm\sigma^2_F$
M × F	$\sigma^2 + c\sigma^2_{MFB} + b\sigma^2_{C(MF)} + bc\sigma^2_{MF}$
Clones (C)/(MF)	$\sigma^2 + b\sigma^2_{C(MF)}$
M × B	$\sigma^2 + c\sigma^2_{MFB} + cf\sigma^2_{MB}$
F × B	$\sigma^2 + c\sigma^2_{MFB} + cm\sigma^2_{FB}$
M × F × B	$\sigma^2 + c\sigma^2_{MFB}$
B × C/(MF)	σ^2

Results and discussion

The trees in the study are growing quite well, averaging 20 m tall after 8 years, and survival (97%) is also quite high. Parental averages for the various traits are presented in Table 3.

The variance due to female parents is non-significant ($p > 0.05$) for all traits; conversely, male parents exhibit significant variation for all traits except HT1 (Table 4). Such a discrepancy is not unexpected given the small sample of parents, and leads to the decision to pool variance components due to male and female parents to obtain a better estimate of genetic parameters.

Several reseachers have also found significant additive genetic variation for *P. deltoides*, although the results were uncertain for one author. Farmer and Wilcox (1966) and Farmer (1970) found significant family variability for first and second year height and second year diameter; while Ying and Bagley (1976) detected significant family differences for seventh year height and diameter as well as for several bark, phenology and stem form traits. Foster (1985), on the other hand, noted significant family variability only for fourth year height in one population; whereas height in a second population as well as diameter, volume and survival in both populations displayed non-significant family variation.

We detected no significant variation for the interaction between male and female parents (Table 4). This finding is consistent with the results of Cooper and Randall (1973) using the same parents. As discussed above, the sample size of parents is small, and the non-significant results may not reflect an accurate estimate of the genetic parameter for the population.

The clone-within-family source of variation is highly significant for all traits in our study (Table 4). Cooper and Randall (1973), Ying and Bagley (1976), and Foster (1985) detected significant variation for clones-within-families for all traits studied with the execption of survival in one population for Foster (1985).

Table 3. Average values for several traits measured in a genetic test of *Populus deltoides*

Trait	Overall	Female parent			Male parent			
		DF1	DF12	DF47	DF16	DF43	ST66	ST107
Height, age 1 (m)	2.95	2.87	3.16	2.81	2.90	2.79	2.99	3.12
Height, age 2 (m)	6.34	6.10	6.71	6.21	6.25	5.78	6.85	6.53
Height, age 4 (m)	13.36	13.05	13.91	13.10	13.22	12.26	14.66	13.31
Height, age 8 (m)	19.97	19.76	20.12	20.03	20.11	18.84	21.29	19.44
Diameter, age 3 (cm)	10.21	10.16	10.63	9.84	9.74	9.67	11.08	10.41
Diameter, age 4 (cm)	13.21	13.19	13.62	12.81	12.69	12.49	14.20	13.49
Diameter, age 8 (cm)	18.57	18.56	18.69	18.45	18.54	17.87	19.53	18.16
Volume, age 4 (m^3)	0.055	0.053	0.062	0.049	0.049	0.042	0.073	0.056
Volume, age 8 (m^3)	0.195	0.192	0.200	0.194	0.197	0.168	0.229	0.182

Table 4. Significance level[a], variance components, and standard errors (in *parentheses*) for sources of variation for several traits in a genetic test of *Populus deltoides*

Source of variation	Variance[b] component	Traits[c]								
		HT1	HT2	HT4	HT8	DBH3	DBH4	DBH8	VOL4	VOL8
Blocks (B)	$\hat{\sigma}^2_B$	0.0000^{NS} (0.0112)	0.0000^{NS} (0.0349)	0.0316^{NS} (0.1078)	0.1558^{NS} (0.1652)	0.0011^{NS} (0.0603)	0.0284^{NS} (0.0671)	0.0441^{NS} (0.0776)	0.0000^{NS} (0.00001)	0.0004^{NS} (0.00007)
Males (M)	$\hat{\sigma}^2_M$	0.0071^{NS} (0.0148)	0.1739** (0.1276)	0.8140** (0.5474)	1.0666** (0.7308)	0.3684* (0.3057)	0.5571** (0.4067)	0.5668** (0.3467)	0.00016** (0.00011)	0.00070** (0.00046)
Females (F)	$\hat{\sigma}^2_F$	0.0325^{NS} (0.0345)	0.0817^{NS} (0.0830)	0.1414^{NS} (0.1308)	0.0000^{NS} (0.0378)	0.0525^{NS} (0.0930)	0.0393^{NS} (0.0800)	0.0000^{NS} (0.0557)	0.00002^{NS} (0.00002)	0.0000^{NS} (0.00004)
M × F	$\hat{\sigma}^2_{MF}$	0.0057^{NS} (0.0092)	0.0149^{NS} (0.0249)	0.0000^{NS} (0.0379)	0.0000^{NS} (0.0789)	0.0887^{NS} (0.1064)	0.0287^{NS} (0.1011)	0.0000^{NS} (0.1407)	0.0000^{NS} (0.00001)	0.0000^{NS} (0.00009)
Clones (C)/(MF)	$\hat{\sigma}^2_{C(MF)}$	0.0245** (0.0075)	0.0536** (0.0169)	0.2280** (0.0461)	1.0725** (0.1815)	0.5431** (0.1081)	0.9812** (0.1718)	3.2891** (0.4645)	0.00015** (0.00003)	0.00221** (0.00031)
M × B	$\hat{\sigma}^2_{MB}$	0.0250* (0.0176)	0.0275^{NS} (0.0327)	0.0907^{NS} (0.0859)	0.1136^{NS} (0.1391)	0.0830^{NS} (0.1006)	0.0154^{NS} (0.0929)	0.0000^{NS} (0.1747)	0.00001^{NS} (0.00002)	0.0000^{NS} (0.00011)
F × B	$\hat{\sigma}^2_{FB}$	0.0341* (0.0230)	0.0656* (0.0499)	0.1393^{NS} (0.1094)	0.0000^{NS} (0.0827)	0.0085^{NS} (0.0641)	0.0041^{NS} (0.0767)	0.0000^{NS} (0.1730)	0.00001^{NS} (0.00002)	0.0000^{NS} (0.00011)
M × F × B	$\hat{\sigma}^2_{MFB}$	0.0253** (0.0126)	0.0769** (0.0357)	0.1809** (0.0825)	0.2704** (0.1543)	0.1994** (0.1111)	0.2269** (0.1374)	0.7852** (0.3984)	0.00004** (0.00002)	0.00043** (0.00023)
B × C/(MF)	$\hat{\sigma}^2$	0.1813 (0.0104)	0.4124 (0.0236)	0.8907 (0.510)	3.0023 (0.1721)	2.0664 (0.1184)	2.9420 (0.1686)	6.0352 (0.3459)	0.00044 (0.00003)	0.00404 (0.00023)

** Significant at $p \leq 0.01$
* Significant at $0.05 \geq p > 0.01$
NS Not significant at $p = 0.05$
[a] Synthetic F tests (Cochran 1951) were used in tesing the Blocks, Males, Females and Male × Female source
[b] The following coefficients were used to derive variance components: b = 3.5; c = 19.8; m = 3.1; f = 2.4; variance components with negative calculated value were assumed to equal zero
[c] Trait codes explained in text

Height growth appears to be conditioned mainly by genes with additive effects (Table 5). Their relative contribution to the total variance is stable over years, as evidenced by similar genetic coefficients of variaton for HT1 – HT8 (Table 5) (Comstock et al. 1958). This pattern differs from that suggested by Atchley (1984): a rapid increase in relative additive genetic variance to an initial peak with a subsequent decline during ontogeny. Small and non-significant levels of dominance variance are found for the first 2 years of height growth (Table 5). Coefficients of variation for dominance effects are 5% and 4% for height for the first 2 years, whereas coefficients for additive effects are 10% and 11% for the 2 years, respectively. Dominance variation for height was not detected after the second year. Estimates of epistatic components of variance are near zero for all height measurements, suggesting that estimates of additive and dominance variance for these traits are not biased. Note that the maximum total contribution for epistatic variance for year 8 height is $4(0.0059) = 0.024$ and is a small fraction of total variance. In their Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] study, Stonecypher and McCullough (1986) found that height at age 2 was governed by equivalent amounts of additive and epistatic variance with half as much dominance variance. Additive variance grew steadily through age 6, while epistasis declined to zero. Dominance variance increased at a faster rate than additive variance, until at age 6 dominance variance was 44% larger than additive variance.

Diameter growth more closely follows Atchley's (1984) model with an initial peak (DBH3) in additive variance for the genetic coefficient of variation (CV) of 9%, which then decreases to 6% by age 8 years (Table 5). While not statistically significant, dominance variance has a peak (genetic CV of 6%) at age 3 and then drops to 0 by age 8 years. Epistatic variance follows the reverse pattern, with a CV of 0 for age 3, increasing to 9% at age 8 years. At age 8 years, epistatic variance for diameter is more than double the additive genetic variance (Table 5). Stonecypher and McCullough (1986) noted a similar pattern with additive and dominance variance for diameter as with height, but with no evidence of epistasis.

Tree volume follows the same general pattern for additive and epistatic variance as for diameter. Additive variance is greatest at age 4 years with a genetic CV of 35% and drops almost in half (CV of 19%) by age 8 years (Table 5). The genetic CV for epistasis is 0 for volume at age 4 years and increases to 20 for age 8 years (Table 5). At age 8 years, the estimates for additive genetic variance and epistasis are essentially equal. Since only a fraction of the epistatic variance is estimated by $\hat{V}_I'$ [refer to equation (7)], total epistasis is actually much larger.

The most obvious weakness of our experimental results is inference based on a small sample of parents. This does not reflect a problem in the proposed experimental method, but does demonstrate that studies designed to estimate complicated genetic parameters will require substantial resources. In addition, the use of a single location in our study causes bias of the genetic parameters by the confounding of the parameters with their interaction with location effects.

There are obvious weaknesses in the procedure, but they are not insurmountable. The critical assumption of no C effects cannot be accepted uncritically and will cause bias in parameter estimation (Burdon and Shelbourne 1974). However, by utilizing the secondary cloning procedure (Libby and Jund 1962), this bias is minimized. Competition among trees in forest stand is likely

Table 5. Estimates of additive ($\hat{V}_A'$), dominace ($\hat{V}_D'$) and epistatic ($\hat{V}_I'$) genetic variance and genetic coefficient of variation (in *parentheses*) for a genetic test of *Populus deltoides*

Genetic parameter[a]	Traits[b]								
	HT1	HT2	HT4	HT8	DBH3	DBH4	DBH8	VOL4	VOL8
$\hat{V}_A'$	0.0792 (0.10)	0.5112 (0.11)	1.9108 (0.10)	2.1332 (0.07)	0.8418 (0.09)	1.1928 (0.08)	1.1336 (0.06)	0.00036 (0.35)	0.00140 (0.19)
$\hat{V}_D'$	0.0228 (0.05)	0.0596 (0.04)	0.0000 (0.00)	0.0000 (0.00)	0.3548 (0.06)	0.1148 (0.03)	0.0000 (0.00)	0.0000 (0.00)	0.0000 (0.00)
$\hat{V}_I'$	0.0000 (0.00)	0.0000 (0.00)	0.0000 (0.00)	0.0059 (0.00)	0.0000 (0.00)	0.2987 (0.04)	2.7223 (0.09)	0.0000 (0.00)	0.00151 (0.20)
$\hat{V}_G$	0.0698[c]	0.3241[c]	1.1834[c]	2.1391	1.0527[c]	1.6063	3.8559	0.00033[c]	0.00291
$\hat{V}_P$	0.3355	0.9065	2.4850	5.5254	3.4100	4.7947	10.6763	0.00083	0.00738

[a] Codes for genetic parameters explained in text

[b] Trait codes explained in text

[c] Difference between sum of genetic parameters and sum of variance components [see Eq. (8) in text for definiton of $\hat{V}_G$] due to calculated negative values for epistatic variance assumed to equal zero in Table 5

to bias genetic parameter estimates (Cooper and Ferguson 1977) as it does for other plants (Hamblin and Rosielle 1978). However, recent advances in genetic test analysis in forestry promise to at least partially remove these biases (Nance et al. 1983).

Standard techniques for evaluating the magnitude of epistasis require knowledge of exact genotype (Mather and Jinks 1977), which is rarely available for forest trees and other predominantly outcrossing crops. Such methods require several generations of controlled matings and are usuallly initiated with inbred lines, to control gene frequencies. Experiments spanning multiple generations are impractical for many long-lived perennial species. Further, application of methods requiring inbreeding of predominantly outcrossing species may result in violation of several important model assumptions. Although our experimental method provides only a partial description of epistatic genetic variance, parameters are estimated within a single generation and can provide useful first approximations for epistatic parameters. The current procedure provides one way to begin assessing non-additive gene action as well as additive gene action through onotgeny.

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References

Atchley WR (1984) Ontogeny, timing of development, and genetic variance-covariance structure. Am Nat 123:519–540

Becker WA (1984) Manual of quantitative genetics, 4th edn. Academic Enterprises, Pullman/WA

Burdon RD, Shelbourne RD (1974) The use of vegetative propagules for obtaining genetic information. NZ J For Sci 4: 418–425

Burton GW, DeVane EH (1953) Estimating heritability in tall fesque (*Festuca arundinacea*) from replicated clonal material. Agron J 45:478–481

Cochran WG (1951) Testing a linear relation among variances. Biometrics 7:17–32

Cockerham CC (1954) An extension of the concept of partitioning hereditary variance for analysis of covariance among relatives when epistasis is present. Genetics 39:859–882

Cockerham CC (1961) Estimation of genetic variances. In: Hanson WD, Robinson HF (eds) Statistical genetics and plant breeding. Proc Natl Acad Sci Natl Res Council USA 982: 53–94

Comstock RE, Kelleher T, Morrow EB (1958) Genetic variation in an asexual species, the garden strawberry. Genetics 43: 634–646

Cooper DT, Ferguson RB (1977) Removal of competition bias from forest genetics experiments. Proc 14th South For Tree Imp Conf, pp 70–77

Cooper DT, Randall WK (1973) Genetic differences in height growth and survival of cottonwood full-sib families. Proc 12th South For Tree Imp Conf, pp 70–77

Falconer DS (1960) Introduction to quantitative genetics. Ronald Press, New York

Farmer RE (1970) Genetic variation among open-pollinated progeny of eastern cottonwood. Silvae Genet 19:149–151

Farmer RE, Wilcox JR (1966) Variation in juvenile growth and wood properties in half-sib cottonwood families. In: Joint Proc 2nd Genetics Workshop of Soc Am For and 7th Lake States For Tree Imp Conf; USDA For Ser Res Paper NC-6, pp 1–4

Foster GS (1985) Genetic parameters for two eastern cottonwood poulations in the lower Mississippi Valley. Proc 18th South For Tree Imp Conf, pp 258–266

Foster GS, Campbell RK, Adams WT (1984) Heritability, gain, and C effects in rooting of western hemlock cuttings. Can J For Res 14: 628–638

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State Universtity Press, Ames/IA

Hamblin J, Rosielle AA (1978) Effect of intergenotypic competion on genetic parameter estimation. Crop Sci 18:51–54

Keller KR, Likens ST (1955) Estimates of heritability in hops, *Humulus lupulus*. Agron J 47:518–521

Libby WJ (1962) Estimation of variance components of internode length in a cloned population of Mimulus guttatus. Genetics 47:769–777

Libby WJ, Jund E (1962) Variance associated with cloning. Heredity 17: 533–540

Mather K, Jinks JL (1977) Introduction to biometrical genetics. Cornell University Press, New York/NY

McKnight JS (1970) Planting cottonwood cuttings for timber production in the South. USDA For Ser Res Paper S0-60, 17 pp

Mohn CA, Krinard FM (1971) Volume tables for small cottonwoods in plantations. USDA For Ser Res Note SO-113, 4 pp

Mohn CA, Randall WK (1973) Interaction of cottonwood clones with site and planting year. Can J For Res 3:329–332

Namkoong G (1979) Introduction to quantitive genetics in forestry. USDA For Ser Tech Bul No. 1588, 342 pp

Nance WL, Land SB, Daniels RF (1983) Concepts for analysis of intergenotypic competition in forest trees. Proc 17th South For Tree Imp Conf, pp 131–145

Searle SR (1971) Linear models. Wiley, New York/NY

Shaw DV, Hood JV (1985) Maximizing gain per effort by using clonal replicates in genetic tests. Theor Appl Genet 71: 392–399

Stonecypher RW, McCullough RB (1986) Estimates of additive and non-additive genetic variance from a clonal diallel of Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco. In: Proc Int Union For Res Org, Joint Mtg Working Parties Breed Theor, Prog Test, Seed Orch. Williamsburg/VA. Publ by NCSU-Industry Coop Tree Imp Pgm, pp 211–227

Wilcox JR, Farmer RE (1968) Heritability and C effects in early root growth of eastern cottonwood cuttings. Heredity 23: 239–245

Ying CC, Bagley WT (1976) Genetic variation of eastern cottonwood in an eastern Nebraska provenance study. Silvae Genet 25:67–73