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THE IMPACT OF MATERNAL EFFECTS ON ADAPTIVE EVOLUTION: COMBINING QUANTITATIVE GENETICS AND PHENOTYPIC SELECTION IN A NATURAL PLANT POPULATION

By

Denise Annette Thiede

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ABSTRACT

THE IMPACT OF MATERNAL EFFECTS ON ADAPTIVE EVOLUTION: COMBINING QUANTITATIVE GENETICS AND PHENOTYPIC SELECTION IN A NATURAL PLANT POPULATION

By

Denise Annette Thiede

When a mother influences the phenotypic expression of traits in her offspring, the direction, rate, and duration of adaptive evolution can be modified from standard Mendelian models. To explore the evolutionary implications of trans-generational maternal effects, I quantified two aspects of evolutionary response: the quantitative genetic basis of maternal inheritance and the magnitude of phenotypic selection at the individual and maternal family level for ten traits expressed at four stages in the life cycle in a winter annual plant, *Collinsia verna*. In a hierarchical quantitative genetic analysis of Mendelian and maternal inheritance, I estimated six additive and environmental causal components of variance: direct (i.e. Mendelian) additive and environmental, maternal additive and environmental, and the direct-maternal additive and environmental covariances. The structure of maternal inheritance changed through the life cycle. Early traits were influenced more by maternal additive than by direct effects, direct and maternal additive effects covaried negatively, and direct-maternal environmental covariance was positive. At subsequent stages, some traits displayed strictly Mendelian inheritance, while others displayed direct and maternal additive genetic effects of the same magnitude and negative direct-maternal covariances. Maternal environmental components were negligible beyond emergence. The negative direct-maternal covariances for all maternally inherited traits resulted in near zero or negative realized heritabilities indicating no or reversed response to selection, respectively. In nature, the magnitude of selection on maternally inherited traits will also determine evolutionary response. I examined phenotypic selection at two levels: individual and maternal. An episodic analysis of individual selection across four stages in the life cycle demonstrated that large fall size and later emergence were directly favored across all episodes, although the magnitude and direction of selection varied among episodes. As a result of positive phenotypic correlations among size traits, selection also indirectly favored heavier seeds and larger initial size. Maternal selection may also affect selection response because substantial among maternal family variance in fitness indicated the opportunity for maternal selection. Maternal effects are likely to have dramatic short-term evolutionary consequences by constraining the selection response, influencing correlated response to selection via the phenotypic variance-covariance matrix, and affecting offspring fitness directly via maternal selection.

In memory of my mother, Irene Niebuhr Thiede.

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INTRODUCTION

The mechanistic basis of contemporary adaptive evolution requires an understanding of ecological factors influencing individual survival and fecundity as well as the genetic propensity for intergenerational changes in the multivariate phenotype. The translation of these short-term dynamics of phenotypic selection and genetic response into observed patterns of adaptive population differentiation requires assumptions about the constancy of the genetic variance-covariance matrix and the magnitude and direction of selection (Lande 1979, 1982). Empirical evidence in natural plant populations shows that patterns of phenotypic selection vary spatially and temporally (Kalisz 1986; Stewart and Schoen 1987; Weis et al. 1992; Kelly 1992; Stratton 1992; Bennington and McGraw 1995b). Furthermore, the genetic variance-covariance matrix is not likely to remain constant due to selection and/or drift (e.g. Shaw et al. 1995). Theoretical models designed to incorporate both environmental and genetic variability are divided into two schools: 1) stochastic demography that incorporates the effect of environmental variability on demographic parameters (Tuljapurkar 1989; Orzack 1993) and 2) evolutionary models that indicate how spatial and temporal variability in selection maintain genetic variation (Haldane and Jaykar 1963; Barton and Turelli 1989). These two schools of thought have yet to be unified in one synthetic theory that incorporates demographic and genetic variability and their interaction in determining how natural selection produces divergence within and among populations observed in nature. One essential component of such a unified theory will be a detailed understanding of short-term evolutionary dynamics.

One important, yet unexplored factor affecting short-term evolutionary dynamics in natural plant populations is the impact of maternal inheritance. Maternal inheritance is one type of non-Mendelian inheritance in which the resemblance between relatives is determined not only by the Mendelian inheritance, i.e. the transmission of one-half of an individual's genes in the fertilization process, but is also influenced by the phenotypic effects of the mother on the attributes of her offspring. These maternal effects on offspring phenotype can have both a genetic and environmental basis. Maternal effects can increase or decrease the similarity between mothers and their offspring, likewise they can increase the similarity between maternal full and half siblings.

Maternal inheritance has a variety of evolutionary consequences. The most striking effect is reversed responses to artificial selection (i.e. Falconer 1965) that can result either from a negative genetic correlation between maternal performance and offspring phenotype or from a negative environmental effects of maternal performance on offspring phenotype. In contrast, positive genetic correlations or environmental effects can enhance selection response. In addition to influencing the direction of selection response, maternal inheritance introduces time lags in the evolutionary response (Kirkpatrick and Lande 1989, 1992). Unlike standard evolutionary models, maternally inherited traits are influenced by phenotypic selection in the previous generation. This time lag is the direct result of phenotypic selection in prior generations altering the distribution of maternal performance phenotypes which directly impact the expression of phenotypes in the generation currently subject to selection. The effect of this time lag in response to selection is twofold: 1) the maximal evolutionary rate of response is approached asymptotically, and 2) the response to selection continues after selection ceases (Kirkpatrick and Lande 1989, 1992). Thus, maternal inheritance can alter the direction, magnitude, rate, and duration of response to selection on a short time scale. Given the observed variability in phenotypic selection in natural populations, these short-term effects may affect the long-term patterns of adaptive divergence that we observe in natural populations.

Quantifying maternal inheritance

Currently, there are two approaches for estimating the genetic component of maternal effects. Models developed by animal breeders to improve response to artificial selection estimate specific causal components of variance by partitioning the phenotypic covariance on a wide variety of relatives (Dickerson 1947; Wilham 1963; Eisen 1967). In contrast, the other approach condenses causal components into single parameters estimating the magnitude of the maternal genetic effect (Falconer 1965; Kirkpatrick and Lande 1989).

Partitioning the phenotypic covariances among relatives

Wilham (1963) derived an equation demonstrating how the offspring phenotypic value (P_{ox}) is a function not only of the direct genotypic (G_{ox}) and environmental values (E_{ox}), but also of the maternal genotypic (G_{mw}) and environmental values (E_{mw}):

$$P_{ox}=G_{ox}+E_{ox}+G_{mw}+E_{mw}.$$

Subsequent derivations of the causal components of genetic variance and covariance for different types of relatives hinges on this construction of maternal effects.

The estimation of the causal genetic components that contribute to phenotypic covariances of different types of relatives when maternal effects are present necessitate phenotypic measurements on a wide variety of different types of relatives (Wilham 1963; Eisen 1967). Because direct additive and maternal additive effects both contribute to the phenotypic covariance when maternal effects are present, their statistical separation is compromised. Cross-fostering offspring post-partum is one technique that has been used to disentangle direct and maternal genetic effects (Riska et al. 1985). This experimental approach allows the estimation of post-natal maternal effects in species exhibiting parental care. Embryo transfer can also be used to explore pre-natal maternal effects (Cowley 1991). In angiosperms, either of these experimental approaches is not currently feasible. Alternatively, Wilham (1980) suggests that perhaps the best types of relatives to use in estimation are different types of first cousins where the correlation between direct and maternal additive effects and their covariance is not as great.

Statistical techniques for estimating these causal (co)variance components fall into two categories: 1) least-squares (Eisen 1967; Cantet et al. 1988; Cantet 1990; Cantet et al. 1992 a,b) and 2) restricted maximum likelihood (Thompson 1976; Meyer 1991, 1992). Because sampling correlations between the various components are large as a consequence of direct and maternal effects being confounded in maternal lineages (Eisen 1967; Thompson 1976; Wilham 1980; Meyer 1992), variance component estimates and heritabilities and genetic correlations derived from them have large variances associated with them (Thompson 1976; Meyer 1992). Even when some of the variance components are eliminated such that models include only the direct and maternal additive and environmental components, large sample sizes are required to overcome this problem (Meyer 1992).

Estimating maternal effect coefficients

Falconer (1965) derived a simplified approach to estimating maternal effects in which maternal effects are not decomposed into specific genetic components, but rather are lumped into one term, m, the maternal effect coefficient. This generalized approach is analogous to the estimates of non-additive maternal effects from reciprocal crosses (Topham 1966; Cockerham and Weir 1977). In this model, Falconer (1965) considered the single case of a maternal trait influencing its expression in the offspring. Kirkpatrick and Lande (1989) have extended this approach to multiple maternal and offspring traits in which specific maternal traits can influence their own expression or the expression of other traits in the subsequent generation. Lande and Price (1989) and Schluter and Gustaffson (1993) have applied this approach to natural populations. This approach requires the assumption that all maternal traits are measured and included in the analysis. It does not provide information about the specific nature of the genetics of maternal effects. Lynch and Walsh (1996) derive the quantitative genetic relationship between this model and the Wilham (1963) approach and demonstrate that m, the maternal effect coefficient, is a function of σ_{AoAm}, σ_{DoDm}, σ_{EoEm}, σ^2_{Am} and σ^2_{Em}. The advantage of this simplified approach is that it can be integrated with estimates of phenotypic selection to predict multivariate response to selection (Kirkpatrick and Lande 1989). The disadvantage is that it does not identify the specific genetic basis of maternal effects.

Estimates of maternal effects in plants

In natural plant populations, diallel breeding designs allow the estimation of maternal effects (Cockerham and Weir 1977). This maternal variance component compares maternal to paternal half-sib offspring and thus includes maternal additive, maternal dominance, maternal environment, direct maternal additive covariance, and direct maternal environmental covariance as well as any cytoplasmic inheritance. A number of studies have found that seed weight, emergence time, and early seedling size display significant maternal variance in artificial or natural environments (Biere 1991a; Mitchell-Olds and Bergelson 1990a; Montalvo 1994; Schmid and Dolt 1994). Most of these studies demonstrate that this general maternal effect in angiosperms is short-lived and does not persist beyond seedling stages. Two of these studies were actually able to compare the relative magnitude of maternal genetic vs. maternal environmental effects by using clonal replicates in the same (Biere 1991a) or multiple environments (Schmid and Dolt 1994), but both found that maternal genetic effects were larger in magnitude than maternal environmental effects.

While these studies suggest that maternal genetic effects are likely to affect the evolutionary potential of juvenile and maternal traits in plants, no study has estimated the contribution of specific causal components to juvenile traits in plants. Thus the importance of the direct maternal additive genetic covariance in enhancing or constraining the evolution of juvenile and maternal traits is unknown.

Quantifying Phenotypic Selection

To evaluate the evolutionary consequences of maternal inheritance, we need information on the nature of phenotypic selection on traits influenced by maternal effects. The methodology for estimating selection on a multivariate phenotype is well developed

(Lande and Arnold 1983; Arnold and Wade 1984 a,b; Phillips and Arnold 1989; reviewed by Brodie et al. 1995). This multiple regression approach can be utilized to estimate the total magnitude of selection on traits even when mortality eliminates individuals before they express all phenotypic traits of interest (Lynch and Arnold 1988). In that respect it differs from the path analytic models of Crespi and Bookstein (1989) in which causal relationships between traits are constructed for each selection episode. In path analytic models, neither the total magnitude of selection nor the total nature of direct and indirect effects of traits can be determined. An alternative approach to estimating selection that allows more complex fitness functions was initially limited by considering only a single trait (Schluter 1988). However, recently this nonparametric approach has been extended to multivariate descriptions of selection (Schluter and Nychka 1994).

In natural plant populations, juvenile traits are likely to display maternal inheritance (see above). Second, juvenile traits like seed size and emergence time can influence early biotic and abiotic interactions and produce a very skewed distribution of fitness (Stanton 1985; Waller 1985). As a result of these early acting selective events manifested both by mortality and differences in individual size, the total magnitude of selection acting on juvenile traits can only be determined by an episodic selection analysis (Arnold and Wade 1984 a,b) modified to account for changes in the phenotypic variance-covariance matrix due to early mortality (Lynch and Arnold 1988). The magnitude of phenotypic selection on traits determines the importance of maternal inheritance either by direct selection on a trait displaying maternal inheritance or by indirect effects of selection on phenotypically correlated traits.

In addition to phenotypic selection on juvenile traits that are likely to be influenced by maternal inheritance, it is possible for maternal traits to directly influence the survival and fecundity of their offspring. This type of maternal effect on offspring fitness has been termed maternal selection. The inclusion of maternal attributes in a selection analysis allows the estimation of maternal selection, a form of group selection (Heisler and Damuth 1987). Like maternal inheritance, maternal selection influences the evolutionary dynamic. Kirkpatrick and Lande (1989) illustrate that maternal selection can result in maladaptive evolution. It is also different from other forms of selection because the magnitude depends on the resemblance between mothers and their offspring. Maternal selection represents the possibility for different levels of selection to influence the evolutionary response for traits that display either standard Mendelian inheritance or maternal inheritance.

Evolutionary consequences of maternal effects

In nature, the phenotypic expression of a trait can be influenced by the phenotype of the individual's mother, maternal inheritance. Furthermore, the survival and fecundity of an individual relative to other individuals in the population can be directly influenced its mother, maternal selection. In this dissertation I explore the evolutionary consequences of these two types of maternal effects. In Chapter 1, I quantify the magnitude of maternal inheritance by carefully partitioning phenotypic covariances among relatives into explicit causal components of genetic variance and covariance. This estimate of the additive genetic variance-covariance matrix for each trait suggests how maternal inheritance influences univariate response to selection. In Chapter 2, I document the nature of phenotypic selection on juvenile traits likely to display maternal inheritance in a natural population. In addition to estimating the total magnitude of selection, I also describe the nature of the indirect effects of selection. How does selection acting on one trait produce responses in phenotypically correlated traits? This multivariate selection analysis indicates how various juvenile traits directly influence survival and fecundity and suggests hypotheses about causal agents of selection. In Chapter 3, I explore the extent to which selection may discriminate not only among individuals offspring, but also among their mothers. Maternal selection, a form of group selection, indicates that mothers can directly influence the fitness of their offspring. I also explore the extent to which spatial variation in selection can influence among family differences in fitness.

These chapters deal explicitly with the two components of the dynamic equations for evolutionary change: inheritance (Chapter 1) and selection (Chapters 2 and 3). In each chapter I explicitly address the evolutionary implications for the component of interest. The synthesis of these components as they are influenced by maternal inheritance and maternal selection remains a challenge. My long-term goal is to unify these estimates into a single evolutionary model to make explicit predictions about response to selection when both maternal inheritance and maternal selection influence offspring phenotype and fitness, respectively. The impact of maternal effects on short-term evolutionary dynamics are likely to be central to our understanding of the adaptive divergence of both juvenile and maternal traits within and among natural populations.

Chapter 1

MATERNAL INHERITANCE AND ITS EFFECT ON ADAPTIVE EVOLUTION: A QUANTITATIVE GENETIC ANALYSIS OF MATERNAL EFFECTS IN A NATURAL PLANT POPULATION.

INTRODUCTION

Phenotypic similarity between mothers and offspring resulting from standard Mendelian inheritance can be altered by phenotypic effects of a mother on traits in her young, termed maternal inheritance (Kirkpatrick and Lande 1989, 1992; Lande and Kirkpatrick 1990). In a classic study of maternal effects in mice, Falconer (1955, 1965) found that large mothers had many small young. At maturity the females from these large litters had only a few large young. The lack of resemblance between mothers and their daughters in litter size and offspring size was mediated by the phenotypic effect of maternal size and its relation to maternal provisioning ability. Interestingly, this maternal effect had short-term evolutionary consequences. Artificial selection on litter size showed a reversed response to selection in a single generation (Falconer 1965). More recently in the collared flycatcher, Schluter and Gustaffson (1993) experimentally quantified how maternal condition and clutch size influenced the resemblance between mothers and daughters in clutch size. In their study, resemblance in clutch size was moderated by the negative effect of maternal clutch size on a daughter's condition and by a positive effect of maternal condition on a daughter's condition. The balance between negative and positive maternal effects led Schluter and Gustaffson (1993) to predict a positive response to selection on clutch size in the first generation. These two examples illustrate how maternal inheritance can impact the evolutionary process. The underlying genetic architecture of maternal inheritance can be described by the genetic basis of traits such as maternal size, condition, or provisioning ability, and their genetic correlation with traits expressed in the juvenile stages such as birth weight or offspring size. It is the underlying genetic architecture of maternal inheritance that determines how trans-generational effects will impact the process of adaptive evolution.

The best model to date for exploring the underlying genetic architecture of maternal inheritance was developed by Dickerson (1947) (hereafter Dickerson's model). Dickerson's model considers two traits, the individual trait of interest and the maternal trait affecting its expression. The goal is to partition the covariance (i.e. resemblance) between mothers and their offspring into explicit Mendelian and maternal components (Table 1; Dickerson 1947; Wilham 1963, 1972; Eisen 1967; Cheverud 1984; Lynch 1987; see Cheverud and Moore 1994; Lynch and Walsh 1996 for current reviews). The nine possible causal genetic and environmental components from Dickerson's model (Table 1) are obtained by the statistical partitioning of phenotypic covariances of the individual trait among different types of relatives generated in a complex breeding design. The maternal trait is a composite trait termed maternal performance and is unobserved. While Dickerson's path analytic approach assumes a causal model of maternal inheritance, the estimates of causal components are correlational in nature because they are derived from covariances.

Table 1. Partitioning the phenotypic (co)variance for each of seven sets of relatives into causal genetic components of variance and covariance (after Eisen 1967; Thompson 1976). Values represent the coefficient for each component. A denotes additive genetic, D denotes dominance, E denotes environmental variance, respectively. The subscript o denotes direct effects due to standard Mendelian inheritance, while m refers to maternal effects due to maternal inheritance. Individual variance shows which components contribute to an individual's phenotypic value.

Phenotypic (Co)variance	Causal Components of Variance and Covariance								
	σ^2Ao	σ^2Do	σAoAm	σDoDm	σ^2Am	σ^2Dm	σ^2Em	σ^2Eo	σEoEm
Paternal half-sibs	1/4	0	0	0	0	0	0	0	0
Full sibs	1/2	1/4	1	0	1	1	1	0	0
Within full sibs	1/2	3/4	0	0	0	0	0	1	0
Dam-offspring	1/2	0	5/4	1	1/2	0	0	0	1
Maternal relative-offspring	1/4	0	3/4	1/4	1/2	0	0	0	0
Sire-offspring	1/2	0	1/4	0	0	0	0	0	0
Paternal relative-offspring	1/4	0	1/4	0	0	0	0	0	0
Individual	1	1	1	0	1	1	1	1	0

In this paper I utilize Dickerson's model to estimate the underlying architecture of maternal inheritance, i.e. the specific causal genetic and environmental components of variance, in a winter annual plant, *Collinsia verna* Nutt. (Scrophulariaceae). My goal is to describe how maternal inheritance affects the magnitude and direction of predicted response to selection for a number of traits expressed at different stages in the life cycle.

The Maternal Inheritance Model

With simple Mendelian inheritance (Figure 1A), the phenotypic value of a trait (P_o) is determined by additive genetic (A_o) and environmental (E_o) components where the subscript o refers to the individual trait of interest. In this two generation path diagram, an offspring in the second generation (x) receives 1/2 of its genes from its mother in the previous generation (w). The translation of additive effects into phenotypic value for this trait is denoted by the direct (i.e. Mendelian) heritability (h_o).

In contrast, in Dickerson's model of maternal inheritance (Figure 1B), the phenotypic value of the trait of interest (P_{ox}) is influenced not only by Mendelian inheritance (described above), but also by the unobserved maternal performance phenotype (P_{mw}), subscripts m and w referring to the maternal performance trait and the maternal generation, respectively. The phenotypic value (P_{mw}) for maternal performance is determined both by additive genetic (A_m) and environmental (E_m) components. Direct and maternal additive effects can be genetically correlated (r_{AoAm}). The resemblance between a mother and her offspring (Figure 1B) can be influenced by four components: 1) maternal additive genetic variance (σ^2_{Am}) and it translation into maternal performance denoted by the heritability (h_m), 2) direct-maternal additive genetic covariance (σ_{AoAm}) standardized

Figure 1. Path diagrams of Mendelian (A) and maternal inheritance (B and C) (after Dickerson 1947; Wilham 1963; Cheverud 1984). Under Mendelian inheritance in model 1 (A), the additive genetic value (A_o) and the environmental value (E_o) determine the phenotypic value (P_o) where the subscript o refers to the offspring trait and w and x refer to the maternal and offspring generations, respectively. In Model 2 (B), maternal inheritance is determined by the phenotypic effects of the maternal performance trait (P_m) and its additive genetic (A_m) and environmental (E_m) components, the subscript m referring to maternal performance. The genetic correlation between direct and maternal traits (r_{AoAm}), and the square root of the direct (h_o) and maternal heritabilites (h_m) are illustrated. The maternal effect coefficient (m) indicates the extent to which the maternal phenotype influences the phenotypic value in the offspring independent of additive genetic effects. In Model 3 (C) maternal inheritance includes the potential correlation between direct and maternal environments (r_{EoEm}).

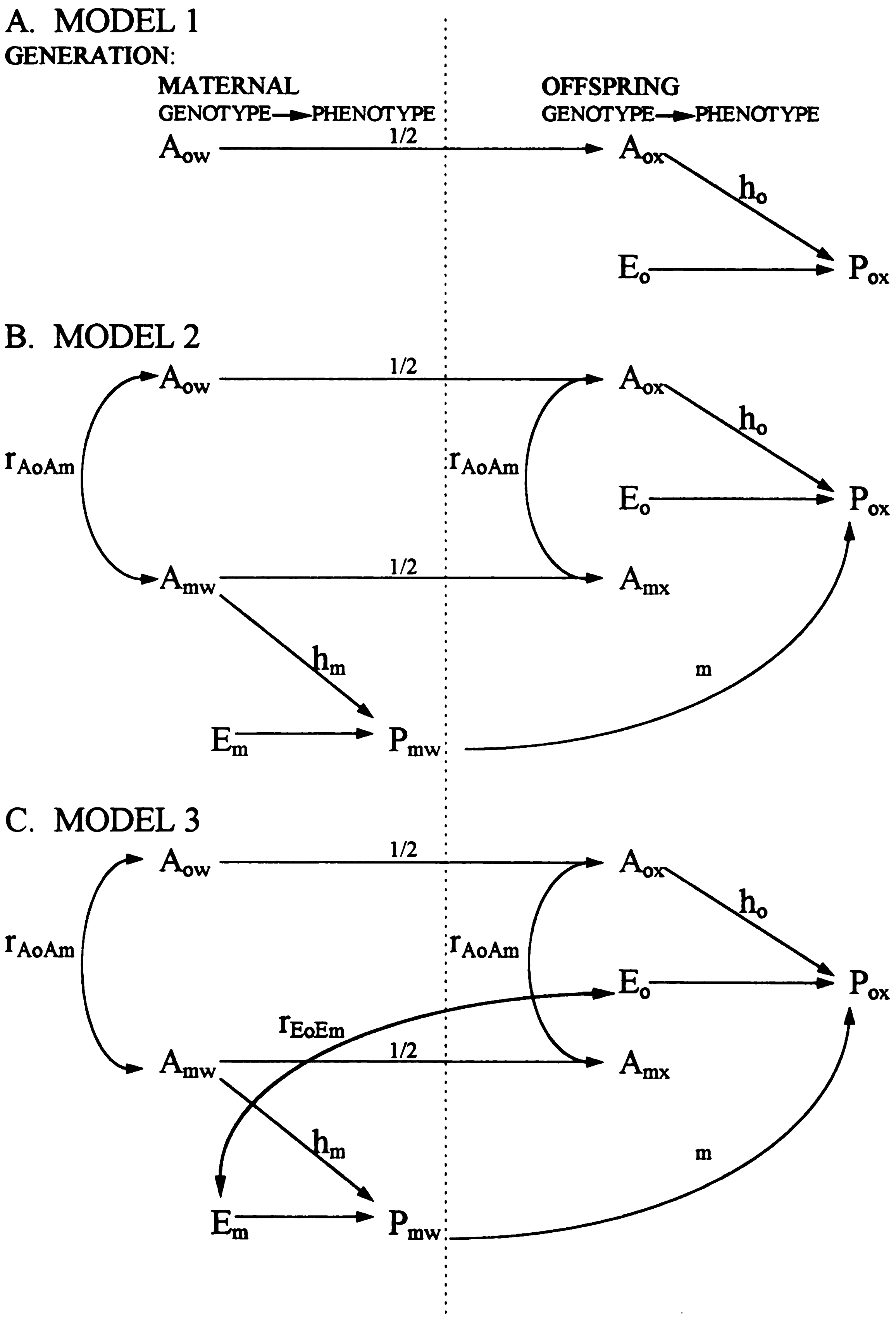

A. MODEL 1
GENERATION:
MATERNAL
GENOTYPE → PHENOTYPE
OFFSPRING
GENOTYPE → PHENOTYPE
A_ow
1/2
A_ox
h_o
E_o
P_ox
B. MODEL 2
A_ow
1/2
A_ox
h_o
r_AoAm
r_AoAm
E_o
P_ox
A_mw
1/2
A_mx
h_m
m
E_m
P_mw
C. MODEL 3
A_ow
1/2
A_ox
h_o
r_AoAm
r_AoAm
E_o
P_ox
r_EoEm
A_mw
1/2
A_mx
h_m
m
E_m
P_mw

Figure 1.

by the additive genetic variances of both the individual and maternal traits as a genetic correlation (r_{AoAm}), 3) maternal environmental variance (σ^2_{Em}), and 4) the purely phenotypic effects of the mother on her offspring (m), termed the maternal effect coefficient. In a second version of Dickerson's model (Figure 1C) a fifth component, the environmental covariance between generations (σ_{EoEm}) standardized as direct-maternal environmental correlation (r_{EoEm}), can also contribute to the resemblance between a mother and her offspring. Thus, relative to standard quantitative genetic models of Mendelian inheritance (Figure 1A), the decomposition of the trait, (P_{ox}), into genetic and environmental components is complicated by the additional paths of maternal inheritance.

The response to selection on the maternally inherited individual trait (P_{ox}) will be determined by the realized heritability (h^2_r) (Dickerson 1947; Wilham 1963; Van Vleck 1970):

$$h_r^2 = (\sigma^2_{Ao} + 3/2\sigma_{AoAm} + 1/2\sigma^2_{Am}) / \sigma^2_P \qquad (1)$$

The realized heritability is a function of the direct additive genetic variance (σ^2_{Ao}), the maternal additive genetic variance (σ^2_{Am}), and the direct-maternal additive genetic covariance (σ_{AoAm}) relative to the total phenotypic variance (σ^2_P). When the direct-maternal genetic covariance (σ_{AoAm}) is negative and $>|2/3\sigma^2_{Ao}+1/3\sigma^2_{Am}|$, the response will be in the opposite direction to selection. Similarly, positive maternal additive genetic variance (σ^2_{Am}) and direct-maternal genetic covariance (σ_{AoAm}) can accelerate response to selection. Thus, the underlying genetic architecture of maternal inheritance influences the direction and rate of adaptive evolution. The time lag in the maternal inheritance can also effect the rate, direction, and duration of the selection response (Kirkpatrick and Lande 1989, 1992; Lande and Kirkpatrick 1990).

Empirical estimates of the causal variance components affecting evolutionary response in domesticated and experimental laboratory species for traits such as litter size, birth weight, and weaning weight show that maternal additive genetic effects can be substantial (e.g. Bondari 1978; Cantet et al. 1988; Shi et al. 1993), can increase from birth to weaning (Shi et al. 1993), and generally display significant negative direct-maternal additive genetic covariances (Figure 2). Maternal effects on a single trait through ontogeny decline after weaning (Atchley 1984; Cheverud et al. 1983).

In contrast in natural populations, empirical estimates of causal variance components determining maternal inheritance are lacking. In plants, the magnitude of maternal effects estimated by less detailed methods also shows a decline through ontogeny. In general, traits expressed early in the life cycle such as seed weight, emergence time, or seedling size are influenced more strongly by maternal genetic effects than direct (i.e. Mendelian) genetic effects (Biere 1991a; Platenkamp and Shaw 1993; Montalvo and Shaw 1994; Schmid and Dolt 1994). The duration of maternal genetic effects beyond the seedling stage is rare (Schmid and Dolt 1994). Maternal genetic effects tend to persist longer in competitive environments (Schmid and Dolt 1994), a pattern analogous to the persistence of initial size differences in more competitive environments (Gross 1984; Stanton 1985; Waller 1985; Weiner 1985, 1990; Stratton 1989; Gross and Smith 1991). While maternal environmental effects are well documented (reviewed by Roach and Wulff 1987) and can persist for multiple generations (Miao et al. 1991; Lacey

Figure 2. Summary of studies estimating direct-maternal additive genetic correlations based on three different estimation models: 1) the animal model included additive and environmental components only, 2) the full model also included dominance components, and 3) cross-fostering models estimated post-natal maternal effects (Bondari et al. 1978; Cantet et al. 1988; Southwood and Kennedy 1990; Shi et al. 1993; Van Sanford and Matzinger 1982; Everett and Magee 1965; Young and Legates 1965).

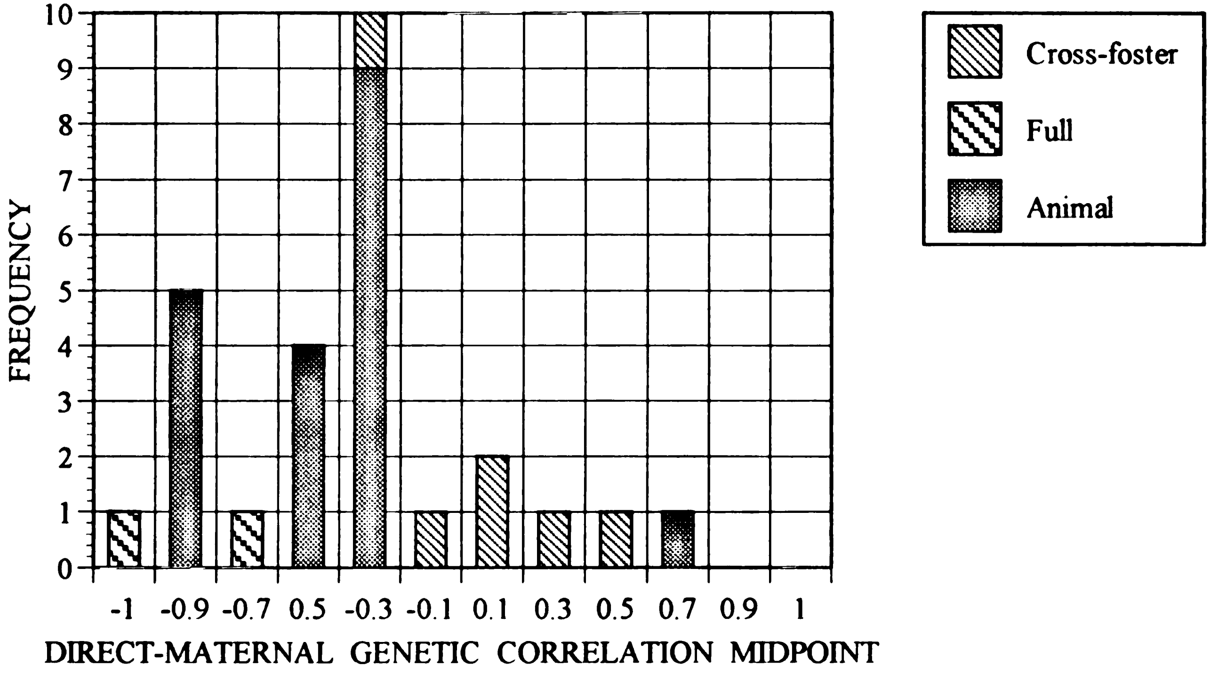

FREQUENCY
10
9
8
7
6
5
4
3
2
1
0
-1
-0.9
-0.7
0.5
-0.3
-0.1
0.1
0.3
0.5
0.7
0.9
1
DIRECT-MATERNAL GENETIC CORRELATION MIDPOINT
Cross-foster
Full
Animal

Figure 2.

1991), studies examining the magnitude of maternal genetic and maternal environmental effects have found that maternal genetic effects predominate over maternal environmental effects for traits expressed early in the life cycle (Biere 1991a; Schmid and Dolt 1994).

In this paper I present the first quantitative genetic analysis of maternal effects, estimating the causal variance components relevant to maternal inheritance in a natural plant population. Causal components are critical for predicting the dynamic role that maternal inheritance plays in adaptive, multivariate evolution. By examining a large number of traits expressed both early and late in the life cycle of the winter annual, *C. verna*, I explore how maternal inheritance changes through ontogeny. In addition, I compare how genetic correlations among traits within a generation differ from the between generation genetic correlations associated with maternal inheritance. The goal is to describe the underlying genetic architecture of maternal inheritance and its implication for evolution by natural selection.

MATERIALS AND METHODS

Study Species

C. verna (Scrophulariaceae), a winter annual plant, germinates in the fall in response to diurnal temperature fluctuations (Baskin and Baskin 1983), overwinters under the leaf litter and snow as a small rosette, and bolts and flowers in mid to late May in the mesic floodplain forests throughout the midwest. Seed and seedling traits vary significantly among maternal families (Thiede, unpublished data) suggesting the likelihood of maternal inheritance early in the life cycle. Seed and seedling traits also strongly influence individual survival and fecundity (Kalisz 1986; Chapter 2). Thus, maternal inheritance is also likely to affect adaptive evolution. In this study, I consider traits expressed at four stages in the life cycle: seed, seedling, overwintering fall rosette, and pre-flowering spring rosette (Table 2). By considering the same trait at multiple stages, I evaluate the magnitude of maternal inheritance through ontogeny.

In Dickerson's model, estimates of causal components of variance and covariance are obtained by partitioning the phenotypic covariances among relatives (Table 1) for traits hypothesized to be influenced by maternal effects. In this approach a single trait is measured in various relatives, while the maternal trait exerting the effect is not quantified. The magnitude of the maternal components is estimated solely by partitioning the covariance of the trait of interest into causal components assuming different models of inheritance. In essence, this design treats the maternal effect as a composite of all maternal traits that influence a particular trait in the offspring (Cheverud 1984; Cheverud and Moore 1994).

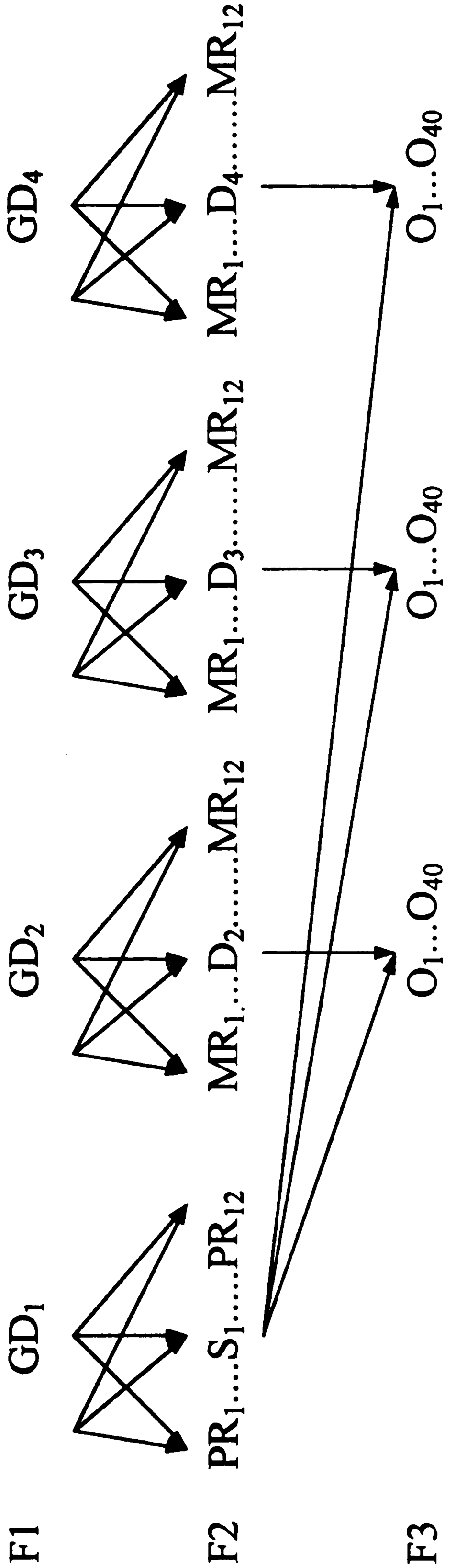
Three Generations

I obtained the seven types of relatives in Table 1 from a three generation breeding design (Figure 3). In the first generation, 100 wild grandmaternal (F1) individuals bearing naturally pollinated seeds were collected every 2 m along a 200 m transect from a natural population of *C. verna* in Kalamazoo County, MI in May, 1991. Twelve seeds from each grandmaternal plant founded the second generation (F2) that was grown to maturity in the greenhouse. The F2 individuals contributed to the estimates in one of two ways: 1) a

Table 2. Mean, standard error, coefficient of variation , and sample sizes for phenotypic traits measured at respective life-cycle stages in both F2 and F3 generations. Parents (F2) were grown in the greenhouse, while offspring (F3) were grown in both the greenhouse and field. For parents cotyledon diameter at two stages is the average of cotyledon length and width at emergence and is equal to fall cotyledon length, while in the offspring cotyledon diameter was consistently measured by a circular template at each of those stages.

Stage	Phenotypic trait	Parents (F2)				Offspring (F3)			
		Greenhouse				Greenhouse			
		x	+ SE	CV	n	x	+ SE	CV	n
Seed	Seed weight (mg)	4.09	0.04	28.57	943	5.50	0.04	24.55	871
	Embryo weight (mg)	3.64	0.05	29.60	447	4.95	0.05	24.91	560
Seedling	Emergence date	36.85	0.45	26.41	458	52.31	0.37	18.05	638
	Cotyledon diameter (mm)	4.98	0.07	31.07	428	5.58	0.04	19.73	589
Fall Rosette	Cotyledon diameter (mm)	9.68	0.14	29.47	437	13.39	0.11	19.16	563
	Basal leaf length (mm)	7.07	0.21	63.40	438	7.55	0.15	48.68	574
	Number of leaves	3.24	0.09	60.27	438	2.80	0.06	51.39	574
Spring Rosette	Cotyledon length (mm)	10.71	0.18	27.65	267	14.86	0.11	17.37	499
	Basal leaf length (mm)	10.47	0.26	40.06	261	16.79	0.15	19.75	500
	Number of leaves	10.29	0.40	65.72	282	22.97	0.46	44.84	504
		Greenhouse				Field			
Seed	Seed weight (mg)	4.09	0.04	28.37	991	5.36	0.04	24.53	1109
Seedling	Emergence week	3.88	0.07	37.48	482	3.13	0.04	35.27	618
	Cotyledon diameter (mm)	5.00	0.07	31.59	451	5.69	0.06	22.29	461
Fall Rosette	Cotyledon diameter (mm)	9.67	0.13	29.38	452	9.69	0.11	21.63	372

Figure 3. Three generation breeding design in which field-collected grandmaternal families (F1, GD_n, n=100) provided seed for the parental generation (F2). Of the twelve seeds planted from each granddam (F1), one was randomly assigned as a sire (S_n, n=24) or dam (D_n, n=72); while the other eleven grandmaternal full-sibs were considered paternal (PR_n) or maternal relatives (MR_n). Greenhouse-raised parents produced up to 40 offspring (F3) that were divided between greenhouse and field environments.


F1
F2
F3
GD$_1$
GD$_2$
GD$_3$
GD$_4$
PR$_1$....S$_1$.....PR$_{12}$
MR$_1$....D$_2$.......MR$_{12}$
MR$_1$....D$_3$.......MR$_{12}$
MR$_1$....D$_4$.......MR$_{12}$
O$_1$...O$_{40}$
O$_1$...O$_{40}$
O$_1$...O$_{40}$

subset of individuals served as parents in the nested breeding design to generate the third generation (see below), 2) the remaining individuals were classified as parental relatives (Figure 3). To determine the coefficients of causal components for parental relatives (Table 1), I assumed that F2 individuals within an F1 grandmaternal family were full-sibs produced by natural outcrossing. This assumption is justified because the outcrossing rate in this population was consistently greater than 0.85 for three years (including 1991). Furthermore, a high estimate of correlated matings suggests that these outcrossed individuals share the same father (Holtsford et al. in prep).

To produce the third generation, one individual (F2) from each grandmaternal family was randomly assigned to serve as a sire or dam in a nested breeding design in May, 1992. Twenty-four sires were crossed to three dams per sire in a standard nested design to generate 24 paternal half-sib and 72 maternal full-sib families (Figure 3). Flowers were emasculated in bud and pollinated within 5 days post-emasculation. Pollinations were performed on all floral whorls to control for position effects. Fruits were harvested as they matured. An accident in the lab eliminated 10 maternal full sib families resulting in a total of 62 maternal full sib families.

The third generation (F3) was planted in a randomized block design in two locations: greenhouse (n=871 offspring from 24 sires and 58 dams) and field (n=1212 offspring from 24 sires and 62 dams). Seeds were planted to a depth of 1 cm in Sunshine seedling mix either in 96 well trays (F2 and F3 in the greenhouse) or in 2 cm long plastic tubes 16 mm in diameter (F3 in the field). In both locations one individual from each maternal full-sib family was planted into each of 20 blocks. In the greenhouse each block consisted of a 96 well tray, all 20 on a single bench in the greenhouse. In the field each block of F3 individuals was divided into three sets of 24 and each set was then randomly assigned to one of three 0.5 m^2 quadrats at one of 20 locations. This planting design was utilized to maintain natural seed/seedling densities in a given quadrat. The 20 blocks spanned the natural habitat and included forest edge and interior.

Traits Measured

To estimate maternal inheritance, I measured the same trait in the F2 and F3 generations: ten traits at four stages in the life cycle in the greenhouse or four traits at three stages in the field (Table 2). Prior to planting, seeds were weighed to the nearest 0.1 microgram. After seedling emergence, seed coats were carefully excavated from the soil, air dried, and weighed (F2 and F3 in greenhouse only). Embryo weight was calculated as the difference between seed weight and seed coat weight. Thus, embryo weight more accurately reflected the diploid genetic composition when compared to seed weight which contained both the diploid embryo, a small amount of residual endosperm, and the diploid maternal seed coat.

Seedling emergence date was scored weekly in the field (F3) and every 3-4 days in the greenhouse (F2 and F3) from September to the beginning of December. Emergence date was defined as the first date when cotyledons were expanded. At emergence, I quantified seedling size by measuring cotyledon diameter using a template of circles of increasing diameter in increments of 0.5 mm (F3). In the F2 generation, cotyledon diameter at emergence was the average of cotyledon length and width.

At two subsequent stages, in late fall prior to overwintering and in early spring prior to flowering, I quantified individual size by measuring three traits: cotyledon diameter, leaf length of the most basal leaf (mm), and number of leaves. Fall rosettes were measured in November (F2) or early December (F3). In December, greenhouse grown plants (F2 and F3) were transferred to a sheltered area outdoors and covered with a thick layer of leaf litter to mimic natural field conditions. Overwintering survival was greater than 90%. In April plants were returned to the greenhouse and I transplanted a random subset of 2-3 individuals per maternal granddam (F2) or all seedlings (F3) into 15 cm^2 pots filled with a 2:1:1 mix of Sunshine seedling mix, perlite, and turface. Size traits were measured on pre-flowering spring rosettes (F2 and F3) after transplanting.

Estimation of Genetic and Environmental Causal Components

I estimated six of the nine causal components relevant to maternal inheritance (Table 1), additive (σ^2_{Ao}, σ^2_{Am}, σ_{AoAm}) and environmental components (σ^2_{Eo}, σ^2_{Em}, σ_{EoEm}), by considering three sequential models of inheritance in a hierarchical approach (Figure 1). The simplest model of inheritance was a purely additive Mendelian model (Figure 1A, hereafter model 1) in which σ^2_{Ao} and σ^2_{Eo} were estimated. In model 2, maternal inheritance was incorporated by estimating three additional components, σ^2_{Am}, σ_{AoAm}, and σ^2_{Em} (Figure 1B). In model 3, all possible additive and environmental covariances were considered by including a sixth component (σ_{EoEm}) (Figure 1C). The hierarchical approach allowed me to ask: 1) Did the more complex estimation model for maternal inheritance better describe the data? 2) Which causal components were significant in each estimation model?

The estimation of additive and environmental components only is often necessary (e.g. Bondari 1978; Meyer 1992; Shi et al. 1993) because obtaining a sufficient number of relatives to estimate all nine components is very difficult (see Cantet et. al. 1988 for an example of the design required for the full model). In addition to standard quantitative genetic assumptions of random mating, linkage equilibrium, and the absence of epistasis and of genotype by environment interactions (Wilham 1963; Eisen 1967; Thompson 1976), the three models, therefore, required the assumption that direct and maternal dominance variances and their covariance (σ^2_{Do}, σ^2_{Dm}, σ_{DoDm}) were zero. To test the assumption of zero dominance variances and covariance, I included them in some preliminary analyses and discuss these results when relevant.

The nature of transmission of maternal inheritance dictates that direct and maternal components are correlated in maternal lineages (Table 1). This biological reality results in a statistical limitation in estimation because causal components are correlated even when numerous types of relatives are considered (Eisen 1967; Thompson 1976; Wilham 1980; Meyer 1992). In this design, correlations among components based on the coefficients in Table 1 showed that σ^2_{Dm} and σ^2_{Em} were perfectly correlated ($p<0.001$). Therefore, only σ^2_{Dm} or σ^2_{Em} or their sum was estimable (Thompson 1976). Maternal component, σ^2_{Am}, was positively correlated with σ_{AoAm} , σ^2_{Dm}, and σ^2_{Em} ($r=0.85$, 0.80, 0.80, respectively, $p<0.05$ for all) and direct components, σ^2_{Eo} and σ^2_{Do}, were also positively correlated ($r=0.94$, $p<0.001$). However, even in more complicated designs involving 10-13 types of relatives, Eisen (1967) found similar correlations among causal components. Thus, the inclusion of more types of relatives did not necessarily decrease sampling correlations among causal components. The inability to estimate all nine causal components and the high sampling correlation between components are both issues that affect the interpretation of the following analysis and are limitations of this approach.

Analysis

To estimate the causal components of variance and covariance, I employed a modified version of a six component restricted maximum likelihood (REML) program (Shaw and Shaw 1992; Shaw 1987). REML provides unbiased estimates, is not sensitive to lack of balance in the data, is flexible in handling non-standard designs, and assumes multivariate normality (Shaw 1987; Thompson and Shaw 1992; Meyer 1992).

Each normally distributed trait was analyzed separately to estimate the causal components related to maternal inheritance. A fixed effect for generation was included in each model because trait means differed between F2 and F3 generations (Table 2) and including a fixed generation effect in the model resulted in smaller likelihoods. The convergence criteria determining the termination of iterations was set at 0.001. Non-negativity constraints on causal component estimates were not imposed because of their adverse effect on significance tests (Shaw 1987).

The log-likelihood ratio test was utilized to evaluate significance in two contexts. First, I evaluated the significance of the models by calculating twice the difference in log-likelihoods for sequential models (1-3). This statistic has a chi-square distribution with degrees of freedom determined by the difference in the number of components estimated in the two models (Shaw and Shaw 1992; Shaw 1987). Second, I utilized this test to evaluate the significance of all components (except E_o) within a given model. To test the significance of each component, I constrained the component of interest to be zero, obtained the log-likelihood of the constrained model, and compared twice the difference in log-likelihood's between the constrained and full models to a chi-square distribution with one degree of freedom.

The estimates of variance components were used to calculate direct and maternal heritabilities and direct-maternal genetic correlations. Resampling methods required to determine the standard errors around these heritabilities and genetic correlations would require inordinate CPU time. Here I indicate the significance of heritabilities and genetic correlations based on the significance of the variance components in the numerator of each respective ratio. Shaw and Platenkamp (1993) used the same approach suggesting that significance in this case reflects the potential for evolutionary response, but not the rate of evolutionary response. The calculation of realized heritability has several components in the numerator (equation 1) and, therefore, no significance is indicated.

An important assumption of this REML analysis is the independence of error terms, i.e. that the contribution of random environmental effects contributing to each individual's phenotype is uncorrelated among individuals and therefore, does not affect their phenotypic covariance. This study was specifically designed to estimate maternal effects which if not included in an analysis can lead to the violation of this assumption. The presence of σ^2_{Do}, σ^2_{Dm}, and σ_{DoDm}, or other factors such as uniparental or cytoplasmic inheritance could have inflated some phenotypic covariances and thus violate the assumption of independent and random error terms. A second bias resulted from not estimating m, the maternal effect coefficient, a scaling factor for maternal phenotypic effects that affected the dam-offspring covariance. A bias in some phenotypic covariances would necessarily result in errors in the estimation of all components because they are estimated simultaneously.

Within-Generation Genetic Correlations

To estimate genetic correlations among traits, I considered each pairwise combination of traits in two hierarchical models, Mendelian inheritance in model 4 (Figure 4A) and maternal inheritance in model 5 (Figure 4B). In model 4, I included only the direct additive (σ^2_{Ao}) and environmental components (σ^2_{Eo}) for each trait as well as their respective covariances (σ_{Ao1Ao2}, σ_{Eo1Eo2}) to estimate direct genetic correlations (r_{Ao1Ao2})(Figure 4A). In model 5, I incorporated the components relevant to maternal inheritance to estimate genetic correlations for direct (r_{Ao1Ao2}) and maternal (r_{Am1Am2}) effects (Figure 4B). However, the structure of the bivariate model depended on the results of the separate analysis of each trait. For example, in figure 4B I show all possible components that would be estimated if both traits were best described separately by model 3 (Figure 1C). If both traits were described separately by model 2 (Figure 1B), then the direct-maternal environmental covariances (σ_{EoEm}) would not be estimated. Thus, the structure of model 5 varied depending on the traits included. I estimated direct and maternal genetic correlations when both traits displayed maternal inheritance or only direct genetic correlations when only one trait displayed maternal inheritance. All covariances were unconstrained (i.e. σ_{Ao1Ao2}, σ_{Eo1Eo2}, σ_{Am1Am2}, σ_{Em1Em2}) except the covariances between traits for direct-maternal additive covariance ($\sigma_{AoAm1AoAm2}$) and direct-maternal environmental covariance ($\sigma_{EoEm1EoEm2}$) components that were constrained to zero.

Figure 4. Path diagrams to estimate genetic correlations between pairs of traits. In Model 4 (A), the genetic correlation between two traits inherited in a Mendelian fashion is denoted by r_{Ao1Ao2}, where 1 and 2 refer to the two traits in the model. All other symbols are identical to Figure 1. In Model 5 (B) both traits are maternally inherited. Within generation genetic correlations between direct additive effects (r_{Ao1Ao2}), and between maternal additive effects (r_{Am1Am2}) are depicted. All possible components are depicted, however, models were simplified based on the best inheritance model determined by the univariate analysis of each trait.

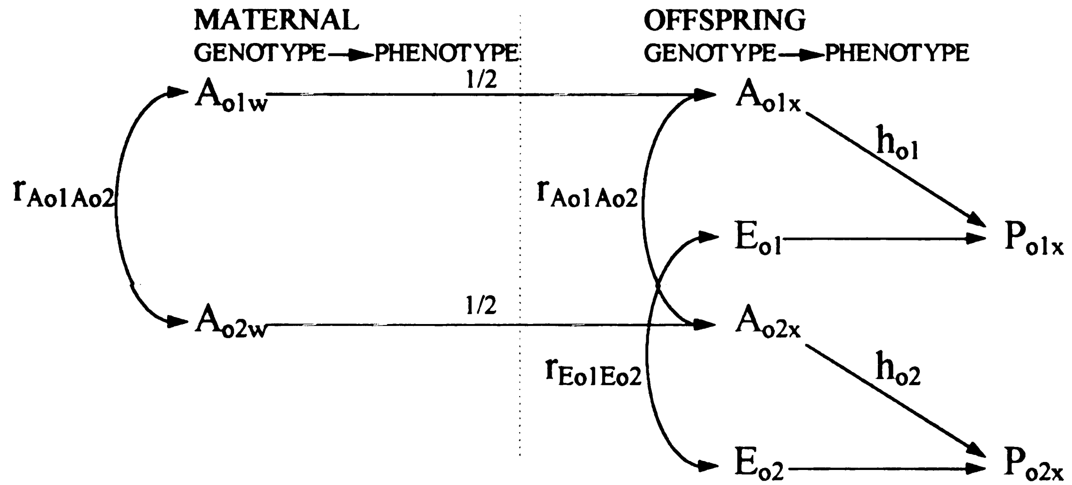
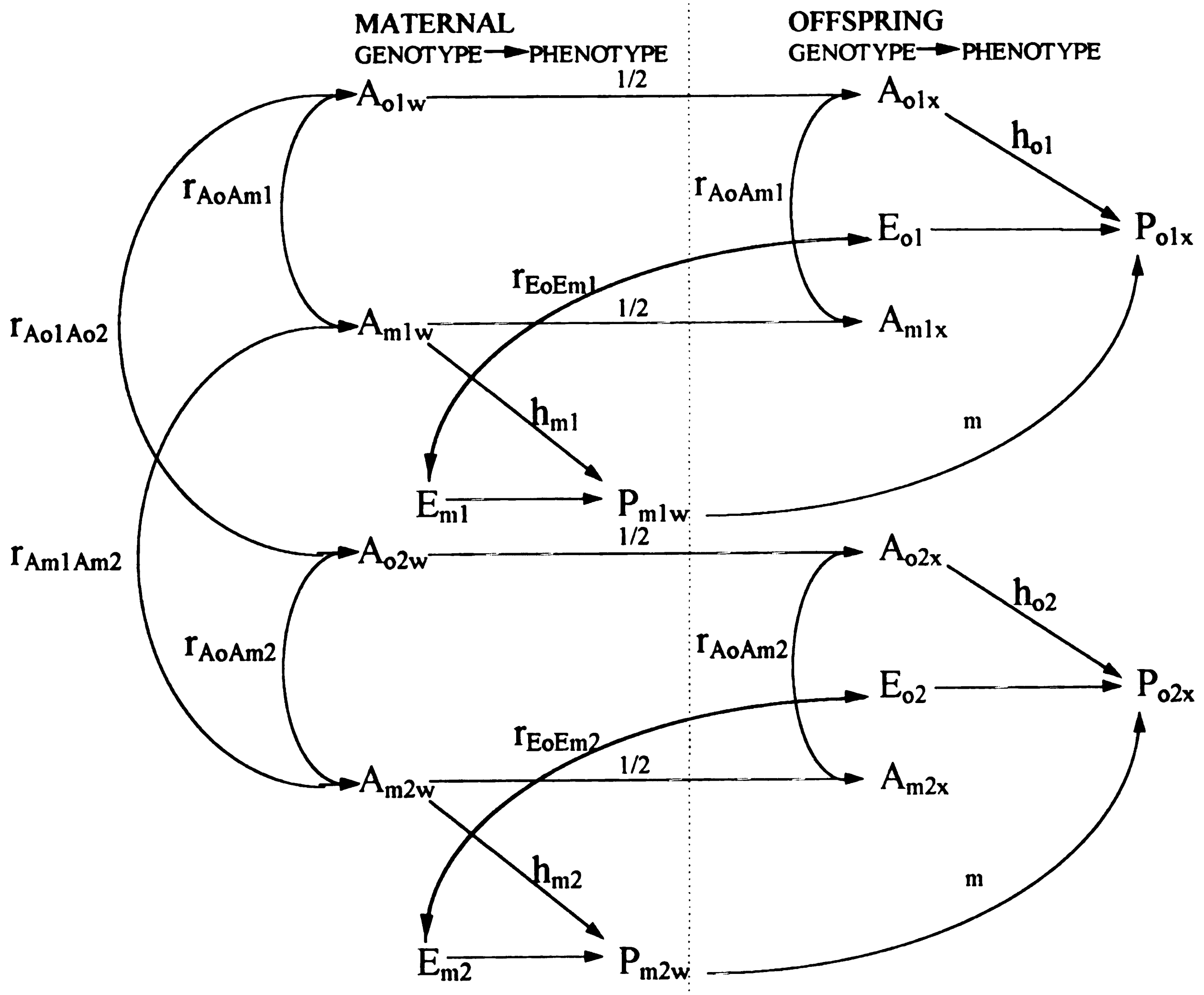

MATERNAL
GENOTYPE→PHENOTYPE
OFFSPRING
GENOTYPE→PHENOTYPE
A_{o1w}
A_{o2w}
r_{Ao1Ao2}
1/2
1/2
A_{o1x}
E_{o1}
A_{o2x}
E_{o2}
r_{Ao1Ao2}
r_{Eo1Eo2}
h_{o1}
h_{o2}
P_{o1x}
P_{o2x}

Figure 4A.


MATERNAL
GENOTYPE → PHENOTYPE
OFFSPRING
GENOTYPE → PHENOTYPE
A_{o1w}
A_{m1w}
E_{m1}
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A_{m1x}
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A_{o2x}
E_{o2}
A_{m2x}
P_{o2x}
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r_{AoAm2}
r_{AoAm2}
r_{Ao1Ao2}
r_{Am1Am2}
r_{EoEm1}
r_{EoEm2}
h_{o1}
h_{o2}
h_{m1}
h_{m2}
m
m

Figure 4B.

RESULTS

Comparison of estimation models

I detected significant σ^2_{Ao} in the simplest inheritance model (1) for 11 out of 14 traits (Table 3). This estimate of σ^2_{Ao} was based on all seven relatives in Table 1 and, therefore, may be inflated by maternal genetic effects in 6 of the 7 phenotypic covariances. Thus, these significant σ^2_{Ao} indicated the potential for 11 traits to be influenced by maternal effects. Lack of significant σ^2_{Ao} for three traits, fall leaf length and number of leaves in the greenhouse and fall cotyledon diameter in the field, suggests that these fall size traits were not influenced either by direct additive or by maternal effects. Two of these traits, fall leaf length and number of leaves in the greenhouse, also had higher coefficients of variation relative to all other traits displaying significant σ^2_{Ao} (Table 2).

In model 2, the addition of three maternal components of variance (σ^2_{Am}, σ^2_{Em}, σ_{AoAm}) significantly improved the likelihood of the estimation model for 8 of the 11 traits that displayed significant σ^2_{Ao} in model 1 (likelihood ratio test, df=3, $p<0.05$; Table 4). Thus, the five component maternal inheritance model better described the data at hand for six traits in the greenhouse, seed weight, embryo weight, cotyledon diameter at emergence and in fall and spring, and spring leaf length and two traits in the field, seed weight and emergence week. Model 2 did not significantly improve the likelihood for three traits (emergence date, spring number of leaves, and field cotyledon diameter at emergence) and did not converge for fall cotyledon diameter in the field.

In model 3 the addition of σ_{EoEm} again significantly improved the likelihood of the estimation model for two traits in the greenhouse, seed weight, and embryo weight (Table

Table 3 ***Model 1***

Table 3. Model 1 restricted maximum likelihood estimates of causal components for greenhouse and field environments. The log likelihood of the full model, magnitude of the fixed generation effect, direct additive (σ^2_{Ao}), direct environmental (σ^2_{Eo}), and total phenotypic variance (σ^2_{p}) are presented. See Table 2 for sample sizes. Significance of each component is noted (*0.1<p<0.05, ** 0.05<p<0.01, ***0.01<p<0.005, ****p<0.005).

Trait	Log likelihood	Fixed effect	σ^2_{Ao}	σ^2_{Eo}	σ^2_{P}
GREENHOUSE					
Seed Weight	-1158.59	1.41	0.92****	0.72	1.64
Embryo Weight	-589.51	1.34	0.64****	0.73	1.37
Emergence Date	-3014.92	15.92	13.40****	78.97	92.37
Cot Diameter	-781.35	0.59	0.15****	1.56	1.71
Fall Cot Diameter	-1486.66	3.63	0.76****	6.49	7.25
Fall Leaf Length	-1922.34	0.47	0.11	16.25	16.36
Fall Number of Leaves	-1034.81	-0.46	0.08	2.74	2.82
Spring Cotyledon Length	-1142.47	4.08	1.03****	6.33	7.36
Spring Leaf Length	-1350.76	6.23	2.14****	10.94	13.08
Spring Number of Leaves	-2134.69	12.49	5.13**	79.30	84.43
FIELD					
Seed Weight	-1362.46	1.28	0.81****	0.81	1.62
Emergence Week	-792.32	-0.67	0.41***	1.22	1.63
Cot Diameter	-785.31	0.68	0.15**	1.90	2.05
Fall Cot Diameter	-1206.70	-0.03	0.23	6.26	6.49

Table 4. Model 2 restricted maximum likelihood estimates of causal components for greenhouse and field environments. The log likelihood of the full model, magnitude of the fixed generation effect, the five direct and maternal causal components, and total phenotypic variance (σ^2_p) are presented. See Table 2 for sample sizes. Significance of the improvement in the log likelihood relative to model 1 (df=3) and the significance of each component are noted (df=1, *$0.1<p<0.05$, ** $0.05<p<0.01$, ***$0.01<p<0.005$, ****$p<0.005$). Traits not converging are indicated by NC.

Trait	Log likelihood	Fixed effect	σ^2_{Ao}	σ^2_{Eo}	σ^2_{Am}	σ^2_{Em}	σ_{AoAm}	σ^2_p
GREENHOUSE								
Seed Weight	-1106.25****	1.42	0.12	1.01	0.06	0.36*	0.03	1.59
Embryo Weight	- 559.47****	1.37	0.33	0.75	0.23	0.23	- 0.19	1.35
Emergence Date	-3012.47	15.92	7.48	80.50	17.88	- 4.44	- 9.45	91.97
Cot Diameter	- 775.36***	0.59	0.49***	1.36	0.67**	- 0.24*	- 0.55****	1.72
Fall Cot Diameter	-1482.17**	3.71	1.98****	5.78	1.85*	- 0.48	- 1.87**	7.26
Fall Leaf Length	-1921.71	0.46	- 0.82	16.54	- 0.12	0.30	0.47	16.36
Fall Number of Leaves	-1034.15	- 0.45	0.09	2.70	0.19	- 0.19	- 0.13	2.83
Spring Cotyledon Length	-1134.55****	4.08	3.30****	4.94	3.32**	- 1.01	- 3.11****	7.44
Spring Leaf Length	-1343.18****	6.31	6.21****	8.90	4.29**	- 1.67	- 4.61****	13.12
Spring Number of Leaves	-2134.23	12.51	3.02	80.49	8.00	- 5.57	- 1.54	84.41
FIELD								
Seed Weight	-1310.48****	1.25	0.11	1.06	0.09	0.31*	- 0.00	1.57
Emergence Week	- 788.44*	- 0.69	0.26	1.26	- 0.22	0.25	0.06	1.62
Cot Diameter	- 784.46	0.69	- 0.07	1.99	- 0.02	0.04	0.11	2.05
Fall Cot Diameter	NC							

Table 5. Model 3 restricted maximum likelihood estimates of causal components for greenhouse and field environments. The log likelihood of the full model, magnitude of the fixed generation effect, six direct and maternal causal components, and total phenotypic variance (σ^2_p) are presented. See Table 2 for sample sizes. Significance of the improvement in the log likelihood relative to model 2 (df=1) and the significance of each component are noted (df=1, *0.1<p<0.05, ** 0.05<p<0.01, ***0.01<p<0.005, ****p<0.005). Traits not converging are indicated by NC.

Trait	Log likelihood	Fixed effect	σ^2_{Ao}	σ^2_{Eo}	σ^2_{Am}	σ^2_{Em}	σ_{AoAm}	σ_{EoEm}	σ^2_p
GREENHOUSE									
Seed Weight	-1102.79***	1.44	0.48	0.83	1.24**	-0.02	-0.93**	0.57***	1.58
Embryo Weight	-557.60**	1.39	0.56**	0.64	1.29*	-0.14	-0.99**	0.45*	1.35
Date	-3012.14	16.13	11.32	78.54	28.87	-8.73	-17.84	6.64	92.15
Cot Diameter	-775.3141	0.59	0.53**	1.34	0.79*	-0.28	-0.65*	0.05	1.73
Fall Cot Diameter	-1482.17	3.70	1.99****	5.77	2.01	-0.53	-1.98*	0.07	7.26
Fall Leaf Length	-1921.70	0.47	-0.86	16.56	-0.30	0.35	0.61	-0.12	16.36
Fall Number of Leaves	-1034.14	-0.45	0.13	2.68	0.26	-0.05	-0.19	0.02	2.83
Spring Cotyledon Length	-1134.55	4.08	3.33****	4.92	3.53	-1.08	-3.26**	0.08	7.45
Spring Leaf Length	-1342.21	6.22	7.01****	8.40	8.72**	-2.92**	-8.06****	1.94	13.16
Spring Number of Leaves	-2133.13*	12.29	5.79	78.86	22.19	-7.94	-14.48	11.37	84.43
FIELD									
Seed Weight	-1309.59	1.26	0.28	0.97	0.59	0.15	-0.43	0.27	1.57
Week	-788.42	-0.69	0.28	1.25	-0.17	0.23	0.03	0.02	1.62
Cot Diameter	-784.46	0.69	-0.07	1.99	-0.03	0.05	0.12	-0.00	2.05
Fall Cot Diameter	NC								

5). Thus, the inclusion of σ_{EoEm} improved the description of the data for traits manifested early in the life cycle.

Significance tests of specific causal genetic components

In model 2, four traits in the greenhouse, cotyledon diameter at three stages (emergence, fall and spring), and spring leaf length, displayed significant σ^2_{Ao}, σ^2_{Am}, and negative σ_{AoAm} (Table 4). Maternal environmental variance, σ^2_{Em}, was significantly positive for greenhouse and field seed weight, significantly negative for greenhouse cotyledon diameter at emergence, and not significantly different from zero for all other traits. A negative σ^2_{Em} is outside the range of possible values.

In model 3, the significance of causal components for two greenhouse traits, seed weight and embryo weight, changed substantially from model 2 as expected from the change in likelihood of the estimation model (Table 5). Seed weight displayed significant σ^2_{Am}, σ_{EoEm}, and negative σ_{AoAm}. These components and σ^2_{Ao} were also significant for embryo weight. Although the likelihood for all other traits did not improve in model 3, the significance of causal components changed slightly. Maternal additive genetic variance (σ^2_{Am}) was no longer significant for cotyledon diameter in fall or spring. Maternal environmental variance (σ^2_{Em}) had a significantly negative value for spring leaf length, but was not significantly different from zero for all other traits.

Relative contribution of components to total phenotypic variance

The relative contribution of the variance components to the total phenotypic variance differed among the models (Figure 5). The inclusion of maternal components in model 2 decreased the contribution of σ^2_{Ao} to the phenotypic variance in all field traits and

Figure 5. The relative contribution of each variance component to the total phenotypic variance for models 1, 2, and 3 for greenhouse (A) and field (B) environments. Only 5 components, the direct additive (σ^2_{Ao}), maternal additive (σ^2_{Am}) , direct environmental (σ^2_{Eo}), maternal environmental (σ^2_{Em}), and the direct-maternal additive covariance (σ_{AoAm}) are included for models 2 and 3 because the direct-maternal environmental covariance (σ_{EoEm}) does not contribute to the total phenotypic variance (see Table 1) The model best describing a trait is indicated by an arrow.

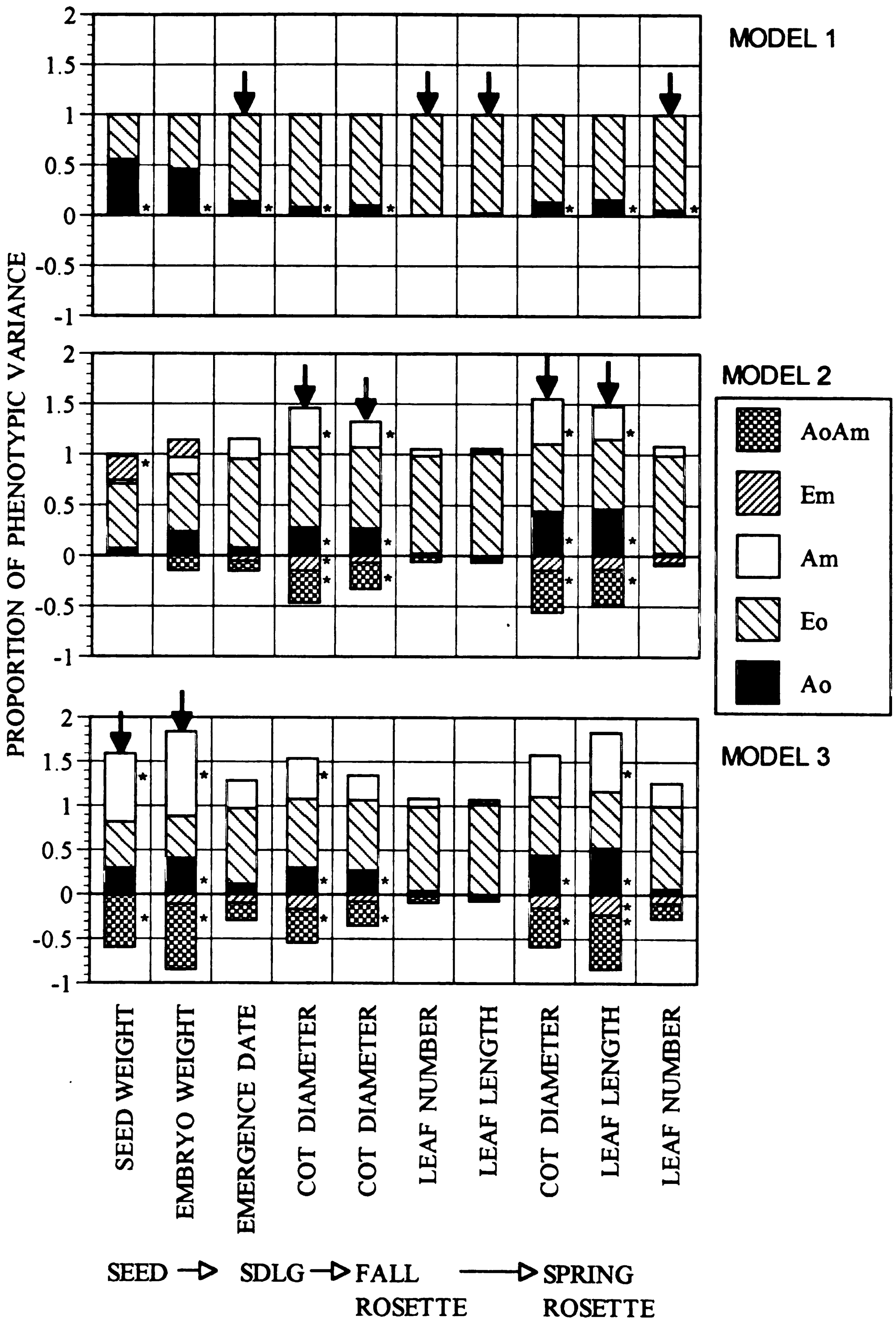

MODEL 1
MODEL 2
MODEL 3
PROPORTION OF PHENOTYPIC VARIANCE
2
1.5
1
0.5
0
-0.5
-1
AoAm
Em
Am
Eo
Ao
SEED WEIGHT
EMBRYO WEIGHT
EMERGENCE DATE
COT DIAMETER
COT DIAMETER
LEAF NUMBER
LEAF LENGTH
COT DIAMETER
LEAF LENGTH
LEAF NUMBER
SEED
SDLG
FALL ROSETTE
SPRING ROSETTE

Figure 5A.

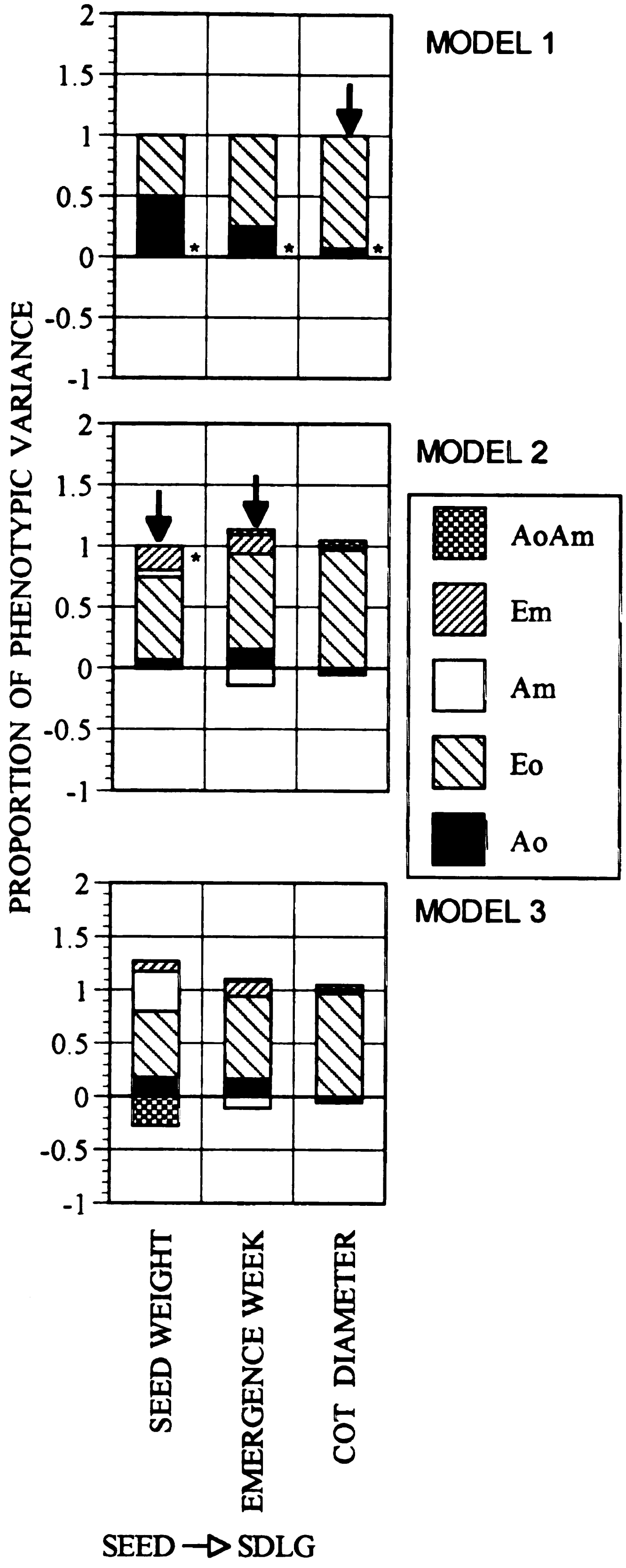

PROPORTION OF PHENOTYPIC VARIANCE
2
1.5
1
0.5
0
-0.5
-1
MODEL 1
MODEL 2
MODEL 3
*
AoAm
Em
Am
Eo
Ao
SEED WEIGHT
EMERGENCE WEEK
COT DIAMETER
SEED —▷ SDLG

Figure 5B.

in greenhouse embryo weight and seed weight. In contrast, the contribution of σ^2_{Ao} appeared to increase for cotyledon diameter at emergence, in fall , and in spring, and spring leaf length relative to model 1. The addition of σ_{EoEm} in model 3 produced little change in the relative contribution of components to the total phenotypic variance between models 2 and 3 for six traits in the greenhouse (emergence date, cotyledon diameter at three stages, fall number of leaves, fall leaf length) and two traits in the field (cotyledon diameter at emergence and in the fall). However, this additional component did change the relative contribution of components for seed weight in both greenhouse and field, embryo weight, spring leaf length, and spring number of leaves. For these traits three components, σ^2_{Ao}, σ^2_{Am}, and σ_{AoAm}, increased in their absolute magnitude and in their contribution to the total phenotypic variance (Tables 4 and 5, Figure 5).

Direct, maternal, and realized heritabilities

Changes in the absolute magnitude and relative contribution of σ^2_{Ao}, σ^2_{Am}, and σ_{AoAm} to the total phenotypic variance among models affected direct, maternal, and realized heritabilities. When maternal effects biased the estimation of σ^2_{Ao} (model 1), a number of traits displayed substantial heritabilities (Figure 6, Table 6). Traits best described by model 2 of maternal inheritance (cotyledon diameter at three stages, spring leaf length, field seed weight, and field emergence week) had significant direct and maternal heritabilities of similar magnitude (Figure 5, Table 6). In contrast, for two traits best described by model 3 (seed weight and embryo weight in the greenhouse), significant maternal heritabilities appeared substantially larger than direct heritabilities. This increase

Figure 6. The direct, maternal, and realized heritabilities for each trait in all models in greenhouse (A) and field (B) environments. The model best describing a trait is indicated by an arrow.

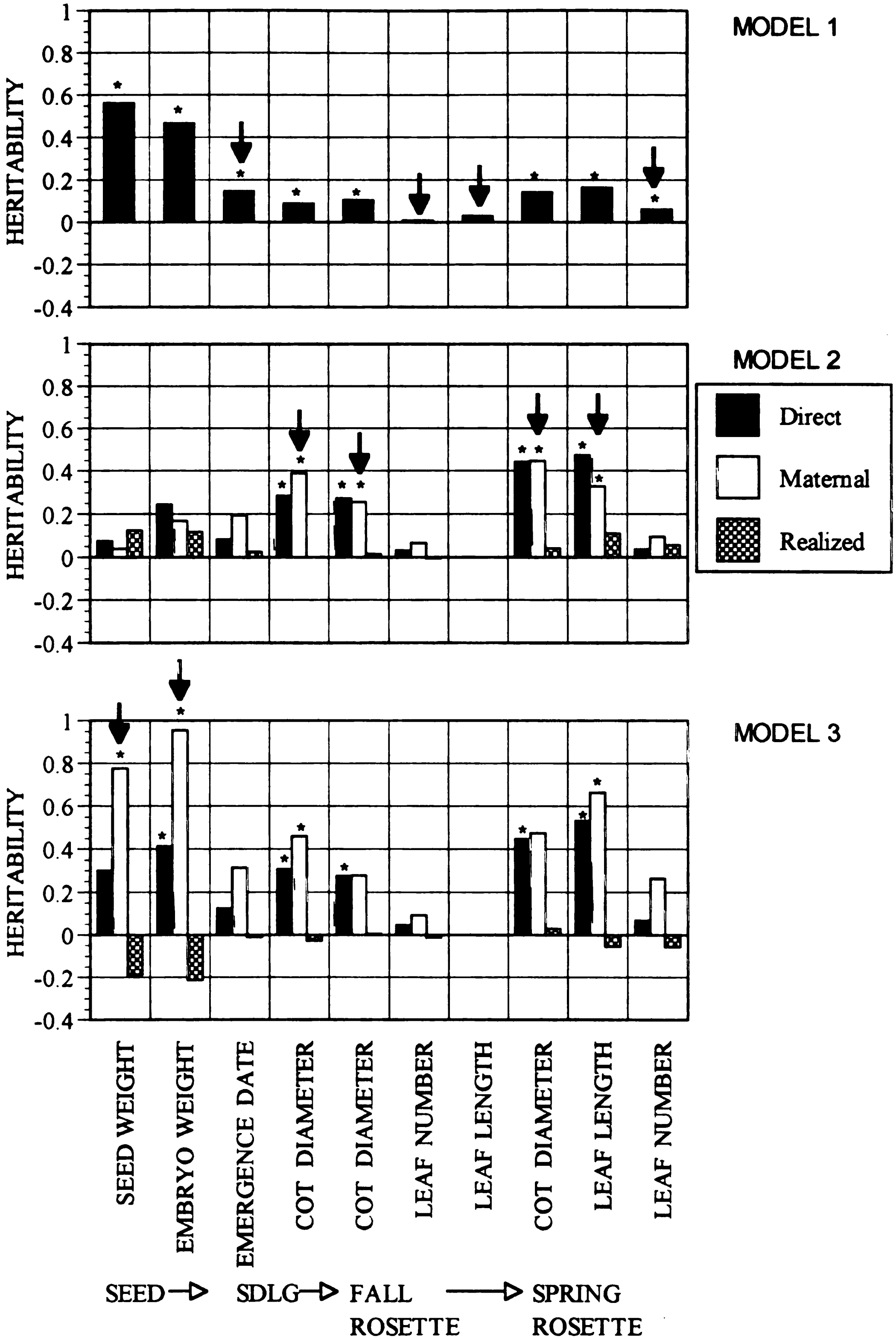

MODEL 1
MODEL 2
MODEL 3
HERITABILITY
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
Direct
Maternal
Realized
SEED WEIGHT
EMBRYO WEIGHT
EMERGENCE DATE
COT DIAMETER
COT DIAMETER
LEAF NUMBER
LEAF LENGTH
COT DIAMETER
LEAF LENGTH
LEAF NUMBER
SEED
SDLG
FALL ROSETTE
SPRING ROSETTE
*

Figure 6A.

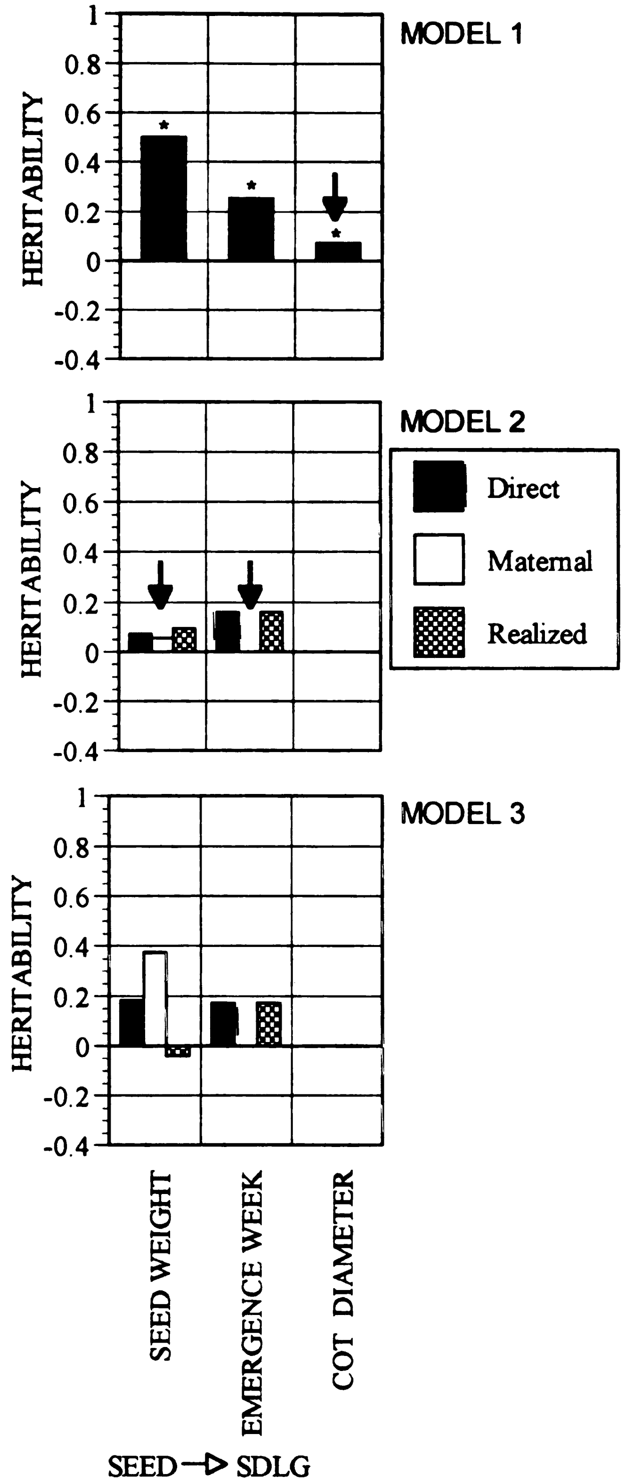

MODEL 1
MODEL 2
MODEL 3
HERITABILITY
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
*
Direct
Maternal
Realized
SEED WEIGHT
EMERGENCE WEEK
COT DIAMETER
SEED→SDLG

Figure 6B.

Table 6. Dire

Table 6. Direct, maternal, and realized heritabilities and direct-maternal genetic correlations for three inheritance models. Significance is based on the significance of the variance component contributing to the numerator (Tables 3-5), indicating the potential for genetic response (Shaw and Platenkamp 1993).

	Model 1	Model 2				Model 3			
Trait	h^2_o	h^2_o	h^2_m	h^2_r	r_{AoAm}	h^2_o	h^2_m	h^2_r	r_{AoAm}
GREENHOUSE									
Seed Weight	0.56*	0.07	0.04	0.12	0.35	0.30	0.77*	-0.19	-1.21*
Embryo Weight	0.47*	0.24	0.17	0.12	-0.69	0.41*	0.95*	-0.21	-1.17*
Emergence Date	0.14*	0.08	0.19	0.02	-0.82	0.12	0.31	-0.01	-0.99
Cot Diameter	0.09*	0.28*	0.39*	0.00	-0.96*	0.31*	0.46*	-0.03	-1.00*
Fall Cot Diameter	0.10*	0.27*	0.25*	0.01	-0.97*	0.27*	0.28	0.00	-0.99*
Fall Leaf Length	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fall Number of Leaves	0.03	0.03	0.06	-0.01	-1.03	0.04	0.09	-0.01	-1.07
Spring Cotyledon Length	0.14*	0.44*	0.44*	0.04	-0.94*	0.45*	0.47	0.03	-0.95*
Spring Leaf Length	0.16*	0.47*	0.33*	0.11	-0.89*	0.53*	0.66*	-0.05	-1.03*
Spring Number of Leaves	0.06*	0.03	0.09	0.05	-0.31	0.07	0.26	-0.06	-1.28
FIELD									
Seed Weight	0.50*	0.07	0.06	0.09	-0.06	0.18	0.37	-0.04	-1.05
Emergence Week	0.25*	0.16	0.00	0.16	0.00	0.17	0.00	0.17	0.00
Cot Diameter	0.07*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

in maternal heritabilites was also observed for greenhouse spring leaf length and field seed weight in model 3.

For maternally inherited traits, realized response to selection is a function of σ^2_{Ao}, σ^2_{Am}, and σ_{AoAm} (equation 1). Despite substantial and significant σ^2_{Ao} and σ^2_{Am} for a number of traits in both models of maternal inheritance, realized heritabilites were near zero or negative (Table 6, Figure 6) because σ_{AoAm} tended to be negative (Tables 4 and 5). Negative covariances resulted in negative genetic correlations for most traits (Table 6) indicating that only alleles that differed in their effects on individual phenotype and maternal performance were maintained. The prediction from the realized heritabilities is that phenotypic selection on a single trait would produce no response. There was an interesting difference in predicted selection response between models 2 and 3 for four greenhouse traits (seed weight, embryo weight, spring leaf length, and spring leaf number, Tables 4 and 5). Because of the changes in the magnitude of the additive components (σ^2_{Ao}, σ^2_{Am}, and σ_{AoAm}) between models, the predicted response to selection is in the same direction as selection in model 2 and in the opposite direction to selection in model 3. For two of these traits, seed weight and embryo weight, model 3 best described the data (Table 5). As a result, seed weight and embryo weight would be expected to show reversed responses to selection in the first generation of selection.

Maternal effects at different stages in ontogeny

For the three size traits quantified in the greenhouse at multiple stages in the life cycle, maternal heritabilites did not decrease through ontogeny (Figure 7). For cotyledon diameter, best described by model 2, significant maternal and direct heritabilities were similar in magnitude from emergence to spring. Leaf length, best described by model 2,

Figure 7. The direct (square), maternal (circle), and realized (triangle) heritabilities for three greenhouse traits measured repeatedly through ontogeny: cotyledon diameter at 3 stages, and leaf length and number of leaves both measured in the late fall and in the spring prior to flowering.

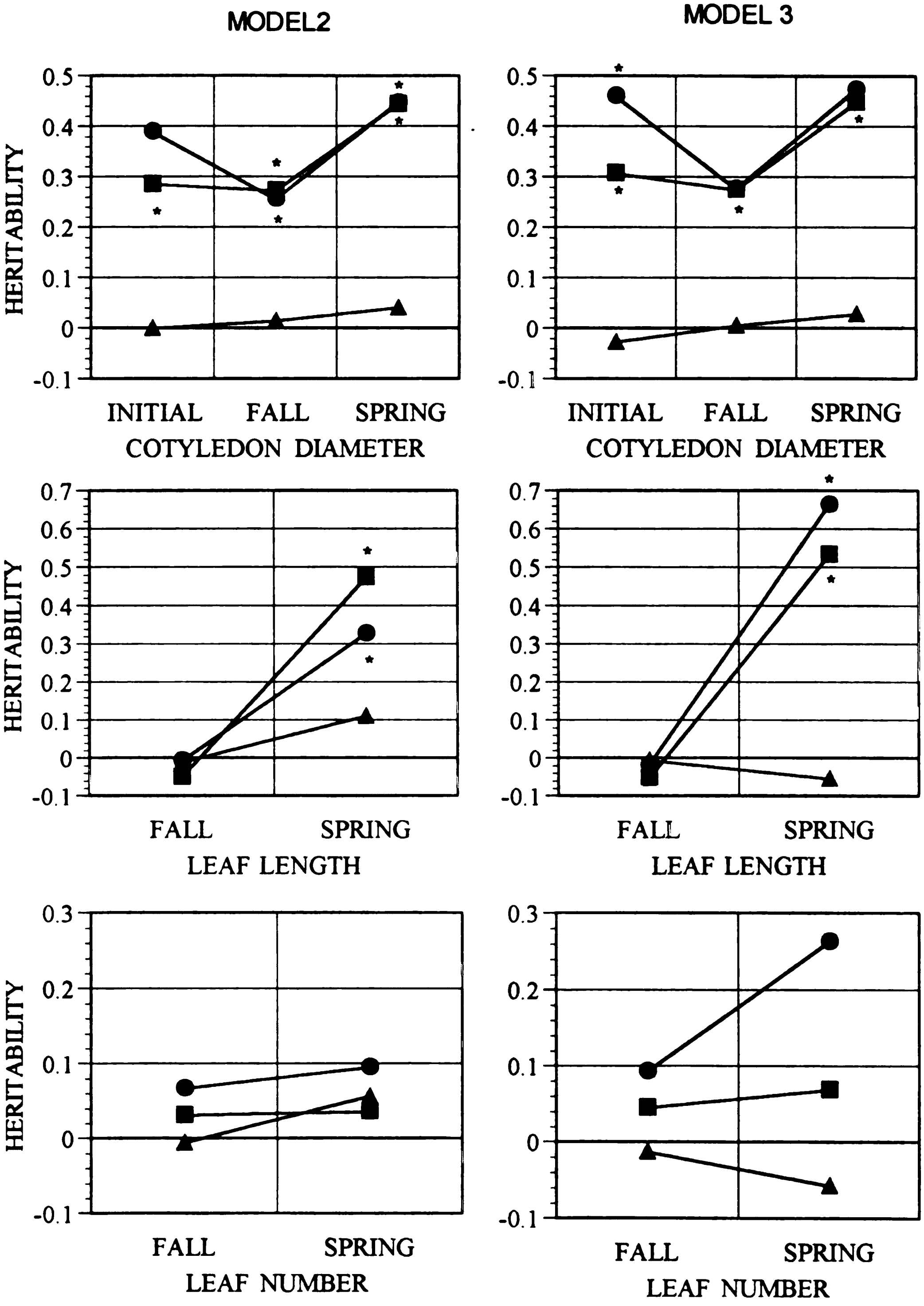

MODEL2
MODEL 3
HERITABILITY
0.5
0.4
0.3
0.2
0.1
0
-0.1
INITIAL
FALL
SPRING
COTYLEDON DIAMETER
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
FALL
SPRING
LEAF LENGTH
0.3
0.2
0.1
0
-0.1
FALL
SPRING
LEAF NUMBER
*

Figure 7.

displayed no heritable variation in the fall and significant direct and maternal heritabilities in the spring demonstrating an increase in maternal inheritance through ontogeny. Number of leaves displayed no heritable variation under any model. Again, the realized heritabilites remained at low values for all three traits at different stages because of the negative σ_{AoAm} at all stages.

Within Generation Genetic Correlations

In contrast to intergenerational genetic correlations (Table 6), within generation genetic correlations calculated from a strictly Mendelian model (1) were positive among a number of size related traits (Table 7). Seed weight and embryo weight were both positively correlated with cotyledon diameter at all three stages in the greenhouse, and seed weight and cotyledon diameter at emergence were also positively correlated in the field. Other traits in the greenhouse showed the following pattern. Cotyledon diameter at emergence was positively correlated with cotyledon diameter at the two subsequent stages with a value close to one. Embryo weight and cotyledon diameter at emergence were also positively correlated with spring leaf length. Emergence date was positively correlated with seed weight, embryo weight, and spring cotyledon diameter and negatively correlated with fall number of leaves, the only significant, negative correlation.

When maternal effects were included in the estimation of genetic correlations (Figure 4b), 31 of 34 estimation models that converged showed improvement in log likelihood. Direct additive genetic correlations were smaller in magnitude and differed in significance from those estimated in a strictly Mendelian model (Table 7). Seed weight was positively correlated with cotyledon diameter at emergence in the field and with fall

Table 7. Direct additive genetic correlations between each pair of traits for greenhouse and field environments. For each bivariate analysis, I estimated genetic correlations from two estimation models: model 4, a strictly Mendelian model (below), and model 5 with maternal effects (above). Model 5 was evaluated only when one or both traits were influenced by maternal effects (------ indicates this pair of traits was not evaluated). Estimation models which did not converge are denoted by NC. When significance tests of the direct additive genetic covariance did not converge, a + follows the value. Bold values indicate model estimation without maternal environmental variances eliminated to obtain convergence. Otherwise significance is as follows: * $0.1<p<0.05$, ** $0.05<p<0.01$, ***$0.01<p<0.005$, ****$p<0.005$.

Trait	SW	EW	ED	CD	FCD	FLL	FLN	SCD	SLL	SLN
GREENHOUSE										
Seed Weight (SW)		NC	0.34	NC	0.52*	0.88	-0.21	0.58***	0.105	0.045
Embryo Weight (EW)	NC		0.27	NC	0.39+	0.54	-0.29	0.48+	**0.06**	-0.00
Emergence Date (ED)	0.41**	0.33*		0.00	0.13	------	-------	0.29*	**0.06**	--------
Cotyledon Diameter (CD)	1.01****	1.00****	0.13		NC	**0.00***	**-0.34**	NC	**0.41+**	**0.17**
Fall Cot Diameter (FCD)	0.75****	0.74****	0.22	1.08****		**0.15**	-0.45	NC	**0.50+**	NC
Fall Leaf Length (FLL)	0.59	0.69	-0.52	0.00**	0.47		-------	-0.22	**-0.12+**	--------
Fall Leaf Number (FLN)	-0.14	-0.01	-0.85**	-0.51	-0.23	0.49		-0.61**	**-0.45**	--------
Spring Cot Diameter (SCD)	0.69****	0.68****	0.48*	1.01****	0.81+	-0.14	-0.64		NC	-0.14
Spring Leaf Length (SLL)	0.24	0.30*	0.21	0.85****	0.57+	0.10	-0.58	0.70+		**0.28**
Spring Leaf Number (SLN)	-0.11	-0.07	-0.40	0.18	-0.03	-0.45	0.57	-0.07	0.02	
FIELD										
Seed weight (SW)			0.53	1.52****	--------					
Emergence week (ED)	0.19			0.34	--------					
Cotyledon diameter (CD)	1.08****		-0.27		--------					
Fall Cot diameter (FCD)	NC		NC	NC						

and spring cotyledon diameter in the greenhouse. Emergence date was also positively correlated with spring cotyledon diameter in the greenhouse. Fall number of leaves and spring cotyledon diameter were negatively correlated in the greenhouse. A number of trait pairs that showed genetic correlations close to a value of one in the simpler Mendelian model did not converge under maternal inheritance (Table 7). Maternal additive genetic correlations were not significant for any estimation model in which they were included. Only one trait pair displayed a large positive value (r_{Am1Am2}=0.73), spring leaf length and fall cotyledon diameter, however significance tests for this component did not converge. Therefore, maternal performance appeared to be genetically uncorrelated in its effects on traits in the subsequent generation.

DISCUSSION

The most significant result in this study is the effect of maternal inheritance on predicted response to selection. Negative genetic correlations between the direct additive and maternal additive effects (r_{AoAm}) result in realized heritabilities near zero for traits expressed at all stages in the life cycle. These negative correlations are so large early in life that traits in the seed stage exhibit negative realized heritabilities. For seed weight and embryo weight, the predicted selection response is in the opposite direction to selection. Thus, the structure of maternal inheritance in *C. verna* is such that trans-generational effects of a mother on her young dramatically constrain the evolutionary response of traits expressed both early and late in the life cycle. It is also interesting that maternal inheritance persists throughout the life cycle in this annual plant. Below I summarize the pattern of maternal inheritance and its consequence for adaptive evolution.

Maternal inheritance

The causal components contributing to maternal inheritance and their magnitude change over the course of development (Figure 1). Four components contribute to phenotypic variation in the seed traits in the greenhouse, seed weight and embryo weight: σ^2_{Ao}, σ^2_{Am}, σ_{AoAm}, and σ_{EoEm} (Table 5). Maternal additive effects are 2-3 times as large as direct additive effects. Maternal environmental effects are small, presumably as a result of relatively uniform environmental conditions in the greenhouse. The positive covariance in environmental effects results from the temporal overlap of environmental conditions in the mother and her young at this stage. For all traits expressed beyond the seed stage, the covariance in environmental effects does not appear to contribute to the resemblance between mothers and offspring, most likely because both parents and offspring were randomized across environmental conditions. The magnitude of these four components is similar for seed weight in the field (Table 5), however, the trait is best described by model 2 (Table 4) in which only the maternal environmental component is significant.

In the seedling stage, three components contribute to the phenotypic value for cotyledon diameter at emergence in the greenhouse: σ^2_{Ao}, σ^2_{Am}, negative σ_{AoAm} (Table 4). Direct and maternal additive effects are more similar in magnitude when compared to seed traits. In contrast in the field, cotyledon diameter is best described by the strictly Mendelian model (Table 3). Emergence time is best described by Mendelian inheritance in the greenhouse and by a marginally significant maternal inheritance model (2) in the field (Table 4). In the latter model , however, none of the components are significant.

The pattern of maternal inheritance for cotyledon diameter in the greenhouse remains the same throughout subsequent stages in the life cycle (Table 4, Figure 6) with three additive genetic components contributing to an individual's phenotypic value. The number of leaves and leaf length show no additive genetic variation when traits are expressed in fall rosettes in the greenhouse (Table 3), however, prior to flowering in the spring, leaf length displays the same pattern of maternal inheritance as cotyledon diameter at all three stages (Table 4). In contrast, the number of leaves displays simple Mendelian inheritance in the spring (Table 3).

This hierarchical analysis clearly shows that the magnitude and structure of maternal inheritance changes throughout the life cycle. Early in life, both genetic and environmental components of maternal performance contribute to the offspring phenotype. At emergence, however, maternal genetic effects predominate. These maternal genetic effects persist throughout the life cycle for cotyledon diameter, while other size related traits show more variation in the model of inheritance. For example, leaf length shows no heritable variation in the fall. In contrast, leaf length in the spring is influenced by maternal genetic effects.

The structure of maternal inheritance appears to differ between the field and greenhouse environments. Seed weight is maternally inherited in both environments, but the best model differs between environments. Emergence week is best described by Mendelian inheritance in the greenhouse and maternal inheritance (model 2) in the field. In the field cotyledon diameter at emergence does not display maternal inheritance, however it does at all three stages in the greenhouse. It is not unusual to obtain different estimates of causal components when offspring are reared in different environments (e.g. Mazer and

Schick 1991; Schmitt et al. 1992; Schmid and Dolt 1994; Platenkamp and Shaw 1993; Montalvo and Shaw 1994). In this study, it is difficult to compare the structure of maternal inheritance between the field and greenhouse environments because the census interval differed between the two environments, emergence time and size at emergence were measured weekly in the field and twice per week in the greenhouse. As a result, I expected and observed larger variances associated with these two field traits (Table 2). More importantly, estimates from the field are compromised by the possibility of genotype by environment interaction. An analysis of paternal half-sib means in the two environments showed little evidence for genotype by environment interactions in the final generation (Thiede, unpublished data). However, other studies have documented that maternal genotypic effects can depend on the environment in which the offspring are raised (Schmitt et al. 1992; Schmid and Dolt 1994). This type of maternal genotype by offspring environment interaction would compromise this quantitative genetic analysis of maternal inheritance. Therefore, the field estimates of maternal inheritance should be viewed with caution.

Maternal performance

What phenotypic traits are likely to contribute to the composite maternal performance phenotype (P_{mw})? Maternal size (Platenkamp and Shaw 1993), maternal nutritional status (Parrish and Bazaaz 1985; Miao et al. 1991), maternal phenology (Lacey 1991), and maternal source-sink relations (Rocha and Stephenson 1990) (see review in Roach and Wulff 1987) could all contribute to maternal performance. The position of seeds in a fruit during development determines source-sink relationships that can affect a number of seed traits, especially seed size (e.g. Rocha and Stephenson 1990). When position effects were included as a fixed effect in the analysis of seed weight, they significantly improved the likelihood of the estimation model indicating that position effects account for a significant amount of the observed phenotypic variation. The within maternal family variation in seed weight accounted for by the fixed effect may be determined by the architecture of the mother. If the architectural traits that determine position effects are genetically based, they may allow the variance in seed weight to evolve as well as the mean (Bull 1987; Carriere 1994). Biere (1991a) suggested similar reasoning for selection on the variance in emergence time in *Lychnis flos-cuculi*. Response of the phenotypic variance to selection may result not only from non-linear components of the selection gradient (Brodie et al. 1995), but also from higher levels of selection such as maternal selection (Thiede, 1996).

Estimation of maternal effects

In natural plant populations, the magnitude of maternal genetic effects have been estimated by three different approaches. First, the "bio-model" from a diallel design (Cockerham and Weir 1977) permits the estimation of maternal and paternal extranuclear effects (e.g Antonovics and Schmitt 1986; Mazer 1987; Biere 1991a; Kelly 1992; Platenkamp and Shaw 1993; Montalvo and Shaw 1994). The estimate of maternal extranuclear effects in the above studies contains a number of specific maternal genetic and environmental causal components, but does not require assumptions about an underlying model of maternal inheritance. The second approach is a nested breeding design in which maternal effects are confounded by dominance, therefore, limiting conclusions about the magnitude of these effects (Mitchell-Olds 1986; Mitchell-Olds and Bergelson 1990a; Schwaegerle and Levin 1991). The final approach uses clonal replicates to experimentally separate maternal genetic, maternal environment, and their interaction as sources of phenotypic variation in offspring traits (Biere 1991a; Schmitt et al. 1992; Platenkamp and Shaw 1993; Schmid and Dolt 1994). Like the diallel, this approach does not provide estimates of specific causal components related to maternal inheritance, but does allow one to compare the magnitude of genetic vs. environmental effects in artificial environments as well as explore the possibility of genotype by environment interactions.

The multi-generation approach that I present here is novel in its detailed partitioning of the phenotypic variance into specific causal components allowing more explicit predictions about evolutionary responses to selection (see below). The general pattern of maternal effects documented in this study is consistent with previous findings. Seed weight and emergence date exhibit low direct heritabilites and substantial maternal effects (Biere 1991a; Platenkamp and Shaw 1993; Montalvo and Shaw 1994; Schmid and Dolt 1994). Subsequent size related traits exhibit moderate direct heritabilities and maternal genetic effects in some studies (Biere 1991a; Schmid and Dolt 1994), but not in others (Montalvo and Shaw 1994). In other studies maternal genetic effects generally decline through the life cycle (Biere 1991a; Schmid and Dolt 1994; Montalvo and Shaw 1994). In contrast in this study, maternal genetic effects continue to contribute significantly to phenotypic variation all the way through the life cycle for two of three traits (Figure 7; for another exception see Schmid and Dolt 1994). The larger magnitude of maternal genetic effects relative to smaller maternal environmental effects in this study is consistent with other studies (Biere 1991a; Schmid and Dolt 1994; Platenkamp and Shaw 1993). However, there is ample evidence that maternal genotype by environment interactions may eliminate the direct maternal genetic effect when maternal genotypes are replicated across contrasting environments (Schmitt et al. 1992; Platenkamp and Shaw 1993; Schmid and Dolt 1994). These genotype by environment interactions for maternal effects should not obscure maternal genetic effects in this study because all mothers were raised under relatively uniform greenhouse conditions. However, the impact of maternal genotype by environment interactions on the evolution of maternally inherited traits awaits the development of theoretical models that incorporate these higher order interactions in the response to selection.

While advantageous for a mechanistic understanding of the evolutionary process, this biometrical approach for estimating maternal effects by partitioning the phenotypic covariances among numerous relatives has limitations (Eisen 1967; Foulley and Lefort 1978; Wilham 1980). The primary limitation is the confounding of direct Mendelian inheritance and maternal effects in maternal lineages that results in large sampling correlations among causal components. In designs such as the one used here, sampling correlations can cause substantial bias in estimation of variance components when not all components are estimable. Experimental approaches that decouple direct and maternal transmission provide an alternative approach. Cross-fostering offspring after birth provides estimates of post-natal maternal effects by separating the maternal effect from the direct effect by using nurse mothers (Riska et al. 1985). Embryo transplantation is another approach that provides estimates of both pre-natal and post-natal maternal effects by decoupling direct and maternal effects (Cowley 1991). Experimental manipulation of maternal attributes such as maternal provisioning can also be utilized to estimate the magnitude of the maternal phenotypic effect separately from genetic contributions (Sinervo 1991). In the absence of similar experimental approaches for detangling maternal and direct effects in plants, the best solution may be to include numerous types of relatives in biometrical analyses. For example, Cantet et al. (1988) were able to estimate all nine variance components in a maternal effects model by utilizing 17 types of relatives. Alternatively, utilizing relatives like second cousins in which direct and maternal effects are less confounded may provide a better approach (Wilham 1980).

A second limitation of this approach is the potential bias that may result from not estimating additional components that may be influencing phenotypic covariances: 1) dominance components, 2) cytoplasmic inheritance, and 3) the maternal effect coefficient. Meyer (1992) indicates that the magnitude of the excluded effect must be quite large (i.e. 30%) to affect the estimates of variance components. To what extent might dominance variance bias variance component estimates in this study? When I included direct dominance in a five component estimation model (σ^2_{Ao}, σ^2_{Eo}, σ^2_{Do}, σ^2_{Am}, σ_{AoAm}), the estimates of the additive components did not change. Furthermore, for seven out of ten traits in the greenhouse, σ^2_{Do} was negative, indicating a value not different from zero. Montalvo and Shaw (1994) also detected no significant dominance variance in similar traits. Therefore, in this study direct dominance variance is unlikely to change the estimates of the direct and maternal additive genetic variance and their covariance. Maternal dominance and maternal environmental variances are perfectly correlated in this design. If one views the estimates of maternal environmental variance as the sum of these two components (suggested by Thompson 1976), it is clear that maternal dominance is also not significantly influencing phenotypic covariances because the maternal environmental variance was not different from zero in most cases (Tables 4 and 5). Therefore, the estimation of variance components in a reduced animal models appears robust to the assumptions of no direct or maternal dominance variances or their covariance in this study.

Resemblance among relatives sharing a common maternal lineage can also be influenced by cytoplasmic inheritance of chloroplast and mitochondrial genomes (reviewed by Gillham 1994; but see Chiu and Sears 1993; Sewell et al. 1993 for exceptions). Lynch and Walsh (1996) suggest how these models could also be extended to include uniparental cytoplasmic and mitochondrial transmission. In the present study, full-sib, dam-offspring, and maternal relative-offspring covariances could include effects due to cytoplasmic inheritance which would inflate estimates of maternal additive, maternal environmental variances, and direct-maternal environmental covariance. Similarly, not estimating the maternal effect coefficient also has the potential to inflate specific variance components (see Cantet et al. 1988).

Evolutionary consequences of maternal inheritance

Previous studies of maternal effects have often suggested that response to selection on juvenile traits such as seed mass or emergence time will be slower (i.e. Antonovics and Schmitt 1986, Roach and Wulff 1987; Biere 1991a) because maternal genetic effects mask the small amount of zygotic genetic variation. Several authors have suggested that selection may act solely on the maternal genetic variation for juvenile traits lacking direct additive genetic variation (Biere 1991a; Platenkamp and Shaw 1993; Montalvo and Shaw 1994; Schmid and Dolt 1994). It is, of course, possible for selection to differentiate among offspring and also among mothers. The resulting response to multiple levels of selection will depend critically on the genetic variance for both offspring phenotype and maternal performance. The strength of the approach presented here is that it allows one to evaluate the response to selection, not only based on direct and maternal additive genetic variance, but also based on their covariance which all other studies in natural populations have not estimated. Accurate predictions about evolutionary responses to selection hinge on this detailed partitioning. This study clearly demonstrates that these direct-maternal genetic covariances will constrain selection response (Figure 6, Table 6).

Intergenerational covariances

Direct-maternal additive genetic covariances between maternal performance and offspring phenotype are consistently negative for 7 of 8 traits displaying maternal inheritance (Tables 4 and 5). Furthermore, the magnitude of this direct-maternal covariance is large enough to result in a predicted reversed response to selection for two traits, seed weight and embryo weight. For all other traits in both models, the negative direct-maternal covariance reduces the predicted response to selection to near zero (Figure 6). Thus, despite substantial direct and maternal additive effects, the evolutionary potential of these traits is limited by the underlying direct-maternal genetic covariances.

Since Dickerson's (1947) seminal paper documenting the evolutionary consequences of maternal effects in domestic hogs, a number of animal breeders and evolutionary biologists have demonstrated negative direct-maternal additive genetic covariances (Figure 2). Others utilizing Falconer's (1965) simplified approach have demonstrated negative maternal effect coefficients. Negative m's have been found for litter size in mice (Falconer 1955,1965), age to maturity in springtails (Janssen at al. 1988) and clutch size and condition in flycatchers (Schluter and Gustaffson 1993). In some cases, the magnitude of these direct-maternal covariances or maternal effects coefficients are large enough to produce reversed responses to selection in the short-term. In theory, long-term responses to consistent selection should asymptotically approach the expected rate in the absence of maternal effects (Kirkpatrick and Lande 1989). In nature, however, spatial and temporal variation in selection (e.g. Kalisz 1986; Kelly 1992; Stratton 1992a) in conjunction with maternal inheritance can be expected to produce complex evolutionary dynamics.

Trade-offs between life history traits have been central in the theory of life-history evolution (e.g. Williams 1957; Lande 1982). In his review of life-history tradeoffs, Stearns (1992) points out that most of the theoretical and empirical literature on life history have dealt with tradeoffs within an individual such as allocation to current vs. future reproduction or current reproduction vs. subsequent survival. However, tradeoffs between generations have received less attention. This analysis of maternal effects in *C. verna* suggests that there is a fundamental, genetically based intergenerational trade-off between maternal performance and offspring phenotype for 7 of 14 traits examined (Table 6). Perhaps the simplest explanation for the existence of antagonistic pleiotropy is that directional selection on maternal performance and/or offspring phenotype has led to the maintenance of alleles that differ in their effect on the phenotype (Falconer 1981). In theory, mutation could supply sufficient variation to prevent the fixation of these differing alleles via selection (Charlesworth 1990), so the explanation for the existence of these negative direct-maternal genetic correlations may require a more complicated model of functional genetic architecture involving pleiotropic effects on allocation and acquisition (Houle 1991). Whatever the mechanistic explanations for these negative genetic correlations, the consequence is that joint evolution of maternal performance and offspring phenotype will be constrained for a number of traits at different stages in the life cycle of *C. verna*.

Within generation covariances

In contrast to intergenerational covariances described above, most of the significant additive genetic covariances between traits within a generation are positive. Under Mendelian inheritance in model 4, these positive additive genetic correlations show substantial pleiotropic effects for traits related to size early in the life cycle. Seed weight is genetically correlated with cotyledon diameter at emergence, but the magnitude of this correlation declines in subsequent measures of this trait (Table 7). Cotyledon diameter is correlated across the three censuses. The only significant negative correlation is between emergence date and fall leaf number. Therefore, in the absence of maternal effects, these estimates of within generation genetic correlations indicate substantial positive pleiotropy among size related traits.

When maternal inheritance is included in the estimation of these genetic correlations, however, the magnitude and significance of direct genetic correlations changes substantially (Table 7). Most correlations remain positive, but many are no longer significant. The inclusion of maternal inheritance in the estimation model reveals decreased pleiotropy. It is common to observe positive correlations among size traits in plants (e. g. Montalvo and Shaw 1994). In general morphological traits tend to show positive genetic correlations (Roff 1996), however, many of these estimates may be inflated by maternal effects. While morphological traits show some pleiotropy, there is no evidence for significant genetic correlations among the unobserved maternal performance traits.

Multivariate evolution

Equations for predicting multivariate evolution require estimates of the additive genetic variance-covariance matrix (**G**) for all traits as well as estimates of the selection gradient (Lande 1982; Lande and Arnold 1983). However, it is not clear how univariate estimates of direct and maternal additive components and bivariate estimates of genetic correlations between traits such as those estimated in this study translate into a multivariate **G**. Currently, evolutionary biologists are technically constrained from obtaining these multivariate estimates with Dickerson's genetic model for estimating maternal effects. An alternative approach for considering the evolutionary consequences of maternal effects in a multivariate framework describes the structure of maternal inheritance by a single term, the mother-daughter covariance (Kirkpatrick and Lande 1989, 1992; Lande and Kirkpatrick 1990; Riska 1991). In a subsequent manuscript I explore the multivariate evolutionary dynamics of maternal inheritance using this simplified covariance approach.

Conclusions

This quantitative genetic analysis demonstrates that maternal inheritance will influence the evolutionary dynamics for a number of traits in this natural plant population. Traits reflecting individual size at the seed, seedling, and adult stages in the life cycle were significantly influenced both by direct and maternal additive genetic variances and their covariance. The persistence of maternal inheritance to later stages in the life cycle is unusual in plants. Perhaps the most significant contribution of this study is the negative estimates of direct-maternal additive genetic covariances, the first demonstration of this evolutionary constraint in a natural plant population. In conjunction with direct and maternal additive genetic variances, this direct-maternal additive covariance clearly results in predicted reversed response to selection for two traits, seed weight and embryo weight, and minimal responses to selection in traits later in the life cycle. In contrast, within generation genetic covariances among size traits are likely to enhance selection response such that direct selection for increased seed or seedling size will result size increases in prior or subsequent traits. The incorporation of within and between generation covariances in a multivariate framework for predicting response to selection remains a challenge. While most authors have suggested that maternal effects may slow the evolutionary response by masking the zygotic genotype, this study illustrates that maternal effects have the potential to enhance or constrain the selection response depending on the sign and magnitude of the direct-maternal additive genetic covariance. In the study population, the joint evolution of maternal performance and individual phenotype is constrained for all traits displaying significant maternal effects suggesting an underlying fundamental trade-off between mothers and their offspring.

Chapter 2

AN EPISODIC ANALYSIS OF PHENOTYPIC SELECTION ON JUVENILE TRAITS IN *COLLINSIA VERNA*: A COMPARISON OF QUANTITATIVE TRAITS DISPLAYING MENDELIAN AND NON-MENDELIAN INHERITANCE.

INTRODUCTION

Studies of evolution in natural populations consider two phases in the evolutionary process: phenotypic selection and Mendelian inheritance. These separate estimates of within generation selection (β and γ) and between generation response to selection based on inheritance (G) can be combined in the standard multivariate equation of evolution to predict the change in the trait mean, $\Delta \bar{z}=GB$, or the trait variance or covariance, $\Delta G=G(\gamma-\beta\beta^{T})G$ (after Phillips and Arnold 1989; Lande and Arnold 1983). However, when an individual's phenotypic value is a function not only of its genotypic value in the environment, but is also influenced by its mother's phenotypic value then evolutionary responses will differ from expectation based on the standard equations.

Kirkpatrick and Lande (1989, 1992) have demonstrated that when traits display maternal or non-Mendelian inheritance, the evolutionary change in a trait mean is a function not only of current phenotypic selection and Mendelian and non-Mendelian inheritance, but also is a function of phenotypic selection in previous generations. Thus, maternal inheritance introduces time lags in the evolutionary process. These time lags influence the rate of evolutionary response such that the maximal rate is approached asymptotically under a constant selection. Furthermore, the response to selection continues after selection ceases and its direction can vary depending on the sign and magnitude of the maternal effect coefficient. Kirkpatrick and Lande (1989) call this evolutionary momentum. In addition to time lags, maternal inheritance can affect the direction of response depending on the sign and magnitude of the direct-maternal additive genetic covariance (Wilham 1963). Thus, predicting the direction and magnitude of evolutionary responses for maternally inherited traits is complicated.

Animal breeders have demonstrated how maternal inheritance can alter predicted responses to artificial selection (Dickerson 1947; Wilham 1963). Both negative direct-maternal genetic covariances (Riska et al. 1985) and negative maternal phenotypic effects (Falconer 1965) can produce reversed responses to selection. In addition to the influence of maternal inheritance on artificial selection, quantitative geneticists have demonstrated that maternal genetic effects on traits like body size decrease through ontogeny (Cheverud et al. 1983; Atchley 1984). While maternal inheritance may decline through the life cycle, it can still influence multivariate evolution in natural populations. If selection acts directly on maternally inherited traits or traits influencing maternal performance, then genetic correlations between these traits will influence their joint evolution.

Like animal breeders, plant population biologists have documented the persistence of maternal effects through ontogeny. In nearly all cases, these studies focus on how maternal **environmental** conditions influence offspring phenotype. Maternal environmental effects have the potential to influence an extensive number of plant traits including seed weight and early size (reviewed by Roach and Wulff 1987). Maternally influenced traits like seed weight, emergence time, and early relative growth rate can determine early size differences and affect intraspecific competitive interactions (Gross 1984; Gross and Smith 1991). These differences in seedling size tend to persist through the life cycle in competitive situations (Fenner 1983; Gross 1984), therefore, maternal effects can be long-lasting in these situations. If juvenile traits influence the outcome of competitive interactions that generate size and consequently fitness hierarchies in plant populations (Waller 1985; Stanton 1985; Weiner 1985, 1990), then maternal effects can directly impact fitness. Thus, maternal environmental effects influence a number of plant traits and their effects can persist to late in life. Lacey (1991) has shown that maternal environmental effects can persist through two generations and influence phenological traits like flowering time.

The demonstration of maternal genetic effects on plant traits is less common. A number of studies have demonstrated a significant maternal genetic component to seed weight (Platenkamp and Shaw 1993; Montalvo and Shaw 1994; Schmid and Dolt 1994; Biere 1991a; Mitchell-Olds and Bergelson 1990a), germination date (Montalvo and Shaw 1994; Schmid and Dolt 1994; Biere 1991a; Mitchell-Olds and Bergelson 1990a), and seedling size (Schmid and Dolt 1994; Biere 1991a). These studies demonstrate that maternal genetic effects decrease through ontogeny with effects being strongest on seed weight, and smaller or non-significant on seedling size. In two studies, maternal genetic effects were larger than maternal environmental effects (Biere 1991a; Schmid and Dolt 1994). Schmitt et al. (1992) demonstrated that maternal genotypes differ in their response to maternal environmental conditions such that maternal genetic effects on offspring are influenced by the maternal genotype by maternal environment interaction. The maternal genetic effects estimated in these studies can not be incorporated into evolutionary models predicting the response of maternally inherited traits because the genetic parameters do not estimate either the maternal additive variance (except Platenkamp and Shaw 1993) or the covariance between direct additive and maternal additive values. Thus, straightforward predictions about the evolutionary role of these maternal genetic effects in natural plant populations are not possible.

In contrast, the nature of phenotypic selection in natural populations has been well documented for a number of these maternally influenced traits. Univariate studies of seed weight and emergence date have documented the effects of these traits on individual survival and fecundity (e.g. Kalisz 1986; Winn 1988; Biere 1991b). In multivariate studies the direct contribution of traits to components of fitness can be separated from indirect effects on phenotypically correlated traits. Multivariate studies including a number of juvenile plant traits have shown that direct selection acts primarily on early size, while seed weight and emergence date contribute mostly indirectly to fitness components via their effect on size (Bennington and McGraw 1995b; Stratton 1992a; Mitchell-Olds and Bergelson 1990b). Thus, traits likely to display maternal inheritance like seed weight, emergence date, and early seedling size can directly or indirectly influence components of fitness in a number of species.

My motivation in this study is to quantify the extent to which maternally inherited traits impact the rate and direction of multivariate evolution by examining the relationship between a number of maternally inherited juvenile traits and fitness. I have found that a number of juvenile traits in the winter annual, *Collinsia verna*, display maternal inheritance. Specifically, three traits, seed weight, cotyledon diameter at emergence, and cotyledon diameter in late fall displayed both significant additive genetic and maternal additive genetic variance as well as negative direct by maternal genetic covariance. A fourth trait, emergence date, displayed only significant additive genetic variance and no maternal effects (Chapter 1). Understanding and predicting the evolutionary response of these maternally inherited traits hinges not only on the nature of phenotypic selection, but also on the observed maternal inheritance.

Here I quantify the magnitude of direct selection on each of these four traits in four episodes of selection (Figure 8). The analysis of selection for sequential episodes in the life cycle is required because early mortality can eliminate individuals before they express all four of the phenotypic traits. Individuals not expressing all traits can not be included in a multiple regression analysis of a single episode spanning the entire life cycle. Therefore, I partitioned the life-cycle into three episodes of viability selection and one episode of fecundity selection (Arnold and Wade 1984a, b). This episodic approach allows me to identify how traits displaying maternal inheritance affect sequential viability and fecundity components of fitness as well as estimate the total magnitude of phenotypic selection on these traits across all episodes (Lynch and Arnold 1988).

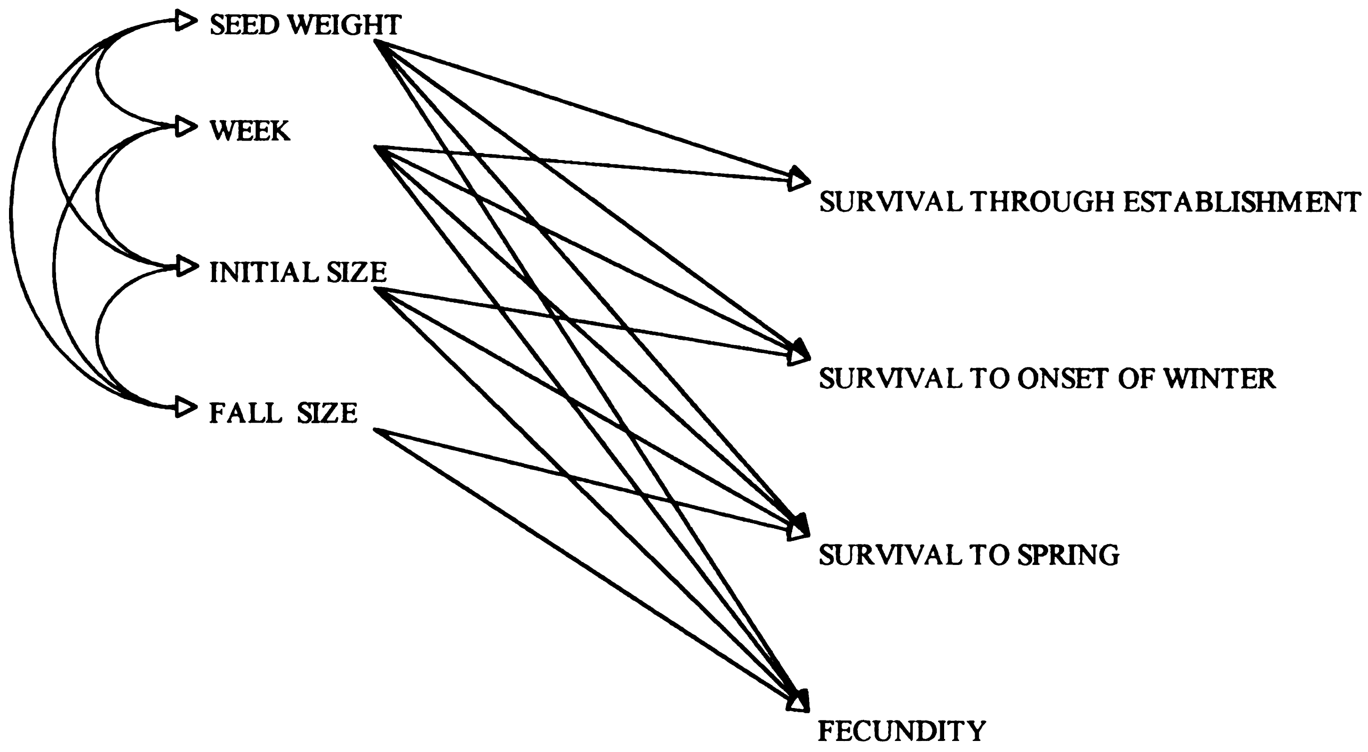


Figure. 8. Path diagram depicting the direct effects of four juvenile traits on four components of fitness, three episodes of survival and final fecundity. Direct effects represented by the single headed arrows allow one to predict the change in the trait mean in the subsequent generation and are estimated as the partial regression coefficient (β) from a multiple regression analysis of all traits on relative fitness. Double-headed arrows between traits denote the phenotypic correlations among traits which mediate indirect effects. Maternal inheritance can influence not only the phenotypic value of a trait, but also the phenotypic correlations between traits. Two traits, seed weight and emergence week, contribute to survival in the first episode. Three traits including initial size contribute to the second episode. All four traits contribute to the third and fourth episodes of selection.

In this study I quantify the nature (linear and non-linear), magnitude, and direction of phenotypic selection on four juvenile traits: seed weight, emergence week, cotyledon diameter at emergence, and cotyledon diameter prior to winter to address the following questions: 1) what is the total magnitude of linear and non-linear selection on these four traits, 2) which episodes are most critical in contributing to the total magnitude of linear and non-linear selection, therefore, suggesting possible hypotheses for the causal agents of selection, 3) what is the relative contribution of direct and indirect effects to the response of particular traits, i.e. do maternally inherited juvenile traits influence survivorship and fecundity directly or indirectly by influencing subsequent traits that then impact fitness?

MATERIALS AND METHODS

Study Site and Species

Collinsia verna Nutt. (Scrophulariaceae) is a winter annual that inhabits mesic forests of the eastern United States (Fernald 1970). Autumn diurnal temperature fluctuations cue germination (Baskin and Baskin 1983; Kalisz 1986) which begins in late September and continues into late November. Seedlings consist of a pair of cotyledons that expand in diameter throughout the fall. In southern Michigan, the first pair of leaves begin to develop in late November or early December, however, most plants overwinter with only cotyledons. These seedlings persist until early spring under a cover of leaf litter and snow. Rapid spring growth leads to rosettes with two to many pairs of true leaves. In May these rosettes initiate flowering which lasts two to three weeks. Fruits mature at the beginning of June and primary dispersal takes place as the plants senesce. While primary dispersal is limited in this species that lacks any specialized dispersal morphology (Thiede, unpublished data), secondary dispersal by surface flow of water is likely to influence seed dispersion because these seeds tend to float.

This study was conducted in a small (≈ 10 hectare) privately owned woodlot on TU Avenue in Kalamazoo County, Michigan. The tree canopy of this mature forest consisted of *Prunus serotina*, *Acer saccharum*, and *Tilia americana*. *C. verna* and *Floerkea proserpinacoides* were the predominant understory herbs in the spring. Other species in the herbaceous community included *Phlox divaricata*, *Laportea canadensis*, *Trillium grandiflorum*, *Arisaema triphyllum*.

The biotic and abiotic environment experienced by *C. verna* at TU Avenue varied spatially and temporally. *C. verna* occurred both in the center and along the edge of the woodlot, reaching highest densities along the edge. Agricultural fields created a sharp boundary at the edge of the woodlot. I observed moderate to severe wilting in early germinating seedlings in some locations along the edge, a sign of drought stress in that location, while wilting was only observed in a few plants in the interior. Therefore, light levels and soil moisture differed between the edge and center of the woodlot. Two herbivores, slugs and deer, consumed *C. verna* at two different times in its life cycle. In the fall primarily after leaf drop, slugs would consume both cotyledons and the apical meristem of seedlings. While the stem and root persisted after slug browsing, the seedling never recovered. In the spring deer browsed the apical meristem of 15-20% of the rosettes each year. As a result of deer browsing, axillary nodes were released from apical dominance and developed branches. Deer browsed seedlings were able to produce flowers and sometimes produced seeds, but their fecundity was very low when compared to unbrowsed plants. Therefore, drought and slug and deer herbivory may be potentially important selective agents in this population. However, the effects of these biotic and abiotic factors varied spatially in the population and temporally in the timing of their effects in the life cycle of *C. verna*.

Quantifying Phenotypic Selection

Data Collection

To quantify patterns of phenotypic selection on traits displaying non-Mendelian inheritance, I monitored survival and reproduction of seedlings at TU Ave from 1992-1994. Along each of two 100 m transects, one on the edge and one in the interior of the woodlot, I marked ten blocks at 10 m intervals for a total of 20 blocks. Within each block I marked eight or three 0.5 m^2 quadrats in 1992 and 1993, respectively. Half m wide aisles were retained between adjacent quadrats. The blocks originated at the same distance along each of the transects in both years. In 1992 the blocks were placed on the north side of the transect and in 1993 the blocks were placed on the south side of the transect, one meter away from the 1992 blocks. In 1992 the quadrats were arrayed in four rows of two columns per row, so the block occupied a 8 by 1.5 meter rectangular area along the transect. In 1993 the quadrats occupied a 0.5 by 2.5 meter area along the transect.

Natural Seedlings

Each fall on a weekly basis I tagged naturally occurring seedlings as they emerged with numbered poultry leg bands (N=13,568 in 1992, N=4,522 in 1993). During each emergence week, I measured cotyledon diameter on a subset of newly emerging seedlings (hereafter referred to as initial size) using a template of circles ranging from 1 to 9 mm in diameter in 0.5 mm increments. Between 1,000 and 1,600 randomly selected seedlings were measured in each census week. All seedlings in early and late censuses were measured for cotyledon diameter because the total number of seedlings emerging in those censuses was less than 1,000. Because the cotyledons grow during the fall, seedlings measured at emergence and surviving to the onset of winter were measured again for cotyledon diameter in early December 1992 and late November 1993 (hereafter referred to as fall size). Most seedlings had not yet begun to initiate true leaves by early December, so cotyledon diameter reflects seedling size. Cotyledon diameter at emergence explained 88% or 55% of the variation in photosynthetic area and total seedling weight, respectively, (photosynthetic area $=-2.74+0.89\pi(\text{diameter}/2)^2$, df=309, p=0.0001 and total seedling weight $=1.28+0.02\pi(\text{diameter}/2)^2$, df=309, p=0.0001). At the onset of winter cotyledon diameter explained 85% and 75% of the variation in these two traits (Photosynthetic area $=15.22+0.82\pi(\text{diameter}/2)^2$, df=95, p=0.0001 and total seedling weight $=-0.75+0.06\pi(\text{diameter}/2)^2$, df=95, p=0.0001). In the spring of 1993 and 1994 prior to seed dispersal, I collected all surviving plants in the quadrats and counted flower, fruit, and seed number for each individual, noting removal of the apical meristem by deer.

Mortality was scored at three stages in the life cycle that reflected different selective episodes. Mortality due primarily to slug herbivory was observed during establishment (1). Slug herbivory resulted in seedlings that lacked cotyledons or an apical meristem and was easily scored. Mortality was also scored at the onset of winter (2) and in the spring (3). As a consequence of mortality during these three episodes of viability selection, not all seedlings were scored for all traits. For example, seedlings that emerged, but were eaten by slugs could only be scored for the trait emergence week. Seedlings that were not eaten by slugs were scored for initial size, and seedlings surviving to the onset of winter were scored for fall size. The final episode of fecundity selection (4) included only those individuals that survived to spring and thus had expressed all three traits. In order to include all seedlings in the multivariate analysis described below, I partitioned the analysis of the magnitude and direction of phenotypic selection into four biologically relevant episodes (Figure 8). This episodic analysis is biologically relevant because the episodes relate to the different postulated selective agents. In the first two episodes, slug herbivory, drought and intraspecific competition were likely sources of mortality. From fall to spring, mortality agents included intraspecific competition, physiological stress, and deer herbivory. Deer herbivory also influenced fecundity in the final episode of fecundity selection. This episodic approach allows me to estimate the total magnitude of phenotypic selection on three traits, emergence week, initial size, and fall size.

Planted Seedlings

Seed weight is another maternally inherited trait that is genetically and phenotypically correlated with emergence week, initial size and fall size (Chapter 1). Seed weight can influence the outcome of competitive interactions (Gross 1984; Gross and Smith 1991) and the genesis of size and fecundity hierarchies in plant populations (Waller 1985; Stanton 1985). To remove the effects of selection on seed weight from other maternally inherited and correlated traits included in the multivariate selection analysis and to determine the extent to which seed weight influences either viability or fecundity components of fitness, I conducted a field experiment with seeds of known weight.

I monitored emergence, survival and fecundity of individually weighed seeds that I had planted back into the field. These seeds originated from natural fruiting maternal plants collected in early June. Seeds were planted in July into moist Sunshine seedling mix to a uniform depth of 1 cm into either 2 cm sections of 15 mm diameter clear plastic tubing in 1992 or 3 cm sections of 7 mm diameter plastic straws in 1993. These seeds were maintained in the greenhouse until August when they were tranplanted into the field prior to natural germination cues and with minimal soil disturbance. In addition to naturally produced seed, I also planted greenhouse produced seeds from the breeding design described in Chapter 1. In 1992 a total of 3180 seeds from field and greenhouse mothers were planted in the population. In 1993 a total 2495 seeds from field collected mothers were planted.

Seeds from maternal families were planted at two spatial scales to address how spatial variation in selection influenced maternal family fitness when seeds from a family were planted locally (i.e. experienced only one selective environment) or when they were planted in numerous blocks across the population (i.e. experienced many selective environments) (see Chapter 3). In addition, to address whether families were better adapted to the location in which they were produced, seeds that were planted locally consisted of two types. The first type of maternal family originated in the block in which it was planted, while the second type was a maternal family that was randomly assigned to that block from the population at large. In this chapter I combine all planted seedlings in one analysis to describe the overall pattern of phenotypic selection in each year.

Data Analysis

I quantified phenotypic selection with two models that differed only in the traits included in the analysis. For natural and planted seedlings, I examined a three trait model that included : 1) emergence week, 2) initial size (cotyledon diameter at emergence), 3) fall size (cotyledon diameter in November). For the planted seedlings, I considered a four trait model that included seed weight. These two models allowed me to evaluate how the inclusion of seed weight affected the estimates of direct selection on other traits in the planted seedlings.

This multivariate selection analysis which quantifies the magnitude and direction of selection acting directly on phenotypic traits by removing the effects of changes in correlated traits can only include observations in which all phenotypic traits have been measured for each individual (Lande and Arnold 1983; see recent review Brodie et al. 1995). When mortality eliminates some individuals, traits expressed later in ontogeny are missing and those individuals must be excluded from the analysis. Arnold and Wade (1984 a, b) developed an episodic approach to selection analysis such that one can estimate the direct effects of particular traits on components of fitness by considering episodes of viability, fecundity, or sexual selection. This analysis by episodes, therefore, allows one to include individuals who die before expressing all phenotypic traits of interest. The estimates of selection resulting from this episodic analysis are conditional because they only provide an estimate of the magnitude of selection if the individual survived to the beginning of the episode being considered. To quantify the total magnitude of selection on a set of traits throughout the life cycle, these conditional measures of selection must be additive. If the phenotypic variance-covariance matrix (P) does not change across all episodes, then conditional selection gradients sum to the total selection gradient (Arnold and Wade 1984 a, b). When P does change across episodes, selection gradients are made additive by weighting conditional gradients by the cumulative change in P to that point in the life cycle (Wade and Kalisz 1989; Kalisz 1986). This approach to additive partitioning of the selection gradient requires that the original P be known at birth, i.e. all traits are measured before selection occurs. When all traits of interest are not expressed at birth, the additive partitioning of the selection gradient requires that the original P be reconstructed (Lynch and Arnold 1988). Reconstruction of the original P requires the assumption that changes in P are due solely to selection and that traits distributions are not changed by selection prior to the time that they are manifested. Bennington and McGraw (1995a) provide an empirical demonstration that this reconstruction can account for changes in P due to selection.

Because mortality eliminated individuals at establishment and during the fall, I employed an episodic analysis to estimate selection for three episodes of viability selection and one episode of fecundity selection (Figure 8). I reconstructed the original P according to Lynch and Arnold (1988) to make conditional selection parameters additive.

Phenotypic selection can produce changes both in the mean and variance of phenotypic traits (Table 8). The conditional selection differential, S_i, measured as the covariance between a trait and relative fitness, describes that change in the trait mean as a result of selection in a given episode. This change may be due to direct selection on the trait as well as changes due to selection on phenotypically correlated traits. The conditional selection gradient, β_i, describes the change in the trait mean due only to direct

Table 8. Phenotypic selection parameters calculated in each episode(i).

	Linear		Non-linear	
Parameter	Response	Symbol	Response	Symbol
Selection differential	Change in trait mean	S_i	Change in (co)variance of trait	C_i
Selection gradient	Change in trait mean due only to direct selection	β_i	Change in (co)variance due only to direct selection	γ_i

effects and is calculated as the partial regression coefficient for a given trait on relative fitness in that episode given all other traits expressed in that episode. In order to quantify changes in the variance, traits values must be expressed as squared deviations from the mean (Lande and Arnold 1983; Brodie et al. 1995). The covariance between these squared deviations and relative fitness in a given episode is the non-linear selection differential, C_i, that describes the change in trait variances due to selection. Changes in the variance due only to direct effects of selection, the non-linear selection gradient, γ_i, is calculated as the partial regression coefficient of the squared deviation trait values when the linear terms are included in the model. Thus, the linear and non-linear selection gradients are determined by two separate multiple regression models, 1) the linear model includes only trait values and 2) the non-linear model includes trait values and their squared deviations. Therefore, non-linear models account for changes in the mean when estimating changes in the variance. In each episode I calculated selection differentials and selection gradients for linear and non-linear components of selection. An analysis of variance inflation factors indicated that these regression models were not compromised by multicollinearity (Neter, Wasserman, and Kutner 1985).

Phenotypic traits were standardized to a mean of zero and a variance of one prior to all analyses so that all differentials and gradients were expressed in units of standard deviation and were comparable among traits and episodes. The covariances describing selection differentials were calculated with a denominator of n rather than n-1 (see Arnold and Wade 1984 b p.726). In each viability episode fitness was either zero or one depending on whether an individual died or survived, respectively. Each fitness was standardized to the mean in that episode to calculate relative fitness. In the final episode of fecundity selection, fitness was the number of seeds produced. Relative fitness was expressed as seed number relative to the population mean in that episode. Relative fitness was not transformed (Lande and Arnold 1983).

Additive linear (β) and non-linear gradients (γ) are calculated by weighting the changes in the phenotypic variance-covariance matrix over all episodes (i) to the original phenotypic variance-covariance matrix (P_0) according to the equations:

$$\beta(i)=P_0^{-1}\,S(i) \quad (1)$$

and

$$\gamma(i)= P_0^{-1}C(i)P_0^{-1} \quad (2)$$

where the linear selection differential, S(i), and the non-linear selection differential C(i) are weighted by the fraction surviving to that episode (Lynch and Arnold 1988). If one assumes that changes in the phenotypic variance-covariance matrix from episode to episode are due only to selection quantified by the linear and non-linear conditional gradients (i.e. not development), then P_0 can be reconstructed by sequentially back calculating variances and covariances for traits not observed in a particular episode according to the following equation:

$$P_i=P_{i-1}+P_{i-1}\gamma P_{i-1}-P_{i-1}\beta_{i-1}[P_{i-1}\beta_{i-1}]^T \qquad (3)$$

(Lynch and Arnold 1988, equation. 2) where β and γ are the conditional linear and non-linear selection gradients in episode i, respectively.

In this study, all four phenotypic traits were measured by the third episode (i=2). So only P_1 and P_0 needed to be reconstructed. In the second episode (1), fall cotyledon diameter was unobserved, so the reconstruction involved solving three simultaneous equations for the variance in fall cotyledon diameter and its covariance with emergence week and initial size based on the observed conditional selection gradients in that episode. Likewise, in the first episode (0) the variance of fall cotyledon diameter and initial cotyledon diameter and covariances of these traits with emergence week were based on the simultaneous solution of five equations. When seed weight was included in the analysis the number of unknowns in each episode increased, so that there were four equations for P_1 and seven equations for P_0. As the number of unknowns and the number of episodes involved in reconstruction increases, error associated with estimation can increase. However, the compounding of errors in reconstruction is likely to be minor in this study because reconstruction involved only two traits in two episodes. In addition, reconstructed estimates of the phenotypic variances and covariances were tested for significance by constructing 95% confidence intervals obtained from the bootstrapping procedure described below.

Significance tests of selection parameters were based on bootstrap resampling methods (Effron 1982; Dixon 1987, Dixon et al. 1993). This approach was required because 1) regression analysis of viability selection is likely to be compromised by non-normality of residuals (Mitchell-Olds and Shaw 1987), and 2) the additive partitioning of the selection differentials and gradients according to Lynch and Arnold (1988) involves the transformation of these regression parameters. Once conditional estimates are transformed into additive estimates, they are no longer associated with significance tests from the regression analysis. My protocol for resampling with replacement was as follows: 1) calculate the covariances between traits (including squared deviations) and relative fitness to estimate linear and non-linear **conditional** selection differentials in each episode 2) estimate linear and non-linear **conditional** selection gradients in each episode via multiple regression analysis, 3) use **conditional** gradients and the phenotypic variance-covariance matrix to **reconstruct** P_0, 4) transform linear and non-linear conditional differentials into **additive** differentials using conditional gradients and weighting by the fraction that survived to that episode, 5) transform the linear and non-linear conditional gradients into **additive** gradients using equations 1 and 2 above. Thus, the 95% confidence intervals of both conditional and additive parameters as well as the original P were obtained by the shift distribution method in which the bootstrapped parameter means are centered on the real value before the confidence intervals are calculated (Noreen

1989). Each resampled data set contained the number of observations originally observed in that data set and 500 bootstrapped estimates were obtained for each parameter.

Survivorship Analysis

Statistical comparisons of the survivorship of natural and planted seedlings through the three episodes of viability selection for two years were based on a failure time analysis using the log-rank chi-square statistic from the lifetable method of the lifetest procedure in SAS (Fox 1993). First, I examined temporal differences across years by combining both natural and planted seedlings within a year. Subsequently, I examined differences between natural and planted seedlings within a given year. I examined temporal variation in fecundity with a nested ANOVA in which treatment (natural vs. planted) was nested within year.

RESULTS

Opportunity for selection

The proportion surviving across episodes did not differ between years (log-rank chi-square, $\chi^2 = 3.13$, df=1, p=0.0768) or between natural and planted seedlings in 1992 (log-rank chi-square, $\chi^2 = 3.43$, df=1, p=0.0639), but did differ between natural and planted seedlings in 1993 (log-rank chi-square, $\chi^2 = 25.95$, df=1, p=0.0001) (Figure 9). Most mortality occurred between the fall and spring censuses. For both natural and planted seedlings in 1992 and 1993, on average 10.4% died during establishment, 28.5% died prior to winter, 39.2% died prior to fruit maturation in late May and early June, only 21.9% survived to the flowering/fruiting stage.

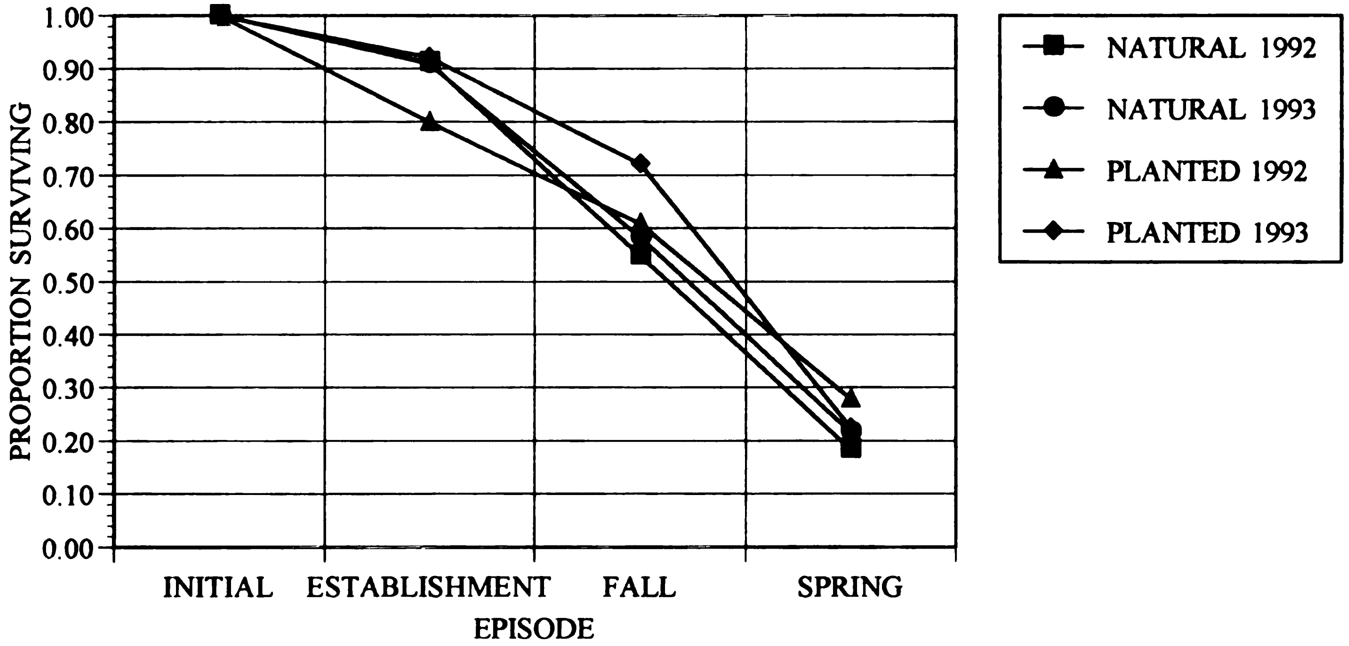


Figure 9. Survival through each episode of viability selection for two years at TU Avenue for natural and planted seedlings. Standard errors are smaller than the symbols.

Female fecundity, the number of seeds produced, was highly variable ranging from 0 to 70, with an overall average of 8.12 seeds per individual. Plants browsed by deer had lower fecundity than unbrowsed plants (Figure 10). Average fecundity differed significantly among years and among treatments (natural vs. planted) within years (Nested ANOVA, df=3,1529, p=0.0001) with lower seed production in 1992 (Figure 10). This variance both in survival and seed production resulted in the greatest opportunity for selection in the spring episodes of viability and fecundity (Figure 11).

Phenotypic correlations among traits

The phenotypic variance-covariance matrix prior to the first and final episodes of selection and their 95% bootstrapped confidence intervals are presented in Table 9. The original matrix, P_0, has been reconstructed based on the conditional selection gradients in the first episode of viability selection according to Lynch and Arnold (1988), while the final matrix, P_3, is based only on individuals that survived to the spring. Emergence week and fall size display a significantly negative covariance through all episodes, initial size and fall size display a significantly positive covariance through all episodes, while the covariance between emergence week and initial size displays positive, negative, and non-significant values in the original matrix depending on the year and treatment (natural or planted). When seed weight was included in the analysis of planted seedlings in both years, seed weight displayed a significant positive covariance with initial size, fall size, however the covariance with emergence week varied from negative in 1992 to positive in 1993.

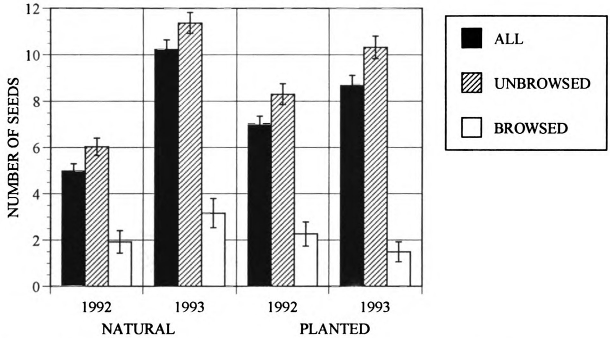


Figure 10. Average fecundity (± 1 standard error) of natural and planted seedlings in both years for all plants, for plants browsed by deer, and plants that were not browsed.

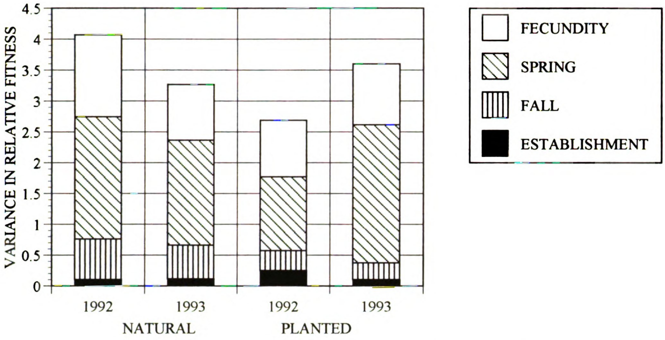


Figure 11. The conditional opportunity for selection presented as the variance in relative fitness for each episode of selection for all individuals surviving the previous episode. The total opportunity is the variance in relative fitness for all individuals across all episodes. The patterns for the episodes of selection apply to all subsequent figures.

Table 9. The phenotypic variance-covariance matrix prior to the original (P_0) and final (P_3) episodes of selection for natural and planted seedlings in 1992 and 1993 for three traits (emergence week (WK), initial size (IS), and fall size (FS) and four trait models (including seed weight (SD)). The original matrix has been reconstructed from conditional selection gradients (see Methods). The 95% confidence intervals for each element are also provided.

TRAIT	P_0	95% CI		P_3	95% CI	
		LOWER	UPPER		LOWER	UPPER
1. NATURAL SEEDLINGS 1992						
WEEK	0.999	0.999	0.999	0.738	0.548	0.934
WK-IS	0.089	0.025	0.158	0.313	0.154	0.497
WK-FS	-0.701	-0.759	-0.636	-0.278	-0.383	-0.174
INITIAL	0.999	0.999	0.999	1.065	0.865	1.332
IS-FS	0.488	0.432	0.542	0.321	0.194	0.446
FALL	0.996	0.970	1.023	0.691	0.586	0.812
2. NATURAL SEEDLINGS 1993						
WEEK	1.000	1.000	1.000	0.875	0.801	0.953
WK-IS	-0.020	-0.059	0.023	0.022	-0.056	0.104
WK-FS	-0.411	-0.450	-0.369	-0.353	-0.428	-0.287
INITIAL	1.000	1.000	1.000	0.961	0.858	1.066
IS-FS	0.600	0.563	0.636	0.519	0.432	0.600
FALL	1.024	1.006	1.042	0.852	0.761	0.934
3. PLANTED SEEDLINGS 1992						
WEEK	0.999	0.999	0.999	0.698	0.610	0.795
WK-IS	-0.106	-0.177	-0.034	-0.075	-0.165	0.010
WK-FS	-0.417	-0.489	-0.343	-0.295	-0.376	-0.218
INITIAL	0.999	0.999	1.000	0.891	0.771	1.010
IS-FS	0.587	0.525	0.647	0.414	0.297	0.523
FALL	1.039	1.011	1.068	0.735	0.636	0.845
4. PLANTED SEEDLINGS 1992 WITH SEED WEIGHT						
WEIGHT	0.999	0.999	0.999	0.841	0.729	0.953
SD-WK	-0.113	-0.179	-0.047	-0.017	-0.109	0.070
SD-IS	0.403	0.342	0.460	0.323	0.234	0.429
SD-FS	0.555	0.479	0.630	0.458	0.366	0.548
WEEK	0.999	0.999	0.999	0.698	0.608	0.798
WK-IS	-0.122	-0.198	-0.058	-0.075	-0.161	0.009
WK-FS	-0.454	-0.526	-0.379	-0.295	-0.364	-0.220
INITIAL	1.003	0.998	1.011	0.891	0.772	1.008
IS-FS	0.601	0.529	0.664	0.414	0.314	0.531
FALL	1.076	1.032	1.125	0.735	0.634	0.840

Table 9 (cont'd).

TRAIT	P_0	95% CI		P_3	95% CI	
		LOWER	UPPER		LOWER	UPPER
5. PLANTED SEEDLINGS 1993						
WEEK	0.999	0.999	0.999	0.746	0.626	0.885
WK-IS	0.043	-0.009	0.098	0.143	0.041	0.242
WK-FS	-0.393	-0.441	-0.347	-0.274	-0.357	-0.198
INITIAL	0.999	0.999	1.000	1.022	0.903	1.149
IS-FS	0.569	0.531	0.611	0.436	0.345	0.540
FALL	1.000	0.987	1.013	0.720	0.638	0.807
6. PLANTED SEEDLINGS 1993 WITH SEED WEIGHT						
WEIGHT	0.999	0.999	0.999	1.018	0.873	1.173
SD-WK	0.154	0.111	0.200	0.135	0.044	0.225
SD-IS	0.475	0.434	0.515	0.504	0.394	0.604
SD-FS	0.463	0.410	0.509	0.464	0.373	0.556
WEEK	0.999	0.999	0.999	0.746	0.618	0.878
WK-IS	0.051	0.001	0.106	0.143	0.042	0.236
WK-FS	-0.376	-0.424	-0.324	-0.274	-0.348	-0.188
INITIAL	0.999	0.997	1.001	1.022	0.896	1.145
IS-FS	0.562	0.522	0.601	0.436	0.343	0.529
FALL	0.986	0.966	1.010	0.720	0.632	0.809

Total magnitude and direction of selection

A. Changes in trait means

Three trait models

The pattern of selection is remarkably consistent among years and between natural and planted seedlings within years: seedlings that are large at establishment and large at the onset of winter have enhanced survival and reproduction. Selection on emergence week is more variable. The total linear selection differential estimating the change in a trait mean due both to direct selection on that trait and indirect selection on phenotypically correlated traits (Table 9) summed across all episodes shows that for natural and planted seedlings in both years, selection favors large initial size and large fall size (Figures 12-15A). In addition to direction, the magnitude of the selection differentials for initial and fall size are similar for natural and planted seedlings in both years (Table 10). In contrast, the magnitude and direction of the total selection differential for emergence week varies among years: in 1992-3 emergence week does not change (Figures 12, 14A), while in 1993-4 late emergence is favored in the natural seedlings (Figure 13A) and early emergence is favored in the planted seedlings (Figure 15A). Total selection differentials on emergence week are of small magnitude in both years, but only significant in 1993-4.

The total selection gradient that reflects changes in trait means due only to the direct effects of selection showed a different pattern from the total selection differential (Figures 12-15B). Later emergence and large fall size were favored for natural and planted seedlings in both years, while mean initial size did not change as a result of direct selection

Figure 12. Phenotypic selection through three episodes of viability and one episode of fecundity selection for natural seedlings in 1992. Viability episodes include survival to establishment (solid), survival to the onset of winter (vertical), survival to spring (diagonal), and the final episode of fecundity selection (clear). The total magnitude of each selection parameter (■) across all episodes and the 95% confidence intervals (●) based on 500 bootstrap samples based on reconstructing the original phenotypic variance-covariance matrix are depicted. Selection parameters include: A) linear selection differential, B) linear selection gradient, C) non-linear selection differential, D) non-linear selection gradient. Trait abbreviations follow Table 9. Significant episodes of selection are denoted by *. Note the difference in scale for non-linear selection parameters.

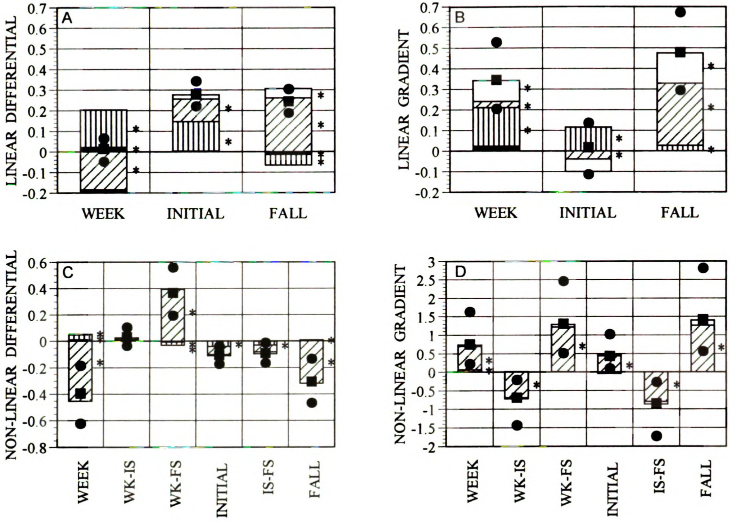

A
LINEAR DIFFERENTIAL
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
WEEK
INITIAL
FALL
B
LINEAR GRADIENT
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
WEEK
INITIAL
FALL
C
NON-LINEAR DIFFERENTIAL
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
WEEK
WK-IS
WK-FS
INITIAL
IS-FS
FALL
D
NON-LINEAR GRADIENT
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
WEEK
WK-IS
WK-FS
INITIAL
IS-FS
FALL

Figure 12.

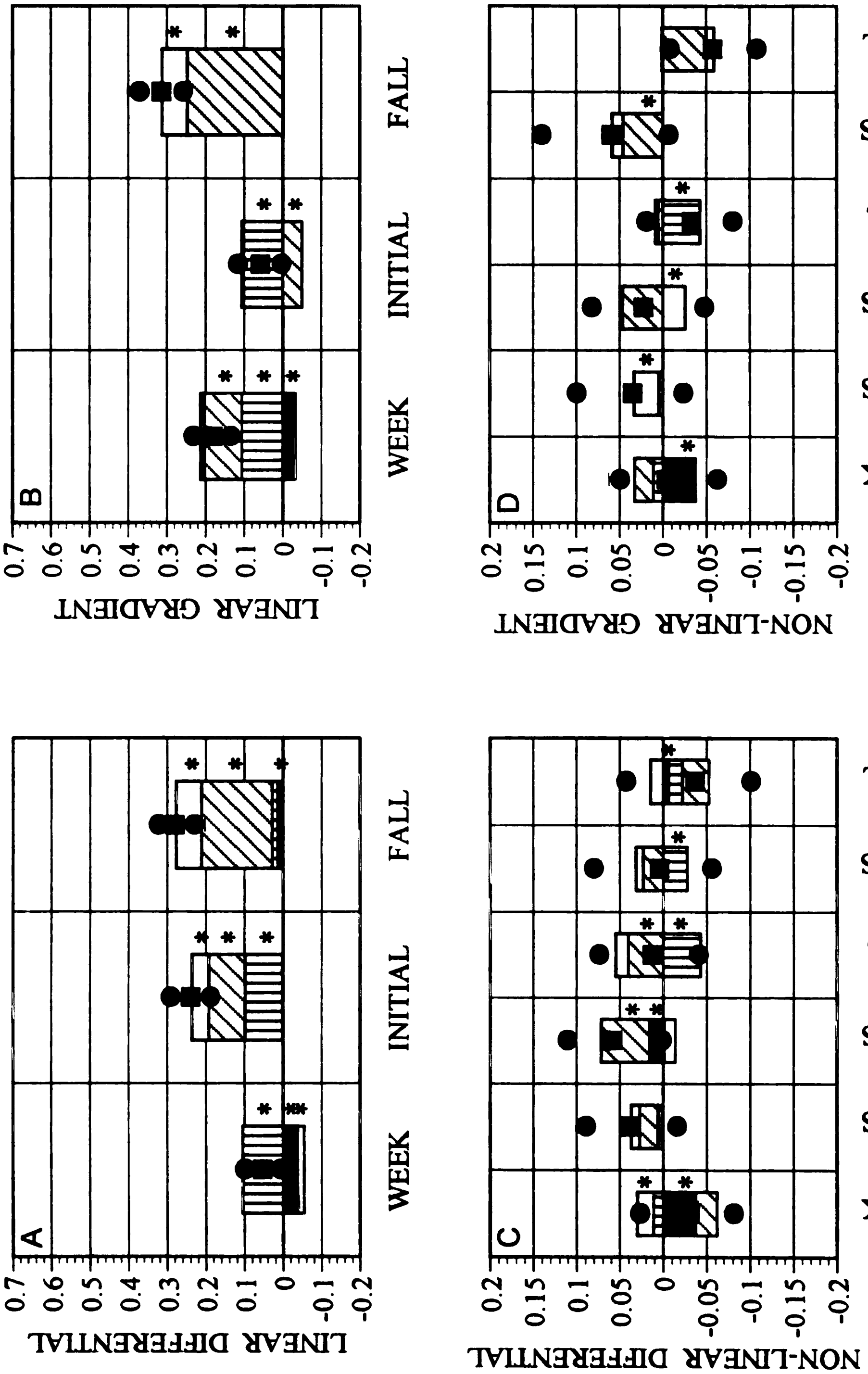


Figure 13. Phenotypic selection through three episodes of viability and one episode of fecundity selection for natural seedlings in 1993. All symbols follow Figure 12.

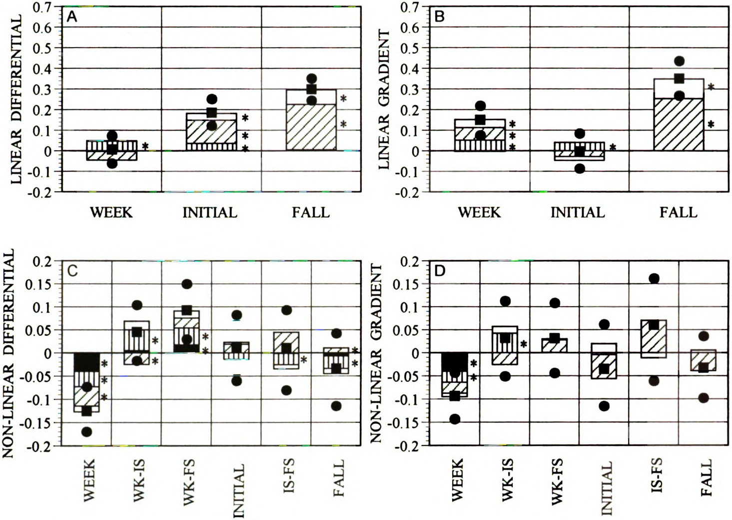


Figure 14. Phenotypic selection through three episodes of viability and one episode of fecundity selection for planted seedlings in 1992. All symbols follow Figure 12.

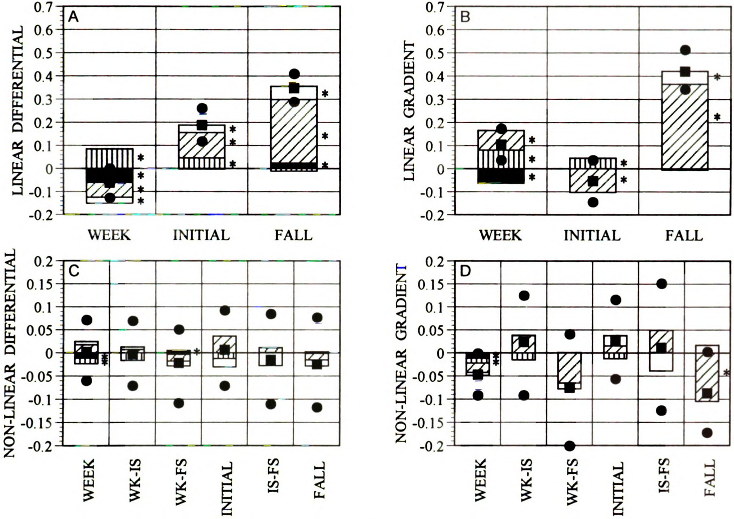


Figure 15. Phenotypic selection through three episodes of viability and one episode of fecundity selection for planted seedlings in 1993. All symbols follow Figure 12.

Table 10. Additive linear selection differentials (A) and gradients (B) for natural and planted seedlings in 1992 and 1993 for three trait or four trait models. Values are given for each of four selection episodes and the total across all episodes. Below each value are the 95% confidence intervals based on bootstrap resampling (n=500). Linear parameters reflect the change in the mean in units of standard deviation. Values significant at $p<0.05$ are bold

	A. LINEAR DIFFERENTIAL				
TRAIT	EPISODE				
	0	1	2	3	TOTAL
1. NATURAL SEEDLINGS 1992-3					
WEEK	**0.022***	**0.181***	**-0.186***	-0.007	0.010
	0.011 0.032	0.151 0.209	-0.228 -0.143	-0.026 0.012	-0.052 0.061
INITIAL	0.002	**0.144***	**0.109***	0.021	**0.276***
	0.000 0.004	0.110 0.181	0.064 0.162	-0.005 0.047	0.217 0.338
FALL	**-0.015***	**-0.051***	**0.260***	**0.046***	**0.239***
	-0.023 -0.008	-0.085 -0.017	0.219 0.300	0.023 0.069	0.183 0.299
2. NATURAL SEEDLINGS 1993-4					
WEEK	**-0.034***	**0.105***	-0.004	**-0.018***	**0.049***
	-0.049 -0.019	0.080 0.133	-0.041 0.030	-0.034 -0.003	0.003 0.094
INITIAL	0.001	**0.096***	**0.094***	**0.044***	**0.236***
	-0.001 0.002	0.070 0.125	0.060 0.131	0.025 0.064	0.184 0.286
FALL	**0.014***	0.014	**0.182***	**0.067***	**0.276***
	0.008 0.020	-0.006 0.034	0.143 0.218	0.050 0.084	0.227 0.318
3. PLANTED SEEDLINGS 1992-3					
WEEK	-0.005	**0.046***	-0.041	0.001	0.002
	-0.039 0.030	0.017 0.077	-0.089 0.006	-0.025 0.025	-0.066 0.068
INITIAL	0.000	**0.035***	**0.113***	**0.032***	**0.180***
	-0.003 0.004	0.003 0.066	0.062 0.163	0.001 0.064	0.117 0.247
FALL	0.002	0.002	**0.220***	**0.071***	**0.295***
	-0.013 0.016	-0.018 0.024	0.176 0.268	0.044 0.098	0.239 0.346
4. PLANTED SEEDLINGS 1992-3 WITH SEED WEIGHT					
SEED	**-0.045***	**0.033***	**0.070***	**0.056***	**0.113***
	-0.077 -0.012	0.002 0.062	0.019 0.117	0.031 0.082	0.041 0.181
WEEK	-0.005	**0.046***	-0.041	0.001	0.002
	-0.039 0.031	0.018 0.077	-0.088 0.009	-0.023 0.026	-0.063 0.064
INITIAL	**-0.017***	**0.035***	**0.113***	**0.032***	**0.163***
	-0.032 -0.004	0.003 0.068	0.062 0.163	0.002 0.064	0.092 0.231
FALL	-0.021	0.009	**0.220***	**0.071***	**0.278***
	-0.046 0.001	-0.016 0.039	0.178 0.265	0.046 0.099	0.221 0.335
5. PLANTED SEEDLINGS 1993-4					
WEEK	**-0.060***	**0.085***	**-0.065***	**-0.025***	**-0.065***
	-0.079 -0.044	0.063 0.109	-0.117 -0.008	-0.046 -0.006	-0.130 -0.004
INITIAL	-0.003	**0.046***	**0.110***	**0.031***	**0.185***
	-0.006 0.001	0.022 0.071	0.047 0.171	0.010 0.053	0.114 0.257
FALL	**0.024***	-0.011	**0.275***	**0.057***	**0.345***
	0.017 0.031	-0.026 0.006	0.222 0.332	0.036 0.077	0.285 0.406
6. PLANTED SEEDLINGS 1993-4 WITH SEED WEIGHT					
SEED	-0.000	**0.084***	**0.077***	**0.041***	**0.203***
	-0.013 0.014	0.060 0.109	0.017 0.138	0.019 0.064	0.133 0.274
WEEK	**-0.060***	**0.085***	**-0.065***	**-0.025***	-0.065
	-0.081 -0.042	0.062 0.109	-0.121 -0.008	-0.045 -0.004	-0.129 0.006
INITIAL	0.001	**0.046***	**0.110***	**0.031***	**0.189***
	-0.006 0.009	0.021 0.069	0.044 0.170	0.006 0.052	0.116 0.254
FALL	**0.028***	0.007	**0.275***	**0.057***	**0.367***
	0.018 0.039	-0.012 0.025	0.221 0.332	0.039 0.076	0.308 0.427

Table 10 (cont'd).

	B. LINEAR GRADIENT									
TRAIT	EPISODE									
	0		1		2		3		TOTAL	
1. NATURAL SEEDLINGS 1992-3										
WEEK	**0.022***		**0.189***		0.029		**0.103***		**0.342***	
	0.011	0.032	0.152	0.228	-0.090	0.171	0.038	0.179	0.201	0.524
INITIAL	0.000		**0.114***		-0.040		**-0.061***		0.014	
	0.000	0.000	0.081	0.152	-0.139	0.065	-0.127	-0.003	-0.117	0.132
FALL	0.000		**0.026***		**0.301***		**0.148***		**0.475***	
	0.000	0.000	0.013	0.043	0.152	0.469	0.065	0.257	0.291	0.668
2. NATURAL SEEDLINGS 1993-4										
WEEK	**-0.034***		**0.106***		**0.096***		0.009		**0.177***	
	-0.049	-0.019	0.082	0.133	0.059	0.135	-0.006	0.025	0.131	0.227
INITIAL	0.000		**0.100***		**-0.051***		0.005		0.054	
	0.000	0.000	0.075	0.128	-0.096	-0.005	-0.020	0.030	-0.001	0.112
FALL	0.000		**-0.003***		**0.246***		**0.066***		**0.310***	
	0.000	0.000	-0.005	-0.001	0.194	0.300	0.043	0.088	0.254	0.367
3. PLANTED SEEDLINGS 1992-3										
WEEK	-0.005		**0.050***		**0.061***		**0.039***		**0.146***	
	-0.039	0.030	0.021	0.081	0.011	0.111	0.013	0.066	0.071	0.214
INITIAL	0.000		**0.040***		-0.029		-0.019		-0.008	
	0.000	0.000	0.008	0.071	-0.098	0.035	-0.062	0.021	-0.091	0.079
FALL	0.000		-0.001		**0.252***		**0.094***		**0.346***	
	0.000	0.000	-0.002	-0.000	0.184	0.320	0.058	0.134	0.264	0.430
4. PLANTED SEEDLINGS 1992-3 WITH SEED WEIGHT										
SEED	**-0.046***		0.027		**-0.072***		0.024		-0.068	
	-0.079	-0.013	-0.007	0.060	-0.133	-0.013	-0.003	0.055	-0.148	0.010
WEEK	-0.010		**0.053***		**0.078***		**0.039***		**0.160***	
	-0.045	0.027	0.024	0.083	0.020	0.133	0.013	0.068	0.088	0.232
INITIAL	0.000		0.030		-0.020		-0.022		-0.011	
	0.000	0.000	-0.004	0.066	-0.089	0.049	-0.065	0.019	-0.097	0.072
FALL	0.000		-0.000		**0.286***		**0.082***		**0.368***	
	0.000	0.000	-0.002	0.001	0.216	0.364	0.047	0.119	0.292	0.451
5. PLANTED SEEDLINGS 1993-4										
WEEK	**-0.060***		**0.081***		**0.084***		-0.003		**0.102***	
	-0.079	-0.044	0.059	0.104	0.024	0.141	-0.021	0.015	0.033	0.171
INITIAL	0.000		**0.046***		**-0.102***		-0.000		-0.056	
	0.000	0.000	0.021	0.069	-0.186	-0.024	-0.035	0.033	-0.148	0.034
FALL	0.000		**-0.005***		**0.366***		**0.056***		**0.417***	
	0.000	0.000	-0.008	-0.002	0.291	0.452	0.026	0.091	0.340	0.510
6. PLANTED SEEDLINGS 1993-4 WITH SEED WEIGHT										
SEED	0.010		**0.069***		**-0.090***		**0.025***		0.014	
	-0.004	0.023	0.042	0.097	-0.161	-0.015	0.002	0.053	-0.073	0.092
WEEK	**-0.062***		**0.072***		**0.107***		-0.012		**0.105***	
	-0.083	-0.043	0.050	0.095	0.044	0.170	-0.031	0.007	0.031	0.188
INITIAL	0.000		0.013		-0.083		-0.005		-0.074	
	0.000	0.000	-0.012	0.037	-0.171	0.005	-0.037	0.023	-0.174	0.027
FALL	0.000		**-0.006***		**0.409***		**0.044***		**0.447***	
	0.000	0.000	-0.010	-0.003	0.322	0.501	0.018	0.070	0.354	0.535

(Figures 12-15B). The magnitude of the selection gradient was similar for fall size across years, while the magnitude for emergence week showed weaker selection for the planted seedlings (Figures 14-15B) when compared to natural seedlings (Figures 12-13B) in both years (Table 10).

The difference between selection differentials and selection gradients indicates that indirect effects of selection on phenotypically correlated traits will influence the overall change in the trait mean. One can partition the selection differential into direct and indirect components. The direct component is analogous to the selection gradient. The indirect component of the differential (Figure 16) shows how selection on phenotypically correlated traits will produce a change in the mean of a given trait. The pattern of indirect selection was consistent: indirect selection favored early emergence and large initial size via direct selection on fall size and decreased fall size (not significant in all cases) via direct selection on emergence week (Table 9).

Four trait models with seed weight

When seed weight is included in this episodic selection analysis, it does not change the sign or magnitude of the total linear selection differentials or gradients observed in the three trait analysis (Figures 17-18AB, Table 10). However, in 1993 the total selection differential on emergence week becomes non-significant. Furthermore, the significance and magnitude of certain episodes contributing to the total gradients does change. In both years the inclusion of seed weight eliminates significant direct selection on initial size in any episode (Figures 17-18B).

As with the other three traits, the total selection differential for seed weight is consistent in sign and magnitude across years favoring an increase in seed weight

Figure 16. Indirect selection differential on three traits for all selection episodes for natural seedlings in 1992 (A) and 1993 (B) and planted seedlings in 1992 (C) and 1993 (D). Viability episodes include survival to establishment (solid), survival to the onset of winter (vertical), survival to spring (diagonal), and the final episode of fecundity selection (clear). The total magnitude of each selection parameter (■) for all episodes and the 95% confidence intervals (●) based on 500 bootstrap samples for the indirect differential are depicted. Trait abbreviations follow Table 9. Significant episodes of selection are denoted by *

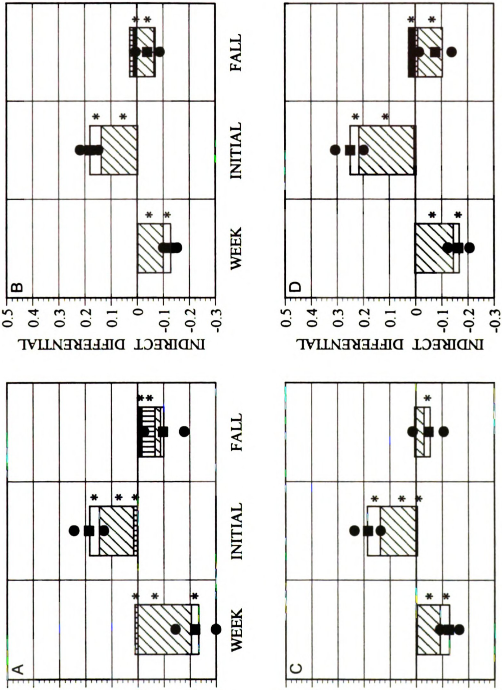

A
B
C
D
INDIRECT DIFFERENTIAL
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
WEEK
INITIAL
FALL

Figure 16.

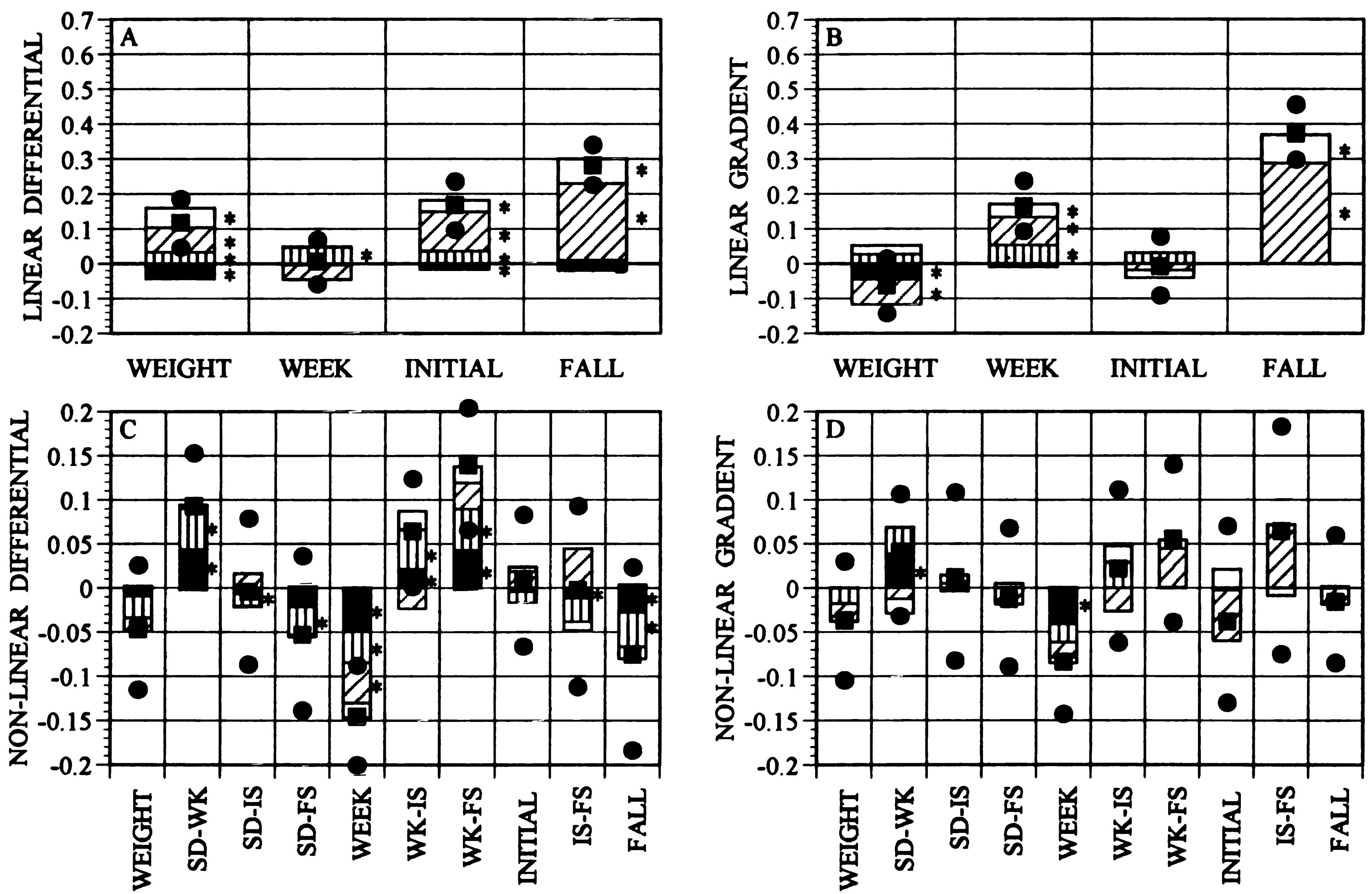


Figure 17. Phenotypic selection on four traits including seed weight for planted seedlings in 1992. All symbols follow Figure 12.

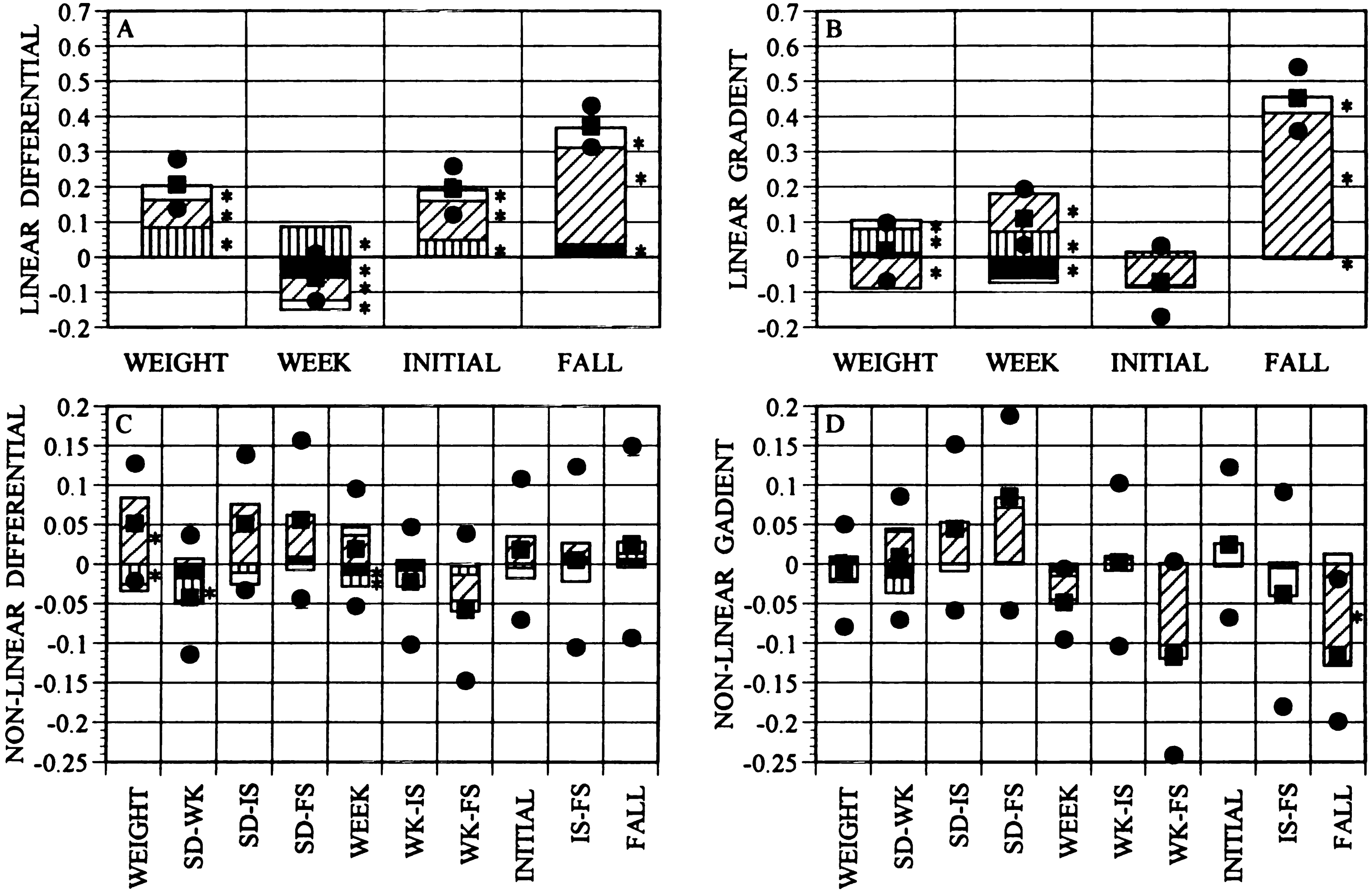


Figure 18. Phenotypic selection on four traits including seed weight for planted seedlings in 1993. All symbols follow Figure 12.

However, the selection gradient showed no significant change in mean seed weight due to direct effects of selection on that trait. Therefore, indirect effects mediated via significant positive covariance with fall size (Table 8) must generate the positive selection differential on seed weight. Indirect effects on seed weight due to its covariance with emergence week opposed the indirect effects due to direct selection on fall size in 1992 and complemented them in 1993 (Table 9).

B. Changes in trait variances and covariances

Three trait models

In contrast to changes in trait means that were relatively consistent in sign and magnitude across years and between natural and planted seedlings, changes in trait variances and covariances due to direct and indirect selection were not consistent (Figures 12-15CD, Table 11). In general the total non-linear selection differentials and gradients only displayed significant values in 1992. While the planted seedlings in that year displayed a few significant total non-linear differentials and gradients, the traits of natural seedlings showed the strongest non-linear effects.

The total non-linear selection differential showed a decreased variance in emergence week, and initial and fall size, a decreased covariance between initial size and fall size and an increased covariance between week and fall size in the natural seedlings in 1992 (Figure 12C). In that year planted seedlings also exhibited decreased variance in emergence week and increased covariance between emergence week and fall size (Figure 14C). The magnitude of these differentials differed between natural and planted seedlings

Table 11. Additive non-linear selection differentials (A) and gradients (B) for natural and planted seedlings in 1992 and 1993 for three trait or four trait models. Values are given for each of four selection episodes and the total across all episodes. Below each value are the 95% confidence intervals based on bootstrap resampling (n=500). Non-linear parameters reflect the change in the variance in units of standard deviation squared. Values significant at p<0.05 are bold.

A. NON-LINEAR DIFFERENTIAL					
TRAIT	EPISODE				
	0	1	2	3	TOTAL
1. NATURAL SEEDLINGS 1992-3					
WEEK	**0.009***	**0.041***	**-0.453***	0.002	**-0.401***
	0.002 0.016	0.020 0.065	-0.681 -0.241	-0.036 0.044	-0.628 -0.191
WK-IS	0.001	0.008	0.004	0.013	0.026
	0.000 0.002	-0.016 0.034	-0.058 0.065	-0.020 0.045	-0.041 0.099
WK-FS	**-0.007***	**-0.025***	**0.392***	-0.000	**0.361***
	-0.012 -0.002	-0.045 -0.005	0.211 0.593	-0.033 0.031	0.189 0.553
INITIAL	0.000	**-0.042***	-0.060	-0.009	**-0.111***
	-0.000 0.000	-0.061 -0.027	-0.120 0.004	-0.047 0.025	-0.178 -0.045
IS-FS	-0.001	**-0.029***	-0.052	-0.014	**-0.095***
	-0.001 -0.000	-0.050 -0.005	-0.126 0.027	-0.048 0.013	-0.171 -0.018
FALL	**0.005***	0.002	**-0.320***	0.002	**-0.312***
	0.001 0.008	-0.021 0.026	-0.488 -0.145	-0.028 0.031	-0.474 -0.141
2. NATURAL SEEDLINGS 1993-4					
WEEK	**-0.038***	0.011	-0.024	**0.019***	-0.033
	-0.052 -0.024	-0.013 0.034	-0.062 0.020	0.003 0.036	-0.083 0.024
WK-IS	0.001	0.005	0.021	0.010	0.036
	-0.001 0.002	-0.021 0.027	-0.019 0.058	-0.009 0.026	-0.017 0.087
WK-FS	**0.016***	-0.001	**0.055***	-0.013	0.057
	0.010 0.022	-0.021 0.018	0.009 0.102	-0.032 0.005	-0.000 0.109
INITIAL	-0.000	**-0.043***	**0.040***	0.014	0.011
	-0.000 0.000	-0.063 -0.026	0.002 0.082	-0.015 0.043	-0.043 0.072
IS-FS	-0.000	**-0.028***	0.023	0.008	0.003
	-0.001 0.000	-0.045 -0.012	-0.025 0.077	-0.022 0.039	-0.058 0.078
FALL	**-0.006***	-0.016	-0.031	0.014	-0.039
	-0.010 -0.004	-0.032 0.000	-0.083 0.028	-0.014 0.044	-0.103 0.040
3. PLANTED SEEDLINGS 1992-3					
WEEK	**-0.041***	**-0.033***	**-0.041***	-0.012	**-0.127***
	-0.070 -0.013	-0.059 -0.007	-0.077 -0.007	-0.033 0.010	-0.172 -0.075
WK-IS	**0.004***	**0.046***	-0.025	0.019	0.043
	0.001 0.009	0.011 0.083	-0.075 0.017	-0.011 0.048	-0.019 0.102
WK-FS	**0.017***	**0.037***	0.021	0.016	**0.091***
	0.005 0.029	0.014 0.063	-0.021 0.064	-0.015 0.045	0.027 0.148
INITIAL	-0.000	-0.014	0.018	0.005	0.008
	-0.001 0.000	-0.042 0.013	-0.037 0.074	-0.029 0.038	-0.063 0.080
IS-FS	-0.002	**-0.024***	0.044	-0.009	0.009
	-0.004 -0.000	-0.049 -0.002	-0.031 0.113	-0.047 0.030	-0.083 0.091
FALL	**-0.007***	**-0.027***	0.010	-0.012	-0.035
	-0.013 -0.002	-0.050 -0.006	-0.057 0.073	-0.045 0.023	-0.117 0.040

Table 11 (cont'd).

A. NON-LINEAR DIFFERENTIAL					
TRAIT	EPISODE				
	0	1	2	3	TOTAL
4. PLANTED SEEDLINGS 1992-3 WITH SEED WEIGHT					
SEED	-0.009	-0.025	-0.016	0.001	-0.049
	-0.038 0.018	-0.054 0.007	-0.066 0.036	-0.031 0.031	-0.117 0.024
SD-WK	**0.043***	**0.046***	0.004	-0.003	**0.091***
	0.011 0.077	0.012 0.079	-0.038 0.051	-0.026 0.020	0.025 0.151
SD-IS	-0.007	-0.013	0.016	-0.002	-0.006
	-0.021 0.005	-0.048 0.026	-0.048 0.083	-0.036 0.029	-0.089 0.077
SD-FS	-0.022	**-0.031***	-0.003	0.001	-0.055
	-0.046 0.001	-0.061 -0.001	-0.067 0.055	-0.030 0.030	-0.140 0.034
WEEK	**-0.050***	**-0.034***	**-0.046***	-0.017	**-0.147***
	-0.083 -0.022	-0.064 -0.006	-0.082 -0.009	-0.043 0.010	-0.202 -0.090
WK-IS	**0.021***	**0.045***	-0.024	0.021	0.063
	0.006 0.037	0.007 0.079	-0.069 0.024	-0.009 0.048	-0.001 0.122
WK-FS	**0.042***	**0.047***	0.030	0.018	**0.137***
	0.018 0.070	0.017 0.078	-0.018 0.081	-0.015 0.049	0.064 0.202
INITIAL	-0.004	-0.013	0.018	0.004	0.006
	-0.012 0.001	-0.042 0.016	-0.044 0.071	-0.029 0.038	-0.068 0.081
IS-FS	**-0.012***	-0.026	0.044	-0.010	-0.004
	-0.026 -0.001	-0.058 0.003	-0.049 0.114	-0.050 0.027	-0.114 0.091
FALL	**-0.028***	**-0.039***	0.003	-0.013	-0.077
	-0.053 -0.008	-0.071 -0.011	-0.082 0.073	-0.054 0.025	-0.185 0.022
E. PLANTED SEEDLINGS 1993-4					
WEEK	**-0.012***	**-0.012***	0.017	0.007	-0.000
	-0.021 -0.002	-0.022 -0.003	-0.034 0.073	-0.012 0.029	-0.062 0.069
WK-IS	-0.001	-0.017	0.007	0.005	-0.005
	-0.002 0.000	-0.036 0.005	-0.056 0.069	-0.017 0.025	-0.073 0.067
WK-FS	**0.005***	-0.004	-0.014	-0.010	-0.024
	0.001 0.008	-0.015 0.007	-0.095 0.060	-0.033 0.011	-0.111 0.049
INITIAL	-0.000	-0.013	0.036	-0.018	0.005
	-0.000 0.000	-0.031 0.004	-0.037 0.119	-0.052 0.015	-0.074 0.090
IS-FS	0.000	-0.000	0.011	-0.028	-0.017
	-0.000 0.001	-0.014 0.013	-0.079 0.109	-0.062 0.007	-0.113 0.083
FALL	-0.002	0.002	-0.014	-0.013	-0.027
	-0.003 -0.000	-0.010 0.013	-0.111 0.082	-0.040 0.014	-0.120 0.075
F. PLANTED SEEDLINGS 1993-4 WITH SEED WEIGHT					
SEED	-0.001	**-0.025***	**0.084***	-0.008	0.050
	-0.010 0.007	-0.046 -0.004	0.016 0.147	-0.041 0.024	-0.023 0.125
SD-WK	-0.019	**-0.028***	0.007	-0.003	-0.044
	-0.038 0.002	-0.055 -0.002	-0.053 0.072	-0.028 0.019	-0.116 0.034
SD-IS	0.000	-0.012	0.075	-0.014	0.049
	-0.004 0.004	-0.040 0.018	-0.001 0.153	-0.051 0.022	-0.035 0.136
SD-FS	0.008	0.000	0.053	-0.008	0.054
	-0.001 0.017	-0.021 0.020	-0.033 0.137	-0.041 0.025	-0.046 0.154
WEEK	**-0.015***	**-0.015***	0.036	0.010	0.017
	-0.027 -0.005	-0.025 -0.004	-0.025 0.095	-0.010 0.033	-0.055 0.094
WK-IS	-0.009	-0.020	0.000	0.004	-0.025
	-0.018 0.001	-0.040 0.002	-0.063 0.067	-0.020 0.026	-0.104 0.045
WK-FS	-0.003	-0.011	-0.033	-0.013	-0.060
	-0.013 0.006	-0.024 0.001	-0.118 0.056	-0.036 0.009	-0.150 0.037
INITIAL	0.000	-0.005	0.034	-0.014	0.016
	-0.002 0.002	-0.029 0.016	-0.044 0.115	-0.045 0.023	-0.073 0.106
IS-FS	0.004	0.004	0.018	-0.022	0.003
	-0.000 0.008	-0.018 0.024	-0.079 0.119	-0.057 0.013	-0.107 0.121
FALL	0.006	0.007	0.015	-0.005	0.023
	-0.003 0.015	-0.011 0.024	-0.092 0.120	-0.037 0.026	-0.095 0.147

Table 11 (cont'd).

B. NON-LINEAR GRADIENT					
TRAIT	EPISODE				
	0	1	2	3	TOTAL
1. NATURAL SEEDLINGS 1992-3					
WEEK	**0.009***	**0.049***	**0.638***	0.035	**0.732***
	0.002 0.016	0.023 0.080	0.182 1.432	-0.198 0.241	0.199 1.603
WK-IS	0.000	0.006	**-0.695***	-0.023	**-0.712***
	0.000 0.000	-0.025 0.034	-1.395 -0.233	-0.213 0.148	-1.457 -0.230
WK-FS	0.000	**0.007***	**1.206***	0.074	**1.286***
	0.000 0.000	0.002 0.014	0.482 2.347	-0.204 0.330	0.490 2.439
INITIAL	0.000	**-0.045***	**0.435***	0.022	**0.413***
	0.000 0.000	-0.066 -0.024	0.087 1.002	-0.073 0.156	0.075 0.997
IS-FS	0.000	0.001	**-0.801***	-0.068	**-0.868***
	0.000 0.000	-0.004 0.005	-1.662 -0.213	-0.275 0.083	-1.756 -0.292
FALL	0.000	0.001	**1.259***	0.135	**1.395***
	0.000 0.000	0.000 0.003	0.480 2.589	-0.114 0.412	0.541 2.788
2. NATURAL SEEDLINGS 1993-4					
WEEK	**-0.038***	0.011	0.022	0.000	-0.006
	-0.052 -0.024	-0.013 0.034	-0.021 0.068	-0.014 0.014	-0.064 0.047
WK-IS	0.000	0.005	-0.000	**0.029***	0.033
	0.000 0.000	-0.021 0.027	-0.050 0.058	0.004 0.051	-0.026 0.097
WK-FS	0.000	-0.000	0.046	-0.026	0.021
	0.000 0.000	-0.001 0.000	-0.015 0.104	-0.051 -0.001	-0.050 0.080
INITIAL	0.000	**-0.043***	0.005	0.003	-0.035
	0.000 0.000	-0.062 -0.025	-0.034 0.044	-0.019 0.028	-0.083 0.017
IS-FS	0.000	-0.000	0.045	0.012	0.058
	0.000 0.000	-0.001 0.001	-0.012 0.111	-0.022 0.046	-0.008 0.138
FALL	0.000	0.000	**-0.050***	-0.009	**-0.059***
	0.000 0.000	-0.000 0.000	-0.093 -0.010	-0.032 0.018	-0.110 -0.010
3. PLANTED SEEDLINGS 1992-3					
WEEK	**-0.041***	**-0.024***	-0.023	-0.008	**-0.095***
	-0.070 -0.013	-0.047 -0.000	-0.065 0.017	-0.026 0.011	-0.146 -0.045
WK-IS	0.000	**0.042***	-0.026	0.015	0.030
	0.000 0.000	0.009 0.078	-0.098 0.040	-0.019 0.047	-0.053 0.110
WK-FS	0.000	0.000	0.027	0.002	0.029
	0.000 0.000	-0.000 0.001	-0.036 0.083	-0.033 0.033	-0.046 0.106
INITIAL	0.000	-0.004	-0.052	0.019	-0.037
	0.000 0.000	-0.029 0.019	-0.117 0.022	-0.027 0.069	-0.118 0.060
IS-FS	0.000	-0.001	0.070	-0.011	0.058
	0.000 0.000	-0.002 0.000	-0.033 0.157	-0.060 0.042	-0.063 0.159
FALL	0.000	-0.000	-0.039	0.005	-0.034
	0.000 0.000	-0.000 0.000	-0.093 0.017	-0.031 0.041	-0.100 0.034
4. PLANTED SEEDLINGS 1992-3 WITH SEED WEIGHT					
SEED	-0.000	-0.018	-0.020	-0.001	-0.039
	-0.027 0.025	-0.048 0.013	-0.069 0.029	-0.024 0.025	-0.106 0.028
SD-WK	**0.038***	0.030	-0.013	-0.016	0.039
	0.006 0.068	-0.005 0.062	-0.064 0.038	-0.044 0.009	-0.034 0.105
SD-IS	0.000	0.005	0.009	-0.004	0.010
	0.000 0.000	-0.037 0.044	-0.067 0.101	-0.043 0.036	-0.084 0.107
SD-FS	0.000	-0.000	-0.018	0.005	-0.014
	0.000 0.000	-0.001 0.001	-0.093 0.053	-0.033 0.041	-0.091 0.066
WEEK	**-0.042***	-0.020	-0.017	-0.007	**-0.086***
	-0.070 -0.015	-0.046 0.005	-0.066 0.029	-0.026 0.012	-0.145 -0.027
WK-IS	0.000	0.029	-0.027	0.018	0.020
	0.000 0.000	-0.007 0.065	-0.099 0.044	-0.022 0.058	-0.064 0.110
WK-FS	0.000	0.000	0.044	0.010	0.054
	0.000 0.000	-0.001 0.001	-0.026 0.117	-0.033 0.044	-0.041 0.138
INITIAL	0.000	-0.003	-0.058	0.021	-0.040
	0.000 0.000	-0.033 0.029	-0.129 0.023	-0.035 0.080	-0.132 0.068
IS-FS	0.000	-0.000	0.072	-0.009	0.062
	0.000 0.000	-0.001 0.001	-0.054 0.171	-0.061 0.038	-0.077 0.181
FALL	0.000	-0.000	-0.019	0.001	-0.018
	0.000 0.000	-0.000 0.000	-0.079 0.045	-0.028 0.035	-0.087 0.058

Table 11 (cont'd).

B. NON-LINEAR GRADIENT					
TRAIT	EPISODE				
	0	1	2	3	TOTAL
5. PLANTED SEEDLINGS 1993-4					
WEEK	**-0.012***	**-0.011***	-0.020	-0.006	**-0.049***
	-0.021 -0.002	-0.018 -0.002	-0.065 0.020	-0.015 0.003	-0.094 -0.004
WK-IS	0.000	-0.016	0.037	0.000	0.022
	0.000 0.000	-0.035 0.006	-0.048 0.127	-0.028 0.029	-0.069 0.123
WK-FS	0.000	0.001	-0.066	-0.012	-0.077
	0.000 0.000	0.000 0.001	-0.182 0.042	-0.044 0.015	-0.203 0.039
INITIAL	0.000	-0.013	0.015	0.022	0.024
	0.000 0.000	-0.030 0.005	-0.060 0.084	-0.014 0.058	-0.059 0.114
IS-FS	0.000	0.001	0.048	-0.039	0.010
	0.000 0.000	-0.000 0.002	-0.066 0.178	-0.084 0.011	-0.127 0.149
FALL	0.000	-0.000	**-0.105***	0.016	-0.089
	0.000 0.000	-0.000 -0.000	-0.188 -0.030	-0.019 0.054	-0.175 0.000
6. PLANTED SEEDLINGS 1993-4 WITH SEED WEIGHT					
SEED	0.005	-0.020	0.003	-0.003	-0.015
	-0.004 0.015	-0.040 -0.000	-0.056 0.064	-0.021 0.016	-0.082 0.048
SD-WK	-0.018	-0.019	0.040	0.004	0.007
	-0.037 0.003	-0.046 0.010	-0.035 0.117	-0.024 0.031	-0.073 0.084
SD-IS	0.000	0.001	0.052	-0.009	0.043
	0.000 0.000	-0.034 0.036	-0.044 0.147	-0.053 0.035	-0.060 0.150
SD-FS	0.000	0.002	0.070	0.012	0.083
	0.000 0.000	-0.001 0.004	-0.042 0.167	-0.030 0.051	-0.036 0.186
WEEK	-0.009	-0.007	-0.030	-0.005	**-0.051***
	-0.020 0.001	-0.016 0.002	-0.072 0.010	-0.017 0.006	-0.098 -0.008
WK-IS	0.000	-0.009	0.006	0.004	0.001
	0.000 0.000	-0.031 0.015	-0.085 0.095	-0.022 0.032	-0.106 0.100
WK-FS	0.000	0.001	-0.103	-0.017	-0.119
	0.000 0.000	-0.000 0.001	-0.224 0.015	-0.050 0.015	-0.243 0.001
INITIAL	0.000	-0.001	-0.002	0.026	0.022
	0.000 0.000	-0.027 0.023	-0.076 0.082	-0.011 0.063	-0.070 0.120
IS-FS	0.000	0.001	-0.005	-0.035	-0.040
	0.000 0.000	-0.001 0.003	-0.126 0.106	-0.080 0.010	-0.182 0.089
FALL	0.000	-0.000	**-0.129***	0.012	**-0.117***
	0.000 0.000	-0.000 0.000	-0.210 -0.043	-0.021 0.045	-0.201 -0.021

in 1992 (Table 11). There were no significant total non-linear selection differentials in 1993.

The pattern of direct selection in the non-linear gradient differed from the non-linear differential. In 1992, the non-linear gradient showed an increased variance in week, initial size, and fall size and increased covariance between week and fall size and decreased covariance between week and initial size and initial size and fall size in the natural seedlings (Figure 12D) and a decreased the variance in week in the planted seedlings (Figure 14D). In 1993 selection acted directly to decrease the variance in fall size in the natural seedlings (Figure 13D) and decrease the variance in week in the planted seedlings (Figure 15D).

The difference between the non-linear selection differential and gradient illustrates that correlated traits also produce changes in variances and covariances. In particular in the natural seedlings in 1992, indirect selection must decrease the variance in all three traits and increase the covariances between traits (compare Figure 12 C to D).

Four Trait Model with Seed Weight

The inclusion of seed weight in the model showed that selection also altered the variance in seed weight and its covariance with other traits in 1992 (Figure 17 CD), but not in 1993 (Figure 18 CD). In 1992 the non-linear differential indicated a decreased variance in emergence week and an increased covariance between seed weight and emergence week and between emergence week and fall size, while the non-linear gradient displayed a decreased variance in emergence week (Figure 17 CD). The number of significant episodes of non-linear selection differentials indicates that there are numerous significant indirect effects. Direct non-linear selection on emergence week (Figure 17 D) generates significant changes in the covariance between seed weight and emergence week and between emergence week and fall size (Figure 17 C) by the negative phenotypic covariances between these traits (Table 9).

Viability and fecundity selection: episodes of selection

The magnitude and direction of selection on all four traits varied among episodes. The selection differential showed significant positive selection for initial size and fall size among all episodes in all years with the one exception being the natural seedlings in 1992 that displayed negative selection for fall size in at emergence and through the fall (Figures 12-15A, Table 10). Thus, in general larger seedlings had higher survival in all viability episodes and greater fecundity. However, selection on emergence week varied substantially from episode to episode (Figures 12-15A). In both years selection favored late emergence for fall survival and early emergence for survival to spring (not significant in all cases), while selection in the initial episode survival to establishment varied among years, from positive in 1992 (Figure 12A) to negative in 1993 (Figures 13, 15A). Selection on seed weight also varied among episodes: plants from heavier seeds performed best in all episodes beyond establishment (Figures 17-18A).

Direct selection favored larger fall size in all episodes (Figures 12-15B) with the spring survival episode contributing most to the total selection gradient. In contrast, large initial size was favored for fall survival and small initial size was favored for spring survival, such that the total selection gradient was non-significant (Figures 12-15B). Plants that emerged later were favored in all episodes beyond establishment (but significance varies from year to year). However, plants that emerged earlier had the highest survival at establishment in 1993-4. The contribution of selection in viability and fecundity episodes to the total selection gradient on emergence week varied among years and among planted and natural seedlings within years. Direct effects on seed weight were also variable (Figures 17-18B). The selection gradient indicated that plants from lighter seeds survived better at establishment and from winter to spring in 1992, while heavier seeds performed better in other episodes (although not significantly) (Figure 17B). In 1993 plants from heavier seeds survived and were more fecund in all episodes except from winter to spring (Figure 18B). Because these episodes differed in sign and magnitude, the total gradient on seed weight was not significantly different from zero in either year.

Indirect effects varied in magnitude among episodes, but were fairly consistent in sign among episodes (Figure 16). In general the total indirect selection differential was influenced most by the effect of all three traits on spring survivorship with two exceptions being fall size in 1992. When seed weight was included in the analysis, indirect effects were also influenced most strongly by survivorship from the onset of winter to spring.

Non-linear selection differentials and gradients also varied among episodes (Figures 12-15CD, Table 11). In 1992 non-linear selection in the spring survival episode contributed most to the total non-linear selection differential and to the total non-linear gradient in the natural seedlings Figure 12CD). While selection differentials and gradients also varied among episodes for the planted seedlings in 1992, the episodes that contributed most to the total values were survival to establishment and to the onset of winter for both the three trait and four trait models (Figures 14, 17 CD). In 1993 the sign and magnitude of selection differentials and gradients varied among episodes and several traits displayed significant episodes of non-linear selection despite the fact that the total differentials and gradients were mostly not significant. In the two cases where the total selection gradient was significant, survival to spring influenced the covariance between initial size and fall size in the natural seedlings (Figure 13D). All episodes influenced the variance in week, although only survival to establishment and to the onset of winter did so significantly.

Conditional vs. reconstructed selection analysis

In order to quantify the total magnitude of selection on these traits across all episodes, selection parameters in a given episode were weighted by the fraction of individuals surviving to that episode (Lynch and Arnold 1988). This weighting means that later episodes contribute less to the total than earlier episodes, therefore, it changes the relative magnitude of the episodes. A comparison of conditional (Figures 19-24, Tables 12 and 13) and additive parameters (Figures 12-15, 17, 18, Tables 10 and 11) shows the decrease in the relative contribution of the spring survival and fecundity episodes to either the total selection differential or gradient when parameters are additive.

In addition, the additive selection parameters are standardized by the original phenotypic variance-covariance matrix to reflect the total change in the phenotypic distributions. Because the initial variances and covariances are not observed at the start of selection, this original phenotypic variance-covariance matrix was reconstructed based on observed selection gradients and the assumption that selection does not act directly on traits before they are expressed. When one weights selection parameters by this reconstructed phenotypic variance-covariance matrix, one can account for the indirect

Figure 19. Conditional selection parameters on three traits through four episodes of selection for natural seedlings in 1992. Viability episodes include survival to establishment (solid), survival to the onset of winter (vertical), survival to spring (diagonal), and the final episode of fecundity selection (clear). Selection parameters include A) linear selection differential, B) linear selection gradient, C) non-linear selection gradient, and D) non-linear selection gradient. Trait abbreviations follow Table 9. All selection parameters are in units of standard deviation. Significant episodes of selection are denoted by *.

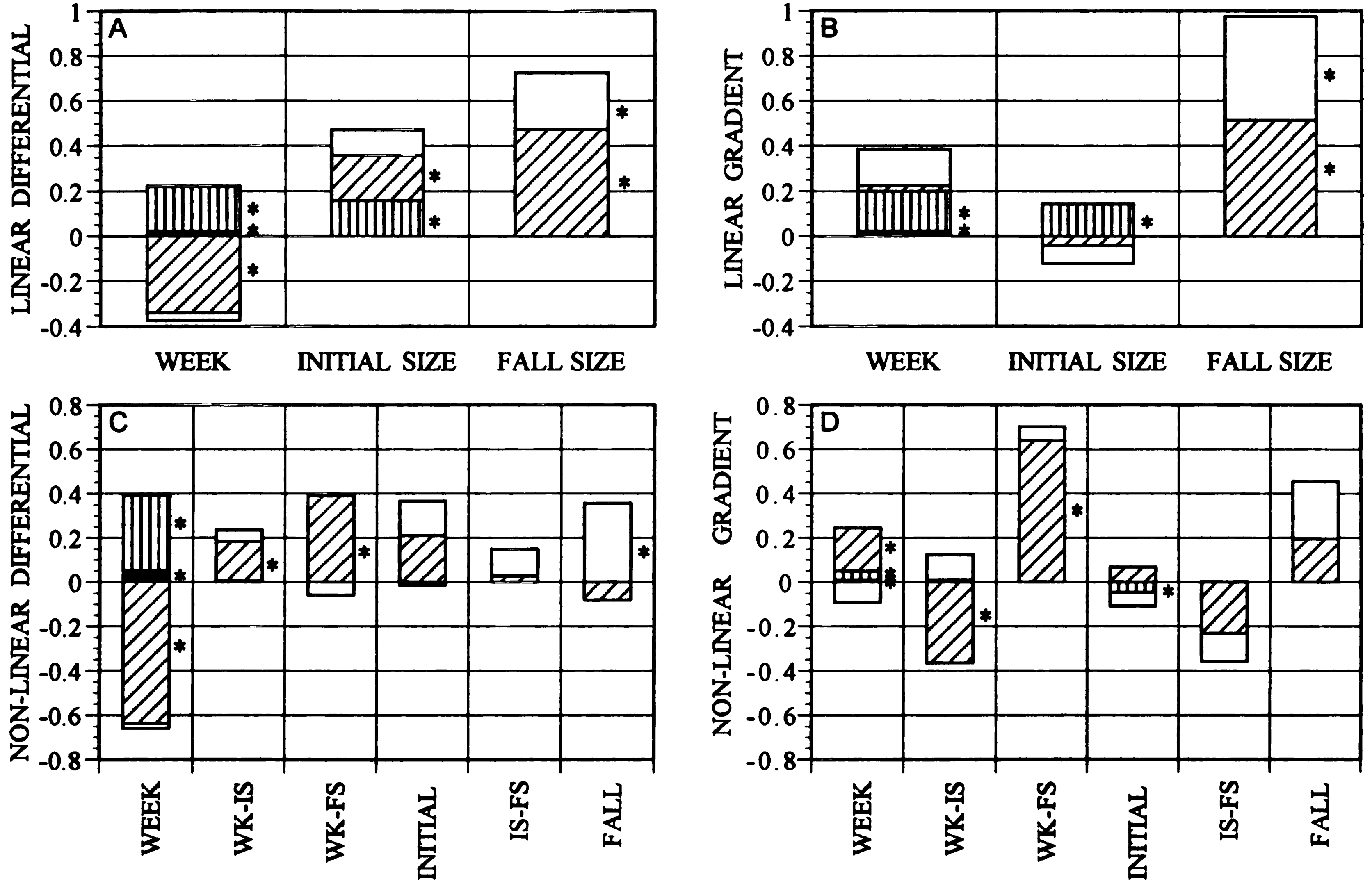

A
LINEAR DIFFERENTIAL
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
WEEK
INITIAL SIZE
FALL SIZE
B
LINEAR GRADIENT
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
WEEK
INITIAL SIZE
FALL SIZE
C
NON-LINEAR DIFFERENTIAL
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
WEEK
WK-IS
WK-FS
INITIAL
IS-FS
FALL
D
NON-LINEAR GRADIENT
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
WEEK
WK-IS
WK-FS
INITIAL
IS-FS
FALL

Figure 19.

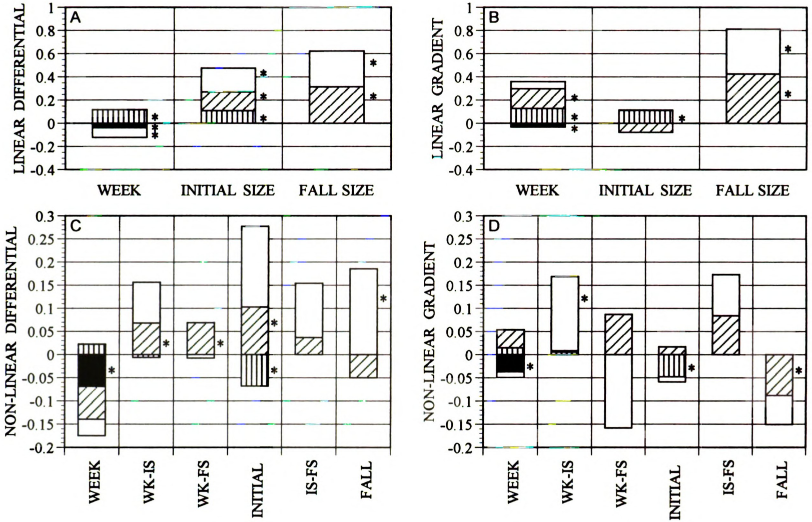


Figure 20. Conditional selection parameters on three traits through four episodes of selection for natural seedlings in 1993. All symbols follow Figure 19.

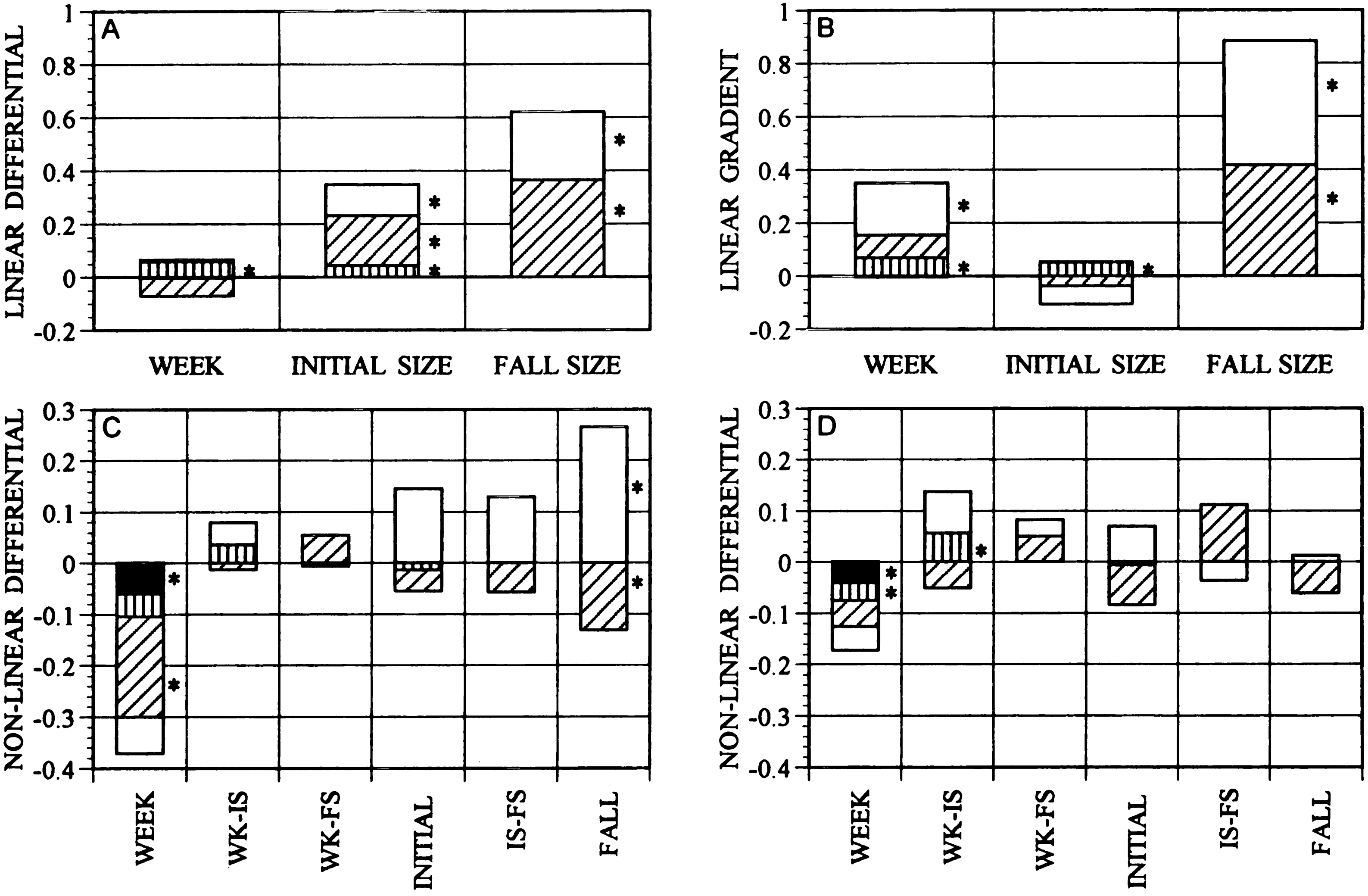


Figure 21. Conditional selection parameters on three traits through four episodes of selection for planted seedlings in 1992. All symbols follow Figure 19.

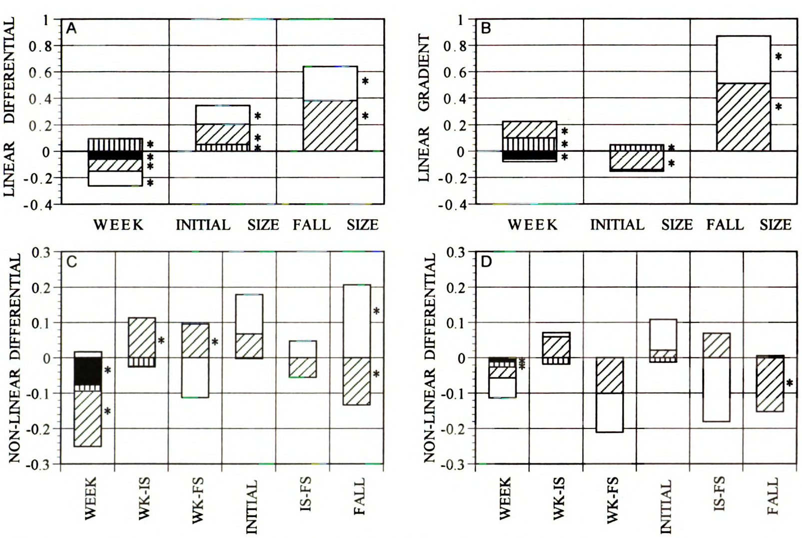


Figure 22. Conditional selection parameters on three traits through four episodes of selection for planted seedlings in 1993. All symbols follow Figure 19.

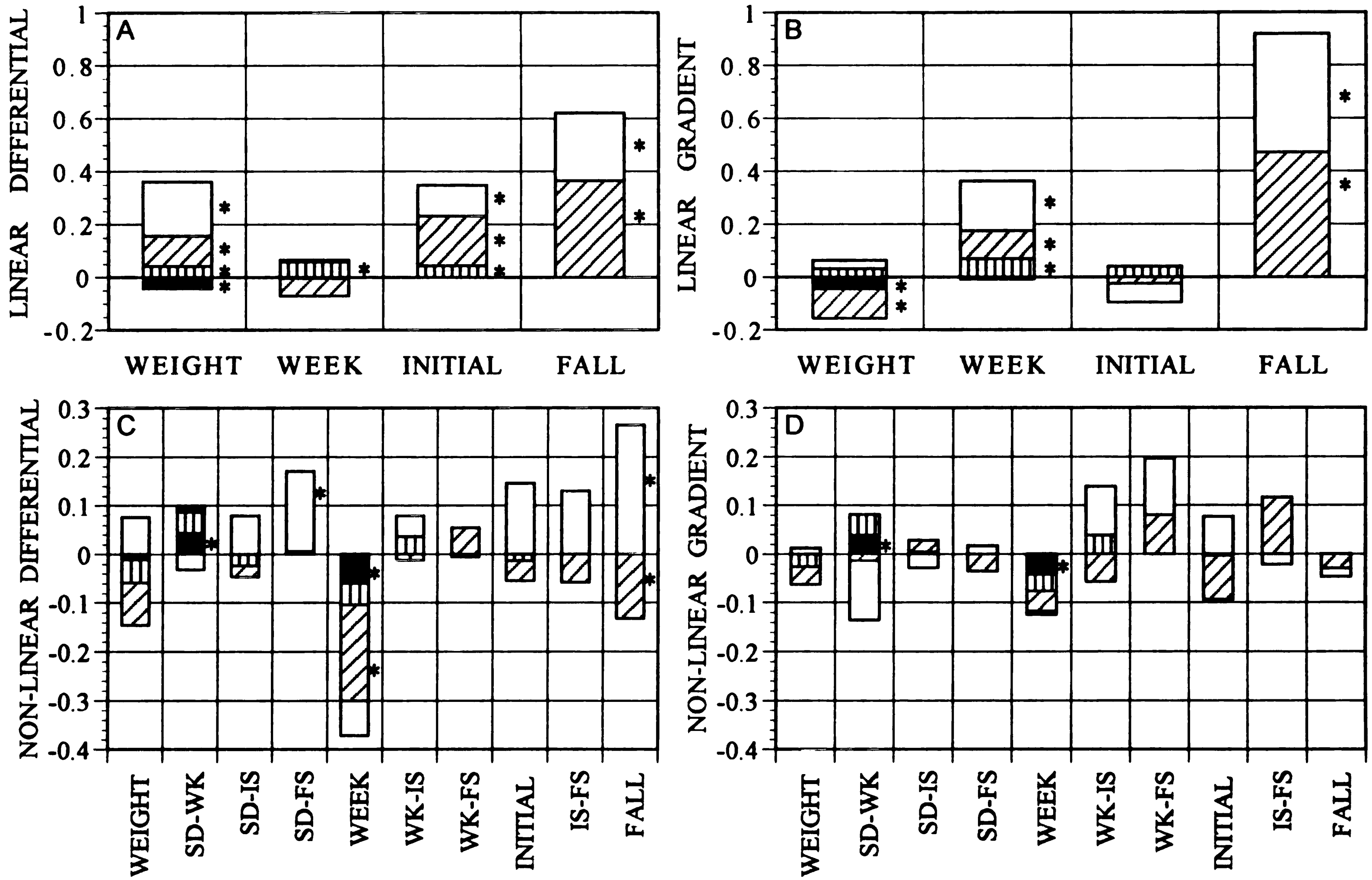


Figure 23. Conditional selection parameters on four traits through four episodes of selection for planted seedlings in 1992. All symbols follow Figure 19.

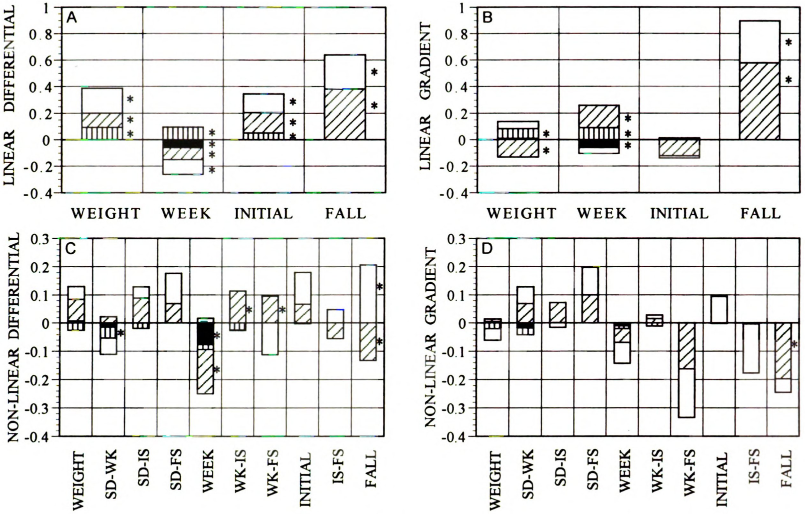


Figure 24. Conditional selection parameters on four traits through four episodes of selection for planted seedlings in 1993. All symbols follow Figure 19.

Table 12. Conditional linear selection differentials (A) and gradients (B) for natural and planted seedlings in 1992 and 1993 for three trait or four trait models. Values are given for each of four selection episodes. Below each value are the 95% confidence intervals based on bootstrap resampling (n=500). Linear parameters reflect the change in the mean in units of standard deviation. Values significant at $p<0.05$ are bold.

	A. LINEAR DIFFERENTIAL							
TRAIT	EPISODE							
	0		1		2		3	
1. NATURAL SEEDLINGS 1992-3								
WEEK	**0.022***		**0.199***		**-0.339***		-0.036	
	0.011	0.032	0.166	0.230	-0.422	-0.259	-0.142	0.068
INITIAL	0.000		**0.158***		**0.199***		0.114	
	0.000	0.000	0.121	0.199	0.118	0.294	-0.026	0.258
FALL	0.000		0.000		**0.474***		**0.249***	
	0.000	0.000	0.000	0.000	0.403	0.548	0.127	0.361
2. NATURAL SEEDLINGS 1993-4								
WEEK	**-0.034***		**0.116***		-0.007		**-0.082***	
	-0.049	-0.019	0.089	0.147	-0.071	0.052	-0.160	-0.014
INITIAL	0.000		**0.107***		**0.162***		**0.206***	
	0.000	0.000	0.079	0.139	0.102	0.221	0.116	0.302
FALL	0.000		0.000		**0.312***		**0.309***	
	0.000	0.000	0.000	0.000	0.243	0.372	0.236	0.393
3. PLANTED SEEDLINGS 1992-3								
WEEK	-0.005		**0.058***		-0.068		0.004	
	-0.039	0.030	0.022	0.095	-0.147	0.010	-0.089	0.089
INITIAL	0.000		**0.043***		**0.187***		**0.117***	
	0.000	0.000	0.003	0.081	0.102	0.268	0.004	0.229
FALL	0.000		0.000		**0.363***		**0.257***	
	0.000	0.000	0.000	0.000	0.293	0.434	0.165	0.354
4. PLANTED SEEDLINGS 1992-3 WITH SEED WEIGHT								
SEED	**-0.045***		**0.041***		**0.115***		**0.204***	
	-0.077	-0.012	0.003	0.078	0.031	0.194	0.113	0.300
WEEK	-0.005		**0.058***		-0.068		0.004	
	-0.039	0.031	0.022	0.098	-0.145	0.015	-0.081	0.093
INITIAL	0.000		**0.043***		**0.187***		**0.117***	
	0.000	0.000	0.003	0.085	0.100	0.272	0.006	0.232
FALL	0.000		0.000		**0.363***		**0.257***	
	0.000	0.000	0.000	0.000	0.296	0.436	0.167	0.358
5. PLANTED SEEDLINGS 1993-4								
WEEK	**-0.060***		**0.094***		**-0.090***		**-0.114***	
	-0.079	-0.044	0.069	0.119	-0.161	-0.010	-0.213	-0.027
INITIAL	0.000		**0.051***		**0.153***		**0.141***	
	0.000	0.000	0.024	0.078	0.064	0.238	0.045	0.240
FALL	0.000		0.000		**0.382***		**0.257***	
	0.000	0.000	0.000	0.000	0.309	0.455	0.169	0.345
6. PLANTED SEEDLINGS 1993-4 WITH SEED WEIGHT								
SEED	-0.000		**0.092***		**0.107***		**0.186***	
	-0.013	0.014	0.065	0.120	0.024	0.192	0.087	0.287
WEEK	**-0.060***		**0.094***		**-0.090***		**-0.114***	
	-0.081	-0.042	0.068	0.119	-0.167	-0.012	-0.205	-0.020
INITIAL	0.000		**0.051***		**0.153***		**0.141***	
	0.000	0.000	0.023	0.076	0.061	0.237	0.029	0.230
FALL	0.000		0.000		**0.382***		**0.257***	
	0.000	0.000	0.000	0.000	0.308	0.462	0.177	0.339

Table 12 (cont'd).

	B. LINEAR GRADIENT			
TRAIT	EPISODE			
	0	1	2	3
1. NATURAL SEEDLINGS 1992-3				
WEEK	**0.022***	**0.177***	0.026	0.158
	0.011 0.032	0.147 0.207	-0.070 0.131	-0.006 0.331
INITIAL	0.000	**0.142***	-0.042	-0.079
	0.000 0.000	0.109 0.181	-0.179 0.095	-0.247 0.099
FALL	0.000	0.000	**0.513***	**0.462***
	0.000 0.000	0.000 0.000	0.361 0.669	0.229 0.695
2. NATURAL SEEDLINGS 1993-4				
WEEK	**-0.034***	**0.127***	**0.168***	0.061
	-0.049 -0.019	0.100 0.159	0.103 0.240	-0.023 0.142
INITIAL	0.000	**0.109***	-0.079	0.004
	0.000 0.000	0.082 0.140	-0.158 0.007	-0.111 0.117
FALL	0.000	0.000	**0.424***	**0.386***
	0.000 0.000	0.000 0.000	0.336 0.514	0.261 0.512
3. PLANTED SEEDLINGS 1992-3				
WEEK	-0.005	**0.067***	0.086	**0.196***
	-0.039 0.030	0.028 0.107	-0.000 0.176	0.068 0.315
INITIAL	0.000	**0.050***	-0.039	-0.069
	0.000 0.000	0.009 0.088	-0.156 0.062	-0.211 0.074
FALL	0.000	0.000	**0.415***	**0.468***
	0.000 0.000	0.000 0.000	0.306 0.523	0.312 0.630
4. PLANTED SEEDLINGS 1992-3 WITH SEED WEIGHT				
SEED	**-0.046***	0.031	**-0.111***	0.030
	-0.079 -0.013	-0.011 0.074	-0.212 -0.012	-0.099 0.172
WEEK	-0.010	**0.068***	**0.106***	**0.188***
	-0.045 0.027	0.030 0.108	0.010 0.201	0.057 0.321
INITIAL	0.000	0.038	-0.027	-0.071
	0.000 0.000	-0.005 0.083	-0.143 0.083	-0.221 0.067
FALL	0.000	0.000	**0.470***	**0.447***
	0.000 0.000	0.000 0.000	0.350 0.593	0.265 0.644
5. PLANTED SEEDLINGS 1993-4				
WEEK	**-0.060***	**0.100***	**0.124***	-0.020
	-0.079 -0.044	0.073 0.127	0.030 0.219	-0.132 0.100
INITIAL	0.000	**0.047***	**-0.141***	-0.011
	0.000 0.000	0.019 0.072	-0.252 -0.032	-0.150 0.125
FALL	0.000	0.000	**0.511***	**0.356***
	0.000 0.000	0.000 0.000	0.409 0.624	0.199 0.539
6. PLANTED SEEDLINGS 1993-4 WITH SEED WEIGHT				
SEED	0.010	**0.073***	**-0.131***	0.053
	-0.004 0.023	0.044 0.103	-0.232 -0.026	-0.069 0.190
WEEK	**-0.062***	**0.090***	**0.167***	-0.042
	-0.083 -0.043	0.064 0.118	0.068 0.268	-0.149 0.082
INITIAL	0.000	0.012	-0.121	-0.017
	0.000 0.000	-0.015 0.040	-0.244 0.002	-0.138 0.109
FALL	0.000	0.000	**0.578***	**0.317***
	0.000 0.000	0.000 0.000	0.454 0.700	0.150 0.480

Table 13. Conditional non-linear selection differentials (A) and gradients (B) for natural and planted seedlings in 1992 and 1993 for three trait or four trait models. Values are given for each of four selection episodes. Below each value are the 95% confidence intervals based on bootstrap resampling (n=500). Non-linear parameters reflect the change in the variance in units standard deviation squared. Values significant at $p<0.05$ are bold.

	A. NON-LINEAR DIFFERENTIAL			
TRAIT	EPISODE			
	0	1	2	3
1. NATURAL SEEDLINGS 1992-3				
WEEK	**0.052***	**0.340***	**-0.639***	-0.023
	0.037 0.065	0.288 0.389	-0.811 -0.458	-0.197 0.179
WK-IS	0.000	0.004	**0.176***	0.053
	0.000 0.000	-0.038 0.050	0.046 0.319	-0.209 0.393
WK-FS	0.000	0.000	**0.390***	-0.059
	0.000 0.000	0.000 0.000	0.293 0.482	-0.220 0.112
INITIAL	0.000	-0.016	0.209	0.157
	0.000 0.000	-0.084 0.051	-0.014 0.470	-0.351 0.742
IS-FS	0.000	0.000	0.026	0.122
	0.000 0.000	0.000 0.000	-0.087 0.154	-0.113 0.402
FALL	0.000	0.000	-0.084	**0.354***
	0.000 0.000	0.000 0.000	-0.194 0.044	0.140 0.589
2. NATURAL SEEDLINGS 1993-4				
WEEK	**-0.070***	0.022	-0.071	-0.035
	-0.101 -0.041	-0.011 0.051	-0.139 0.004	-0.107 0.037
WK-IS	0.000	-0.006	**0.068***	0.088
	0.000 0.000	-0.037 0.022	0.002 0.138	-0.017 0.207
WK-FS	0.000	0.000	**0.069***	-0.008
	0.000 0.000	0.000 0.000	0.006 0.133	-0.095 0.078
INITIAL	0.000	**-0.069***	**0.103***	0.174
	0.000 0.000	-0.113 -0.025	0.009 0.199	-0.044 0.418
IS-FS	0.000	0.000	0.036	0.118
	0.000 0.000	0.000 0.000	-0.045 0.112	-0.061 0.304
FALL	0.000	0.000	-0.051	**0.185***
	0.000 0.000	0.000 0.000	-0.137 0.037	0.005 0.394

Table 13 (cont'd).

	A. NON-LINEAR DIFFERENTIAL							
TRAIT	EPISODE							
	0		1		2		3	
3. PLANTED SEEDLINGS 1992-3								
WEEK	**-0.060***		-0.044		**-0.196***		-0.071	
	-0.107	-0.018	-0.087	0.002	-0.292	-0.105	-0.164	0.022
WK-IS	0.000		0.036		-0.012		0.043	
	0.000	0.000	-0.008	0.078	-0.088	0.054	-0.042	0.131
WK-FS	0.000		0.000		0.054		-0.006	
	0.000	0.000	0.000	0.000	-0.020	0.126	-0.104	0.074
INITIAL	0.000		-0.014		-0.041		0.145	
	0.000	0.000	-0.075	0.047	-0.160	0.075	-0.077	0.365
IS-FS	0.000		0.000		-0.058		0.129	
	0.000	0.000	0.000	0.000	-0.141	0.042	-0.057	0.337
FALL	0.000		0.000		**-0.132***		**0.265***	
	0.000	0.000	0.000	0.000	-0.232	-0.019	0.072	0.459
4. PLANTED SEEDLINGS 1992-3 WITH SEED WEIGHT								
SEED	-0.012		-0.048		-0.086		0.076	
	-0.056	0.030	-0.102	0.001	-0.188	0.015	-0.044	0.202
SD-WK	**0.043***		0.043		0.008		-0.032	
	0.009	0.080	-0.001	0.085	-0.077	0.085	-0.108	0.046
SD-IS	0.000		-0.024		-0.022		0.079	
	0.000	0.000	-0.062	0.013	-0.105	0.064	-0.048	0.202
SD-FS	0.000		0.000		0.005		**0.165***	
	0.000	0.000	0.000	0.000	-0.082	0.093	0.038	0.301
WEEK	**-0.060***		-0.044		**-0.196***		-0.071	
	-0.106	-0.019	-0.096	0.005	-0.296	-0.107	-0.160	0.019
WK-IS	0.000		0.036		-0.012		0.043	
	0.000	0.000	-0.008	0.082	-0.087	0.053	-0.041	0.136
WK-FS	0.000		0.000		0.054		-0.006	
	0.000	0.000	0.000	0.000	-0.021	0.128	-0.100	0.084
INITIAL	0.000		-0.014		-0.041		0.145	
	0.000	0.000	-0.070	0.043	-0.154	0.076	-0.045	0.361
IS-FS	0.000		0.000		-0.058		0.129	
	0.000	0.000	0.000	0.000	-0.154	0.043	-0.063	0.335
FALL	0.000		0.000		**-0.132***		**0.265***	
	0.000	0.000	0.000	0.000	-0.234	-0.024	0.074	0.469

Table 13 (cont'd).

	A. NON-LINEAR DIFFERENTIAL			
TRAIT	EPISODE			
	0	1	2	3
5. PLANTED SEEDLINGS 1993-4				
WEEK	**-0.077***	-0.017	**-0.156***	0.016
	-0.120 -0.038	-0.055 0.018	-0.275 -0.016	-0.120 0.169
WK-IS	0.000	-0.024	**0.113***	-0.003
	0.000 0.000	-0.050 0.002	0.032 0.199	-0.117 0.110
WK-FS	0.000	0.000	**0.095***	-0.112
	0.000 0.000	0.000 0.000	0.008 0.167	-0.277 0.020
INITIAL	0.000	-0.003	0.067	0.112
	0.000 0.000	-0.041 0.034	-0.060 0.199	-0.042 0.289
IS-FS	0.000	0.000	-0.055	0.047
	0.000 0.000	0.000 0.000	-0.150 0.040	-0.100 0.193
FALL	0.000	0.000	**-0.133***	**0.206***
	0.000 0.000	0.000 0.000	-0.230 -0.025	0.057 0.376
6. PLANTED SEEDLINGS 1993-4 WITH SEED WEIGHT				
SEED	0.007	-0.025	0.076	0.047
	-0.013 0.026	-0.062 0.013	-0.074 0.226	-0.136 0.238
SD-WK	-0.014	**-0.039***	0.022	-0.058
	-0.033 0.003	-0.065 -0.015	-0.049 0.094	-0.148 0.036
SD-IS	0.000	-0.019	0.088	0.039
	0.000 0.000	-0.051 0.011	-0.009 0.183	-0.095 0.182
SD-FS	0.000	0.000	0.069	0.107
	0.000 0.000	0.000 0.000	-0.026 0.153	-0.022 0.241
WEEK	**-0.077***	-0.017	**-0.156***	0.016
	-0.130 -0.037	-0.058 0.018	-0.279 -0.035	-0.129 0.179
WK-IS	0.000	-0.024	**0.113***	-0.003
	0.000 0.000	-0.049 0.002	0.031 0.198	-0.119 0.111
WK-FS	0.000	0.000	**0.095***	-0.112
	0.000 0.000	0.000 0.000	0.020 0.177	-0.266 0.022
INITIAL	0.000	-0.003	0.067	0.112
	0.000 0.000	-0.040 0.030	-0.057 0.202	-0.057 0.296
IS-FS	0.000	0.000	-0.055	0.047
	0.000 0.000	0.000 0.000	-0.144 0.034	-0.084 0.175
FALL	0.000	0.000	**-0.133***	**0.206***
	0.000 0.000	0.000 0.000	-0.238 -0.034	0.059 0.365

Table 13 (cont'd).

	B. NON-LINEAR GRADIENT							
TRAIT	EPISODE							
	0		1		2		3	
1. NATURAL SEEDLINGS 1992-3								
WEEK	**0.009***		**0.039***		**0.195***		-0.091	
	0.002	0.016	0.019	0.063	0.076	0.331	-0.312	0.113
WK-IS	0.000		0.009		**-0.367***		0.112	
	0.000	0.000	-0.018	0.036	-0.604	-0.136	-0.234	0.463
WK-FS	0.000		0.000		**0.639***		0.061	
	0.000	0.000	0.000	0.000	0.326	0.982	-0.398	0.518
INITIAL	0.000		**-0.048***		0.067		-0.062	
	0.000	0.000	-0.069	-0.029	-0.072	0.188	-0.211	0.115
IS-FS	0.000		0.000		-0.233		-0.125	
	0.000	0.000	0.000	0.000	-0.462	0.042	-0.502	0.113
FALL	0.000		0.000		0.193		0.260	
	0.000	0.000	0.000	0.000	0.034	0.367	0.042	0.480
2. NATURAL SEEDLINGS 1993-4								
WEEK	**-0.038***		0.014		0.039		-0.012	
	-0.052	-0.024	-0.016	0.044	-0.038	0.129	-0.100	0.080
WK-IS	0.000		0.005		0.002		**0.160***	
	0.000	0.000	-0.025	0.033	-0.095	0.114	0.026	0.286
WK-FS	0.000		0.000		0.086		-0.159	
	0.000	0.000	0.000	0.000	-0.023	0.189	-0.325	0.001
INITIAL	0.000		**-0.048***		0.017		-0.011	
	0.000	0.000	-0.069	-0.028	-0.056	0.086	-0.112	0.101
IS-FS	0.000		0.000		0.083		0.089	
	0.000	0.000	0.000	0.000	-0.026	0.206	-0.111	0.288
FALL	0.000		0.000		**-0.089***		-0.062	
	0.000	0.000	0.000	0.000	-0.165	-0.019	-0.223	0.117

Table 13 (cont'd).

B. NON-LINEAR GRADIENT								
TRAIT	EPISODE							
	0		1		2		3	
3. PLANTED SEEDLINGS 1992-3								
WEEK	**-0.041***		**-0.035***		-0.050		-0.047	
	-0.070	-0.013	-0.067	-0.001	-0.131	0.025	-0.187	0.075
WK-IS	0.000		**0.056***		-0.051		0.081	
	0.000	0.000	0.012	0.103	-0.175	0.069	-0.101	0.253
WK-FS	0.000		0.000		0.049		0.033	
	0.000	0.000	0.000	0.000	-0.066	0.151	-0.213	0.245
INITIAL	0.000		-0.006		-0.078		0.069	
	0.000	0.000	-0.037	0.024	-0.183	0.038	-0.101	0.234
IS-FS	0.000		0.000		0.111		-0.037	
	0.000	0.000	0.000	0.000	-0.054	0.256	-0.268	0.180
FALL	0.000		0.000		-0.061		0.012	
	0.000	0.000	0.000	0.000	-0.146	0.024	-0.172	0.197
4. PLANTED SEEDLINGS 1992-3 WITH SEED WEIGHT								
SEED	-0.000		-0.026		-0.036		0.012	
	-0.027	0.025	-0.066	0.013	-0.121	0.048	-0.127	0.163
SD-WK	**0.038***		0.043		-0.013		-0.122	
	0.006	0.068	-0.005	0.086	-0.105	0.077	-0.316	0.052
SD-IS	0.000		0.004		0.023		-0.028	
	0.000	0.000	-0.048	0.055	-0.107	0.179	-0.208	0.161
SD-FS	0.000		0.000		-0.036		0.017	
	0.000	0.000	0.000	0.000	-0.166	0.088	-0.208	0.250
WEEK	**-0.042***		-0.034		-0.042		-0.007	
	-0.070	-0.015	-0.070	0.003	-0.124	0.039	-0.155	0.138
WK-IS	0.000		0.038		-0.057		0.101	
	0.000	0.000	-0.010	0.086	-0.177	0.068	-0.096	0.311
WK-FS	0.000		0.000		0.080		0.116	
	0.000	0.000	0.000	0.000	-0.047	0.205	-0.231	0.394
INITIAL	0.000		-0.004		-0.090		0.076	
	0.000	0.000	-0.043	0.036	-0.210	0.032	-0.114	0.278
IS-FS	0.000		0.000		0.117		-0.022	
	0.000	0.000	0.000	0.000	-0.091	0.282	-0.277	0.236
FALL	0.000		0.000		-0.030		-0.017	
	0.000	0.000	0.000	0.000	-0.119	0.066	-0.239	0.212

Table 13 (cont'd).

	B. NON-LINEAR GRADIENT			
TRAIT	EPISODE			
	0	1	2	3
5. PLANTED SEEDLINGS 1993-4				
WEEK	**-0.012***	**-0.014***	-0.031	-0.056
	-0.021 -0.002	-0.024 -0.002	-0.106 0.035	-0.162 0.026
WK-IS	0.000	-0.019	0.059	0.011
	0.000 0.000	-0.041 0.007	-0.074 0.191	-0.150 0.194
WK-FS	0.000	0.000	-0.101	-0.110
	0.000 0.000	0.000 0.000	-0.271 0.063	-0.381 0.123
INITIAL	0.000	-0.013	0.021	0.087
	0.000 0.000	-0.032 0.007	-0.078 0.117	-0.048 0.215
IS-FS	0.000	0.000	0.069	-0.181
	0.000 0.000	0.000 0.000	-0.091 0.249	-0.444 0.094
FALL	0.000	0.000	**-0.152***	0.005
	0.000 0.000	0.000 0.000	-0.269 -0.040	-0.205 0.219
6. PLANTED SEEDLINGS 1993-4 WITH SEED WEIGHT				
SEED	0.005	-0.020	0.009	-0.040
	-0.004 0.015	-0.044 0.001	-0.073 0.094	-0.159 0.073
SD-WK	-0.018	-0.024	0.069	0.058
	-0.037 0.003	-0.056 0.013	-0.044 0.179	-0.150 0.270
SD-IS	0.000	0.001	0.071	-0.015
	0.000 0.000	-0.036 0.039	-0.059 0.203	-0.200 0.159
SD-FS	0.000	0.000	0.100	0.099
	0.000 0.000	0.000 0.000	-0.061 0.240	-0.192 0.387
WEEK	-0.009	-0.010	-0.050	-0.074
	-0.020 0.001	-0.022 0.002	-0.119 0.017	-0.213 0.048
WK-IS	0.000	-0.011	0.016	0.012
	0.000 0.000	-0.038 0.018	-0.127 0.153	-0.154 0.179
WK-FS	0.000	0.000	-0.163	-0.172
	0.000 0.000	0.000 0.000	-0.344 0.023	-0.456 0.119
INITIAL	0.000	-0.001	-0.001	0.094
	0.000 0.000	-0.029 0.025	-0.103 0.113	-0.043 0.224
IS-FS	0.000	0.000	-0.003	-0.175
	0.000 0.000	0.000 0.000	-0.175 0.159	-0.430 0.103
FALL	0.000	0.000	**-0.198***	-0.047
	0.000 0.000	0.000 0.000	-0.322 -0.070	-0.284 0.206

effects of selection on traits in episodes prior to their expression. Accounting for these indirect effects means that the total additive linear and non-linear selection differentials include selection on the traits before they are expressed. In this study, linear selection differentials for fall size are significant for survival to establishment and to the onset of winter episodes (i.e. Figures 12, 13, 15A) as a result of its correlation with emergence week and initial size. Non-linear differentials on fall size are also significant in episodes prior to the onset of winter (i.e. Figures 12, 13, 14C). Thus, both the mean and variance in fall size can change due to selection prior to the actual manifestation of the trait. Furthermore, the covariance between fall size and other traits can also change due to selection prior to the onset of winter on phenotypically correlated traits, i.e. covariance between week and fall size in the first two episodes (Figures 12-13C).

This reconstruction makes the assumption that selection does not act directly on traits before they are manifested. Thus, reconstruction should not alter the linear and non-linear selection gradient (compare Figures 12-15, 17, 18 with Figures 19-24). However, when gradients are weighted by the original phenotypic variance-covariance matrix, small (<0.03 standard deviation units), but statistically significant selection gradients occur for fall size in the first episode in three of the four cases (Figures 12, 13, 15, Table 10), probably a result of compounding errors in reconstruction.

Thus, making the selection parameters additive changes the relative magnitude of selection in later episodes and sign and magnitude of the linear and non-linear selection differentials in several episodes by accounting for unobserved changes in the phenotypic variance-covariance matrix. The consequence is that both differentials and gradients were of smaller magnitude because later episodes contributed less to the total. Second, the differentials now reflected indirect selection in early episodes that was not accounted for by conditional differentials.

DISCUSSION

Changes in Trait Means

In this two year study of phenotypic selection on four juvenile traits, I found that the direction of linear selection was consistent across years. The total selection differential, the sum of direct selection and selection on phenotypically correlated traits, indicated that plants with heavier seeds, larger initial size, and larger fall size had higher fitness, while emergence week was unrelated to fitness. When the indirect effects of correlated traits are removed, the direct effects are quantified by the selection gradient which indicated that plants that emerged earlier and were larger at the onset of winter had higher fitness, while seed weight and initial size did not directly affect fitness. The magnitude of these changes varied among traits and years. The magnitude of the predicted change in the mean due to direct selection ranged from 0.1 to 0.47 standard deviations. The largest of these predicted changes was for fall size where a 0.47 standard deviation shift is equivalent to a 1.05 mm change in mean fall cotyledon diameter.

If one assumed that fall size was inherited in a Mendelian fashion, then one could predict the selection response according to the equation, $R= h^2s$. With a selection differential of 0.24 on fall size in the natural seedlings in 1992, the predicted change in the mean would be from 8.27 to 9.32 mm in diameter. Selection of this magnitude could generate a substantial change in the trait from one generation to the next. In a multivariate framework, this change will be affected by phenotypic and genetic correlations with other traits such that the response will be a function of the genetic variance-covariance matrix and the vector of selection gradients ($\Delta \bar{z}=G\beta$). If fall size were negatively genetically correlated with another trait, its response might be constrained by selection on that trait. In this study fall size was positively genetically correlated with seed weight and initial size (Chapter 1) neither of which were directly selected in the natural population. Thus, its response will not be influenced by these genetic correlations. However, because of these genetic correlations seed weight and initial size should respond to direct selection on fall size if one only considers Mendelian inheritance.

Maternal Inheritance

Response to selection will be more complicated than either the univariate or multivariate equations would predict because three of these four traits display maternal inheritance (Chapter 1). Seed weight, and initial and fall cotyledon diameter display significant maternal additive variance and significant negative direct-maternal covariance, while emergence week displays only standard Mendelian inheritance. The magnitude of the negative direct-maternal covariance suggests that the evolution of these traits will be strongly constrained by maternal inheritance. Direct selection may favor large fall size , but this direct selection produces a correlated genetic response such that changes in maternal performance will lead to smaller fall seedlings. Thus, the response to selection may be in a direction opposite to selection depending on the integration of these maternal inheritance parameters. Animal breeders have generally examined responses of single traits and thus, base their expectations on a calculated realized heritability that incorporates maternal genetic variances and covariances in the response. In Chapter 1 I showed that this realized heritability for fall size is near zero in the greenhouse. Multivariate predictions about evolutionary response require the incorporation of maternal inheritance in a specific evolutionary model. Currently, there are two possible approaches to integrating phenotypic selection and maternal inheritance: 1) a covariance approach suggested by Kirkpatrick and Lande (1989) that simplifies quantitative genetic parameters in one term and 2) index selection incorporating maternal inheritance (Van Vleck 1970).

When traits display maternal inheritance the response to direct selection involves both traits expressed at the juvenile stage in the life cycle and traits expressed in the parental stage in the life cycle. While I have not documented phenotypic selection on maternal traits in this population, the quantitative genetic analysis in Chapter 1 demonstrates that direct selection on maternally inherited juvenile traits will produce evolutionary responses in the maternal phenotypic traits (unobserved) that determine the nature of the observed maternal effects. Thus maternal inheritance results in a response to selection for traits expressed much later in ontogeny. Direct phenotypic selection on those maternal traits would also influence the evolutionary response of maternally influenced juvenile traits. Thus, the genetic and phenotypic correlations between maternal and offspring traits influence the joint evolution of these traits. One consequence of this fact is that early-acting phenotypic selection can influence the distribution of maternal traits and late-acting phenotypic selection on maternal traits influences the evolution of juvenile traits. As Kirkpatrick and Lande (1989) point out, one interesting aspect of maternal inheritance is that traits that are not under direct selection influence the evolutionary response. When traits are inherited in a Mendelian framework, this does not occur.

Direct and Indirect Effects

Equations for multivariate evolution incorporate indirect effects of selection by incorporating the phenotypic variance-covariance matrix indicated by the correlations among traits in Figure 8. Differences between the selection differential and the selection gradient suggest how important these indirect effects will be for trait evolution. In this study, I found that although larger seed size and larger initial size were favored (selection differential), there was no direct selection on these traits (selection gradient). Therefore, changes in these traits are due solely to the operation of direct selection on other traits. It appears that strong positive directional selection on fall size indirectly selects for large seed size and large initial size via the positive phenotypic correlations between these traits. In addition, direct selection for later emergence week is countered by indirect effects mediated via the negative phenotypic correlation between fall size and emergence week. As a result, no change in emergence week is expected because although later emergence directly enhances fitness, direct selection for larger fall size decrements the direct change in emergence week via the negative phenotypic correlation.

The linear selection differentials observed in this study are consistent with univariate studies of these juvenile traits: heavier seeds (Wulff 1986; Winn 1988), earlier emerging seeds (Kalisz 1986; Miller 1987; van der Toorn and Pons 1988; Biere 1991b), and larger seedlings (Ross and Harper 1972) all experience enhanced fitness, although these relationships can vary spatially and temporally (i.e. Kalisz 1986). Multivariate studies that include emergence time and early seedling size also emphasize spatial and temporal variation in selection (Kelly 1992; Stratton 1992b; but see Mitchell-Olds and Bergelson 1990b). The general pattern observed in these multivariate studies is that variation in seedling size generates most of the variation in fitness, with larger seedlings displaying higher survivorship and/or fecundity (Bennington and McGraw 1995b; Stratton 1992b; Kelly 1992; Mitchell-Olds and Bergelson 1990b). Although emergence time directly influences early survival (Kelly 1992; Stratton 1992), most selection on emergence time and seed weight is indirect via correlated traits expressed later in ontogeny (Bennington and McGraw 1995b; Stratton 1992b; Mitchell-Olds and Bergelson 1990b). My study documents the same pattern: fall size is the critical trait and once direct selection on it is included in the model, then predictions about the changes in other traits are a function of their correlation with fall size. Early traits like seed weight, emergence date, and initial size influence survival in the early episodes, however, once fall size is expressed selection acts only indirectly on seed weight and initial size. Emergence week continues to directly influence survivorship and in some cases fecundity even after fall size is expressed.

Episodic Analysis

Path analysis represents an alternative approach to the episodic analysis of selection that allows one to test alternative causal models (Crespi and Bookstein 1989; Crespi 1990; Kingsolver and Schmeske 1991). In their multivariate study of juvenile traits and early growth, Mitchell-Olds and Bergelson (1990b) constructed a specific path analytic model to address the relative magnitude of direct and indirect effects of early traits on fitness by allowing early traits to directly influence the expression of later traits as fitness. In my analysis indirect effects are accounted for by the correlation structure alone. One limitation of path analysis is the inability to account for early mortality eliminating individuals from the analysis before they express all the relevant traits. In these cases, one would be unable to quantify the total magnitude of selection in a path analytic framework because individuals not expressing all traits would be excluded from the analysis. Thus, for my purposes an episodic analysis offers the most complete view of phenotypic selection in this species with substantial mortality early in the life cycle. It also permits an examination of selection through ontogeny and allows me to examine the nature of direct and indirect effects in early vs. late episodes of selection, to identify if traits act directly in early episodes, and only indirectly in later episodes.

A second advantage of the episodic approach is that it allowed me to examine the association between selective agents observed in particular episodes relative to the total change in the trait mean, i.e. it can suggest the relative impact of selective agents associated with particular episodes. For instance, in this study slug herbivory was a major source of mortality at establishment and likely to be one from establishment to the onset of winter. An examination of selection gradient (Figures 12-15, 17, 18B) in the first two episodes shows that slugs generally select for earlier emergence in the first episode (significant only in 1993), but later emergence in the second episode. This corresponds to observations of slug activity. Slug activity prior to leaf drop is low, but once a thick layer of leaf litter covers the seedlings, many more slugs were observed. Thus, seedlings emerging early avoided slug herbivory at establishment, while seedlings that emerged after leaf drop were consumed by slugs as they emerged. In late fall leaf litter begins to freeze and slug activity decreases. In the second episode from establishment to the onset of winter, earlier emerging seedlings were available for slug consumption when slugs were most active i.e. after leaf drop and prior to cold temperatures, while later emerging seedlings were more likely to avoid slug consumption after establishment.

In the spring viability episode, direct selection acted primarily on fall size favoring large individuals, but strong direct non-linear selection in this episode (Figure 12D) suggests that while large seedlings survived best, intermediately sized seedlings were lost from the population. Deer herbivory is one possible mechanism for this disruptive selection if deer consume medium-sized plants more frequently than the rare small or large plants. This effect of deer herbivory on spring survivorship could not be quantified because herbivory was scored only on those plants that survived. However, 20.7% and 15.9% of plants surviving to spring were browsed by deer in 1992 and 1993, respectively and browed plants produced fewer seeds than unbrowsed plants (Figure 10). Fall size was significantly smaller in unbrowsed plants, but no more variable than the larger browsed plants. Thus, deer did have an impact on fecundity selection that contributed significantly to the total linear selection differentials and gradients, but not significantly to the non-linear parameters.

Reconstruction

The reconstruction of the phenotypic variance-covariance matrix in this analysis is a powerful technique because it allowed me to estimate the total forces of selection on particular phenotypic traits as well as examine the indirect and direct effects of selection through ontogeny. The larger context of the present study is the integration of maternal inheritance of juvenile traits and phenotypic selection on them in order to illustrate how maternal inheritance may affect the evolution of juvenile traits in this natural population. Conditional estimates of selection derived directly from the multiple regression analysis would not have allowed me to predict how direct and indirect selection on these traits through the life cycle would generate total change in traits means and variances.

My comparison of conditional and additive estimates demonstrates that additive estimates differ in the relative contribution of later episodes to the total selection gradient. Second, additive differentials incorporate indirect effects that were essentially unobserved in the conditional analysis, thereby providing a more complete picture of phenotypic selection.

Conclusions

Phenotypic selection acts on phenotypic values and produces changes in means, variances, and covariances (Figure 8). The trans-generational response to these phenotypic changes depends on the underlying inheritance and genetic correlations. In a separate study, I demonstrated that each of these traits displays significant additive genetic variance. Three traits, seed weight, and initial and fall cotyledon diameter display significant maternal additive variance and significant negative direct-maternal covariance, while the fourth trait, emergence week, displays only standard Mendelian inheritance. In addition, seed weight is positively genetically correlated with the other three traits and initial size and fall size are positively genetically correlated. Thus, while the genetic correlations among traits will enhance the response to selection via indirect effects (i.e. direct selection on fall size will generate concomitant increases in seed weight and initial size), the negative direct-maternal genetic covariances will constrain the responses. This constraint is due to direct selection on offspring traits altering the distribution of maternal phenotypes and their subsequent impact on offspring in the next generation. The integration of phenotypic selection and maternal inheritance requires the multivariate analysis of maternal inheritance suggested by Kirkpatrick and Lande (1989). Once that analysis is completed, I will be able to integrate this complex interaction between maternal inheritance and phenotypic selection and make quantitative predictions about response to selection. Clearly, in this natural plant population, maternal inheritance will have an impact on the evolution of four juvenile traits related to individual survival and fecundity.

Chapter 3

THE OPPORTUNITY FOR MATERNAL SELECTION IN A NATURAL POPULATION OF *COLLINSIA VERNA* (SCROPHULARIACEAE).

INTRODUCTION

The biological complexity of hierarchically structured populations generates the opportunity for selection at multiple levels of organization (Wilson 1975,1980; Wade 1978, 1982, 1985). While evolutionary biologists have argued that individual selection is most parsimonious (Williams 1966; reviewed by Sober 1984), much theoretical work in population and quantitative genetics has focused on how the differential extinction or proliferation of groups, group selection, may also contribute to changes in allele frequencies (Wilson 1975, 1980; Wade 1978, 1980, 1985; Breden 1990) or phenotypic distributions (Yokoyama and Felsenstein 1978; see references in Cheverud 1984). Empirical demonstrations of the components of group selection utilize artificially constructed populations in which hierarchical levels of interaction can be easily manipulated (Wade 1978; Breden and Wade 1989; Goodnight 1990ab; except see Stevens et al. 1995; Kelly 1996). In contrast, in natural populations hierarchical interactions can be more complex (e.g. Brandon 1990).

One approach to incorporating multiple levels of biological complexity is partitioning the covariance between phenotype and fitness, a measure of phenotypic selection, into within and among group components (Wade 1978, 1980). In this approach the term phenotypic selection refers only to the relationship between phenotype and fitness which is distinct from Endler's (1986) definition of natural selection that incorporates both phenotypic selection, heritability, and between generation response in the term natural selection (Lande and Arnold 1983; Brodie et al. 1995 distinguish these two different processes as natural selection and evolution, respectively). Utilizing Price's (1970, 1972) covariance partitioning approach, Wade (1985) demonstrated the relationships between hard selection, soft selection, group selection, and kin selection by illustrating how these different selection models affect the within and among group components of selection as well as the genetic variance between groups. Thus, the covariance approach provides a generalized framework for describing multiple levels of selection given different assumptions about the nature of selection, i.e. hard vs. soft selection. Furthermore, Wade (1985) derived the relationship between the within and among group covariances and corresponding partial regression coefficients. This link between covariances, selection differentials, and partial regression coefficients, selection gradients, paved the way for a new approach to the analysis of group selection, contextual analysis (Heisler and Damuth 1987; Goodnight et al. 1992). Contextual analysis is an extension of Lande and Arnold's (1983) multiple regression approach to selection analysis that allows one to include contextual traits i.e. both aggregate and emergent characters of groups as well as individual traits. In hierarchically structured populations this statistical technique allows one to identify the magnitude of selection at various scales i.e. to compare group and individual selection. One advantage of the regression approach relative to the covariance partitioning approach is the ability to disentangle direct from indirect effects of selection at different levels of organization.

In nature, family groups often interact. For example, the effect of a mother on her offspring is one of the most ubiquitous interactions with the potential to generate group structure in natural populations. These family interactions can be considered as kin selection, a type of group selection in which interactions among related individuals have fitness consequences (Hamilton 1964; Wade 1980; Kelly 1994 and many others). Cheverud (1984) explored the effect of pleiotropy on kin selection between mothers and their offspring to illustrate how genetic constraints may prohibit the evolution of altruism. Kirkpatrick and Lande (1989, 1992) termed this type of kin selection when mothers directly affect the survival or fecundity of their offspring maternal selection and demonstrated that maternal selection can differ from individual selection in two ways (Kirkpatrick and Lande 1989, 1992). First, like maternal inheritance, maternal selection can result in maladaptive evolution. Using the Karn and Penrose (1951) example of stabilizing selection on human birth weight as a model of a maternal selection, Kirkpatrick and Lande (1989, 1992) demonstrated that traits influencing maternal selection could evolve maladaptively when stabilizing selection acted more strongly on the maternal trait than on the offspring trait, when there was strong correlational selection between a mother and her offspring, and when heritability was low. Second, they showed that maternal selection was unlike other forms of selection because the magnitude depended on the resemblance between parents and offspring.

Maternal selection is analogous to family selection utilized by animal and plant breeders (Falconer 1981; England 1977). When heritability is low, family size is large, and common environmental effects do not affect resemblance among family members, family selection yields better response to selection than individual selection by providing a more accurate assessment of individual breeding values (Falconer 1981). It is interesting to note, however, that the combination of individual, family, and within family selection (i.e. index selection) can produce greater responses to selection than individual selection alone in certain cases. Thus, these artificial forms of selection, analogous to individual and group selection in nature, demonstrate the possibility that different forms of selection are likely influence evolutionary change in natural populations.

Maternal selection can include both pre-natal provisioning traits or post-natal parental care traits by which a mother directly impacts her offspring's survival in the juvenile period and beyond. When mothers differ in their influence on the subsequent generation, they create the *opportunity* for selection among families because group membership can influence both individual phenotype and/or individual fitness components. Several factors may contribute to the among maternal family group variance: 1) maternal inheritance, the contribution of maternal phenotypic traits to phenotypic attributes in her offspring that may cause maternal family groups to differ phenotypically, 2) maternal selection, the contribution of maternal phenotypic traits to the fitness of her offspring, and 3) spatial variation in the environment that can influence phenotypic expression and/or the nature of phenotypic selection. In this chapter, I present a preliminary investigation of maternal selection in which no maternal phenotypic attributes are included, and I explore the *opportunity* for maternal selection as the variance among maternal families in relative fitness. This among family variance is the first prerequisite for maternal selection to occur. Furthermore, I evaluate the extent to which family membership may influence individual relative fitness. This family effect indicates the maximum amount of variance that any particular maternal trait may explain (Heisler and Damuth 1987).

In natural populations, variance among maternal families in relative fitness can exist when mothers differ in their effects on individual offspring fitness or when maternal families experience different selective environments. In plants limited seed dispersal means that offspring from a maternal individual may experience selection on a local scale. Evidence for fine scale variation in phenotypic selection in natural plant populations is extensive. Phenotypic selection can vary spatially on a scale from meters to centimeters (Kalisz 1986; Stewart and Schoen 1987; Scheiner 1989; Kelly 1992; Stratton 1992a). Stratton (1994, 1995) has demonstrated that relative fitness of different *Erigeron* genotypes varies at a scale of 20 cm suggesting fine-scale spatial variation in selection in experimental populations. In their extensive study of variation in selection on gall size in *Solidago*, Weis et al. (1992) note that variable selection can result from: 1) variation in the relationship between phenotype and fitness, termed fluctuating fitness function, 2) variation in the underlying phenotypic distribution, or 3) from a combination of these two components. Thus, at the population level, variance among maternal families can be influenced both by maternal effects on fitness and by spatial variation in selection. In contrast, paternal effects on fitness, paternal selection, would likely be manifested over larger spatial scales and integrate fine scale variation in phenotypic selection because pollen flow tends to be more widespread than seed dispersal (Levin and Kerster 1974).

In this chapter I evaluate the *potential* for maternal selection in a natural plant population in which a number of juvenile traits display significant maternal inheritance (Chapter 1) and are subject to individual phenotypic selection at various stages in the life cycle (Chapter 2). I explore empirically maternal selection at two spatial scales that reflect how different processes might contribute to among family variance. At the scale of the population, families may differ in fitness either because offspring differ in their phenotypic attributes or because maternal effects on offspring fitness differ among families. At this scale, all families are planted into all environments and thus experience the average selective environment. I refer to this average selective environment as the global scale. However, in nature families typically experience only a single environment due to limited seed dispersal distance. In this case, in addition to the two factors above, among family variance can also be influenced by spatial variation in selection. I refer to this local selective environment as the local scale. In this chapter I address the following questions:

1) is there opportunity for selection by maternal family when families experience the average selective environment,
2) for these families, can the variation in individual fitness be attributed to family membership,
3) is there opportunity for selection among maternal families experiencing single environments,
    a) does the direction and magnitude of linear selection vary spatially,
    b) if so, at what spatial scale does it vary,
    c) what factors might account for spatial variation in selection,
4) on a local scale can the variation in individual fitness be attributed to maternal family membership?

This study is the first to examine the potential for maternal selection on two spatial scales in a natural plant population.

MATERIALS AND METHODS

Study Site and Species

Collinsia verna Nutt. (Scrophulariaceae), a winter annual inhabiting mesic forests of the eastern United States (Fernald 1970), displays substantial phenotypic variation in juvenile traits including seed weight, emergence time, initial cotyledon diameter (hereafter initial size), and fall cotyledon diameter (hereafter fall size) in a natural population in Kalamazoo County, Michigan. In previous chapters I have demonstrated that seed weight, initial size and fall size are maternally inherited (Chapter 1) and that phenotypic selection favors earlier emergence week and larger fall size in the natural population (Chapter 2).

To explore the opportunity for maternal selection, I document among family variance in relative family fitness. To explore the factors influencing among family variance in mean family fitness, I planted offspring at two spatial scales: global and local. For the global scale, offspring from a single mother were planted at random across multiple blocks (n=12 blocks) in the population. Families planted at this global scale allow me to examine the first two factors independent of spatial variation in phenotypic selection. For the local scale, offspring from a single mother were planted back into a single block in the population. These single environment (hereafter local) offspring consisted of two types: 1) home offspring were planted back into the block from which their mother was collected and 2) away offspring were planted into a randomly chosen block. In preliminary analyses, home and away families paired by block showed no significant difference in any fitness component in 1992 and a significant difference in only two of four components of fitness in 1993 (Wilcoxin sign rank test). Therefore, home and away offspring were combined in all subsequent analyses.

The study site and life cycle of *C. verna* were previously described (Chapter 2). Naturally emerging and planted seedlings were individually tagged and scored for three phenotypic traits, emergence week, initial size, and fall size. In addition, the planted individuals were scored for seed weight. The measurement of these traits allowed me to assess survivorship at three stages in the life cycle: 1) survival to establishment, 2) survival to the onset of winter, and 3) survival to spring. The number of seed produced served as an estimate of fecundity on all individuals that survived to the spring.

Spatial and temporal variation in biotic and abiotic factors influencing survival and fecundity previously described in Chapter 2 suggest that spatial variation in phenotypic selection may affect the opportunity for maternal selection. The most notable spatial variation in this population resulted from a sharp boundary between the woods and an adjacent agricultural field that resulted in higher light levels along the edge and presumably greater drought stress as well. I did observe wilting in the first few emergence censuses along this edge. Seedling densities were also highest along this forest edge. In order to account for this spatial variation in biotic and abiotic factors influencing survival and fecundity, I censused naturally emerging and planted individuals along two transects, one along the edge and the other 25 m away in the interior. Ten blocks were arrayed along each 100 m transect at 10 m intervals. To ensure adequate sample sizes in the analyses described below, spatial variation was assessed at two spatial scales transect and block nested within transect.

To document the opportunity for selection among maternal families, I monitored the survival and reproduction of locally planted offspring from 77 and 191 naturally produced maternal families in 1992 (n=844 seeds) and 1993 (n=1882 seeds), respectively, and of globally planted offspring from 56 naturally produced maternal families in 1993 (n=552 seeds). Relatedness of offspring from a maternal family must fall between 0.25 and 0.5 for half-sibs and full-sibs, respectively. At TU Ave. the outcrossing rate was consistently greater than 0.85 with a high probability of correlated mating (Holtsford et al. in prep) suggesting that these maternal families have an average relatedness close to 0.5. Relatedness between mothers and offspring was also likely to be 0.50 given the infrequency of selfing in this population. Twelve seeds were planted per family, while all seeds were planted for smaller families. In 1993 some families had more than 12 seeds planted to fill in the planting array. On average 10.96 ± 0.20 (± 1 SE) and 9.86 ± 0.18 seeds per local family were planted in 1992 and 1993, respectively, and 9.86 ± 0.0.29 seeds per global family in 1993. This approach eliminated initial differences in the number of offspring per family, hence among family differences are conservative with respect to initial family size.

Analysis

Maternal selection at a global scale

For global families relative family fitness was calculated as the mean of individual offspring fitness in a maternal family standardized by the grand mean of family mean fitness for survival in each of three viability episodes and family mean fecundity in the final episode of reproduction. The among family variance in relative family fitness is the opportunity for maternal selection.

Differences in survivorship among global families were examined by failure time analysis using the life table method in the LIFETEST procedure in SAS (Fox, 1993; SAS Institute, Inc., 1994). Subsequently, variation in survivorship in each selection episode was analyzed by one-way analysis of variance for all families with at least two offspring in a given episode. Significance tests for these analyses are likely to be compromised by non-normality of residuals. Variation among families in phenotypic traits was determined by a multivariate analysis of variance.

To evaluate the effect of family membership on individual fitness, family was added to univariate models of phenotypic selection for global seedlings in each of the four selection episodes. Heisler and Damuth (1987) suggested including group membership as a class variable in an analysis of covariance as an indicator of the potential for group selection. In this study a statistically significant family effect indicates the maximum amount of variance that any maternal or family group attribute may explain (see Firebaugh 1979 in Heisler and Damuth 1987). First, I evaluated whether the relationship between the trait and fitness component might vary among families testing for heterogeneity among slopes by including an interaction term last in a sequential sums of squares analysis. If there was no indication of heterogeneous slopes, a reduced model including only the trait and family were subsequently analyzed by partial sum of squares. Significant heterogeneity of slopes in the full model or family effects in the reduced model both indicate the *potential* for maternal selection. These analyses of covariance for each selection episode are termed conditional because analyses were based only on individuals surviving to the beginning of a particular episode.

Maternal selection at a local scale

The opportunity for maternal selection was calculated in the same way for local families as for global families and therefore includes spatial variation in relative fitness.

Spatial variation in fitness components

Spatial variation in survivorship and fecundity in each of four episodes among the edge and interior transects and among blocks within transects was analyzed in a separate nested analysis of variance for each episode (GLM, SAS Institute Inc. 1994). Transects and blocks nested within transects were treated as random effects with appropriate F-tests calculated using the Random statement in SAS using a type III sum of squares. Residuals from these analyses were not normally distributed because of the bivariate nature of the survivorship data and the non-normal distribution of fecundity. While ANOVA is robust to departures from normality (Neter, Waserman, and Kutner 1985), significance tests may be compromised. Stratton (1995) found that significance for randomization tests did not deviate substantially from ANOVA F-tests for similar survivorship data. These ANOVA results are preferable in this case to non-parametric tests such as Kruskal-Wallis because transect and block can not be treated as random factors nor can those spatial effects be appropriately nested in non-parametric approaches.

Spatial variation in phenotypic traits

The extent of spatial variation in the phenotypic traits, seed weight, emergence week, initial size, and fall size was determined by a multivariate nested analysis of variance using GLM (SAS Institute Inc. 1994). MANOVA is preferable to separate univariate analyses because it does not inflate type I error. Secondly, it evaluates not only differences in multivariate means, but also in correlation structure (Scheiner 1993).

Spatial variation in phenotypic selection

An episodic analysis of phenotypic selection for three viability and one fecundity selection episode according to the reconstruction techniques of Lynch and Arnold (1988) described in Chapter 1 was used to estimate spatial variation in the total linear selection differentials and total linear selection gradients for natural and planted seedlings in 1992 and 1993. Only linear components of selection were examined because small sample sizes at the block scale made estimates on non-linear components unreliable. Furthermore, only the total magnitude of selection is described here for simplicity. This spatial analysis was performed at two spatial scales in separate analyses i.e. at the transect and block levels with the number of bootstrap resampling rounds at 250 per transect and 50 per block, respectively. The number of observations in the resampled data sets matched the original number of observations from that transect or block.

Local family effects

ANCOVA models for each selection episode were utilized to evaluate the potential contribution of families to local variation in individual relative fitness. These models included all phenotypic traits and a class variable, family. Separate analyses were performed by block. Slopes were not tested for heterogeneity. In addition a single nested ANCOVA that included block and family nested within block as well as all phenotypic traits in a given episode were utilized to explore the effect of family on fitness when spatial variation could be accounted for by the inclusion of block in the analysis. This hierarchical statistical model is a preliminary approach to analyzing selection at multiple levels of biological organization indicating the potential for phenotypic selection at individual, family, and block level in this population.

RESULTS

Maternal selection at a global scale

For globally planted seedlings in 1993 the mean number (± standard deviation) of offspring in a family (n=56) decreased from 9.85 ± 2.19 at planting, 6.73 ± 2.58 from germination to establishment, 6.09 ± 2.35 from establishment to the onset of winter, 4.66 ± 2.18 from winter to spring, to 1.58 ± 1.28 in the final fecundity episode when only 43 families were represented. A comparison of survivorship among families did not differ for the three survivorship episodes (log-rank chi-square, χ^2=60.07, df=55, p>0.2973), but differed when all planted seeds were included such that seeds not germinating were censored in the analysis (log-rank chi-square, χ^2=108.85, df=55, p>0.0001) suggesting that differences in dormancy among families contributed to among family differences in survivorship curves. Mean survivorship only differed among families in the second episode, survival to the onset of winter (Table 14). The opportunity for selection among globally planted families was greatest in survivorship to spring and fecundity episodes (Figure 25).

Global families differed significantly in phenotype (MANOVA, Wilk's λ=0.24, numerator df=220, denominator df=809.3, p<0.0001). Univariate analyses for each of the four phenotypic traits showed that only seed weight differed significantly (ANOVA, F=2.53, df=55, 205, p<0.0001), while emergence week (ANOVA, F=1.37, df=55, 205, p<0.0586) and fall size (ANOVA, F=1.36, df=55, 205, p<0.0644) were marginally significant.

Did family membership explain variation in individual survivorship and fecundity? In Chapter 2 the conditional selection differentials for all planted seedlings in 1993-4, i.e. both local and global, showed significant selection on all traits except seed weight in the first episode (see Chapter 2, Table 12). A separate analysis for global seedlings only showed significant linear selection on emergence week in the first two survivorship episodes, on fall size in survivorship to spring, on seed weight, initial size, and fall size in the fecundity episode (Table 15). The magnitudes of the selection coefficients were similar between analyses based on all planted seedlings and only on globally planted seedlings. The ANCOVA including family and its interaction showed that the relationship between each trait and fitness components varied among families for two traits, emergence week during survival to establishment (Table 16A) and seed weight during survival to the onset of winter (marginal interaction term) (Figure 26). These early episodes provided the most power for testing for heterogeneous slopes because they had more observations per family than later episodes (see survivorship above). When slopes were not heterogeneous among families, one trait, initial size, approached significance for family effect on survival to the onset of winter (Figure 28, Table 16B), while other traits showed no significant family effects (Figure 27, Table 16B).

Maternal selection at a local scale

Spatial variation in biotic and abiotic factors can affect among family differences in phenotypic traits, fitness components, and in the relationship between phenotype and fitness at two scales: transects (25 m apart) and blocks (adjacent pairs separated by 10 m).

Table 14. One-way analysis of variance of family variation in survivorship for globally planted seedlings in 1993 at TU Ave for four episodes: 1) survival through establishment (A), 2) survival to the onset of winter (B), 3) survival to spring (C), and 4) final fecundity (D). Only families with two or more offspring in a given episode were included in this analysis. Degrees of freedom are for the numerator and denominator, respectively.

Episode					
Source	R^2	df	MS	F	P
A. Survival to establishment					
Family	0.12	51, 321	0.08	0.91	0.6490
B. Survival to the onset of winter					
Family	0.20	51, 336	0.24	1.38	0.0538
C. Survival to spring					
Family	0.17	48, 205	0.20	0.88	0.6892
D. Fecundity					
Family	0.32	27, 45	92.65	0.81	0.7213

Figure 25. The opportunity for maternal selection portrayed as the among family variance in relative fitness in each episode for locally and globally planted seedlings. Relative family fitness is calculated as mean family fitness standardized by the grand mean of family fitnesses for the sample population.

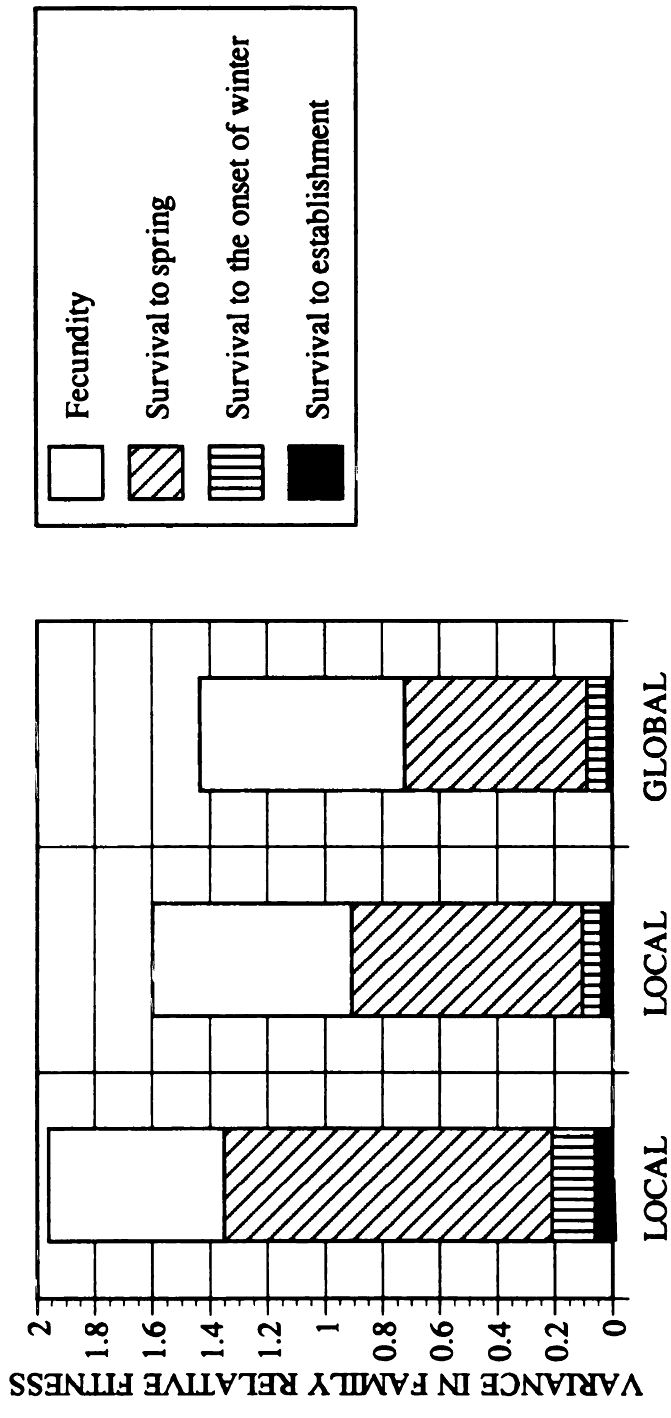

VARIANCE IN FAMILY RELATIVE FITNESS
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LOCAL
1992
LOCAL
GLOBAL
1993
Fecundity
Survival to spring
Survival to the onset of winter
Survival to establishment

Table 15. Univariate regression models for globally planted seedlings in 1993 in four selection episodes: 1) survival through establishment (A), 2) survival to the onset of winter (B), 3) survival to spring (C), and 4) final fecundity (D). Significant linear selection coefficients (β) are bold.

Source	R^2	df	β	F	P
A. Survival to establishment					
Seed weight	0.00	1, 375	0.01	0.13	0.7208
Emergence week	0.05	1, 375	**-0.07**	20.16	0.0001
B. Survival to onset of winter					
Seed weight	0.01	1, 339	0.05	2.40	0.1223
Emergence week	0.02	1, 339	**0.08**	6.70	0.0100
Initial size	0.01	1, 339	0.05	2.92	0.0886
C. Survival to spring					
Seed weight	0.01	1, 259	0.14	2.36	0.1257
Emergence week	0.01	1, 259	-0.12	1.83	0.1770
Initial size	0.00	1, 259	0.07	0.60	0.4400
Fall size	0.05	1, 259	**0.33**	15.25	0.0001
D. Fecundity					
Seed weight	0.08	1, 86	**0.38**	8.00	0.0058
Emergence week	0.00	1, 86	0.01	0.00	0.9530
Initial size	0.09	1, 86	**0.38**	8.91	0.0037
Fall size	0.13	1, 86	**0.47**	12.45	0.0007

Table 16. Two ANCOVA models for evaluating the potential for maternal selection for global seedlings in 1993. The full model included the interaction term to evaluate heterogeneity of slopes (A). Significance was based on Type 1 sum of squares, i.e. sequential sum of squares (SAS Institute Inc., 1994). Reduced models for all traits not displaying heterogeneity of slopes among families (B) were tested for significance of family effect using Type III sum of squares, i.e. partial sum of squares.

Source	Episode											
	Survival to establishment			Survival to onset of winter			Survival to spring			Fecundity		
	R^2	df	P	R^2	df	P	R^2	df	P	R^2	df	P
A. Heterogenous slopes												
Seed weight	0.24	1, 269	0.7288	0.39	1, 233	0.1031	0.39	1, 156	0.1290	0.82	1, 17	**0.0106**
Family		55, 269	0.8151		55, 233	0.0664		55, 156	0.7713		42, 17	0.7098
SW*FAM		51, 269	0.8707		51, 233	0.0548		48, 156	0.3036		27, 17	0.2468
Emergence week	0.36	1, 269	**0.0001**	0.32	1, 233	**0.0099**	0.31	1, 161	0.2019	0.47	1, 23	0.9668
Family		55, 269	0.5990		55, 233	0.2082		55, 161	0.8122		42, 23	0.9963
EW*FAM		51, 269	**0.0139**		51, 233	0.7391		43, 161	0.9692		21, 23	0.9997
Initial size				0.37	1, 233	0.0775	0.37	1, 156	0.4505	0.66	1, 19	0.0326
Family					55, 233	0.0605		55, 156	0.7754		42, 19	0.9867
IS*FAM					51, 233	0.2747		48, 156	0.5616		25, 19	0.9186
Fall size							0.40	1, 157	**0.0002**	0.61	1, 18	**0.0269**
Family								55, 157	0.7451		42, 18	0.9992
FS*FAM								47, 157	0.6077		26, 18	0.9925

Table 16 (cont'd).

Source	Episode											
	Survival to establishment			Survival to onset of winter			Survival to spring			Fecundity		
	R^2	df	P	R^2	df	P	R^2	df	P	R^2	df	P
B. Family effects												
Seed weight	0.13	1, 320	0.8037				0.19	1, 204	0.7261	0.44	1, 44	**0.0229**
Family		55, 320	0.7680					55, 204	0.8108		42, 44	0.9079
Emergence week				0.20	1, 284	0.2581	0.20	1, 204	0.0977	0.37	1, 44	0.6534
Family					55, 284	0.1697		55, 204	0.6990		42, 44	0.9387
Initial size				0.21	1, 284	**0.0495**	0.19	1, 204	0.8661	0.42	1, 44	0.0546
Family					55, 284	0.0707		55, 204	0.7663		42, 44	0.9557
Fall size							0.23	1, 204	**0.0005**	0.41	1, 44	0.0947
Family								55, 204	0.7251		42, 44	0.9873

Figure 26. The phenotypic distribution of seed weight for all globally planted seedlings (A), the distribution of family means (■) in standard deviation units with 10, 25, median, 75, and 95 percentiles depicted (B), and the overall linear relationship between seed weight and survival to the onset of winter (C).

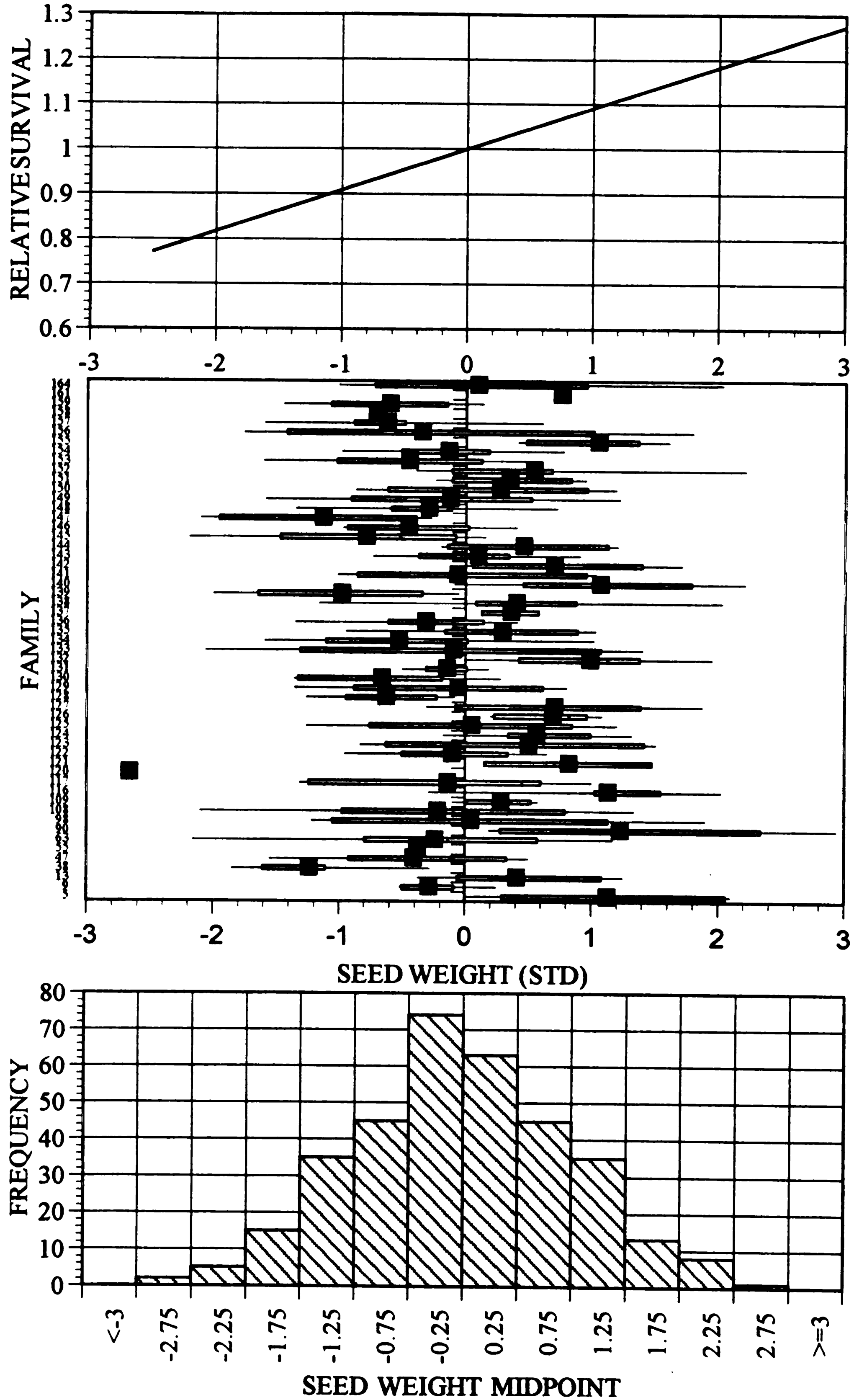

RELATIVE SURVIVAL
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
-3
-2
-1
0
1
2
3
FAMILY
SEED WEIGHT (STD)
FREQUENCY
80
70
60
50
40
30
20
10
0
<-3
-2.75
-2.25
-1.75
-1.25
-0.75
-0.25
0.25
0.75
1.25
1.75
2.25
2.75
>=3
SEED WEIGHT MIDPOINT

Figure 26.

Figure 27. The phenotypic distribution of emergence week for all globally planted seedlings (A), the distribution of family means (■) in standard deviation units with 10, 25, median, 75, and 95 percentiles depicted (B), and the overall linear relationship between emergence week and survival to the onset of winter (C).

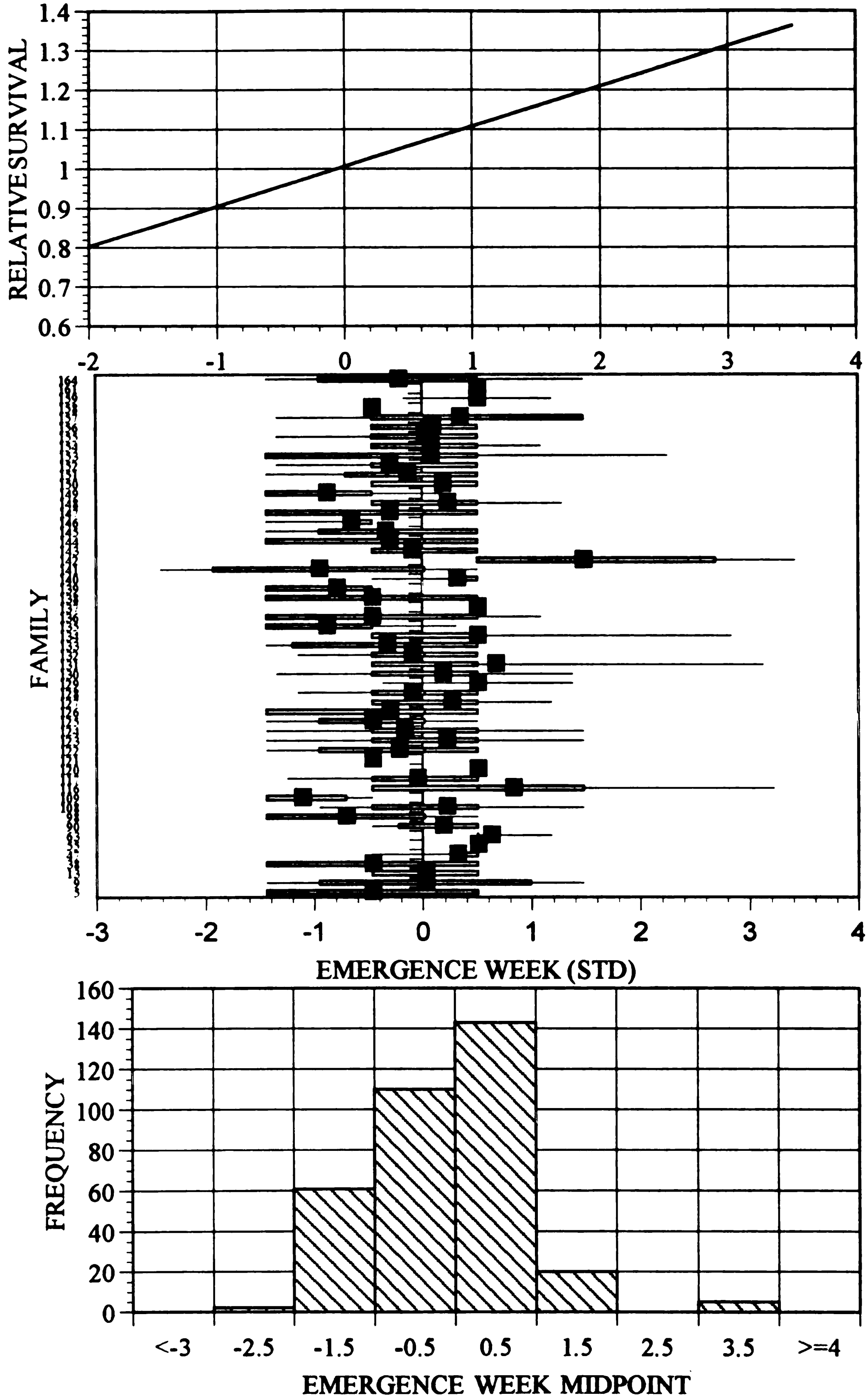

RELATIVE SURVIVAL
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
-2
-1
0
1
2
3
4
FAMILY
-3
-2
-1
0
1
2
3
4
EMERGENCE WEEK (STD)
FREQUENCY
160
140
120
100
80
60
40
20
0
<-3
-2.5
-1.5
-0.5
0.5
1.5
2.5
3.5
>=4
EMERGENCE WEEK MIDPOINT

Figure 27.

Figure 28. The phenotypic distribution of initial size for all globally planted seedlings (A), the distribution of family means (■) in standard deviation units with 10, 25, median, 75, and 95 percentiles depicted (B), and the overall linear relationship between initial size and survival to the onset of winter (C). The two lines in (C) are for overall regression and weighted average of within family regressions, respectively.

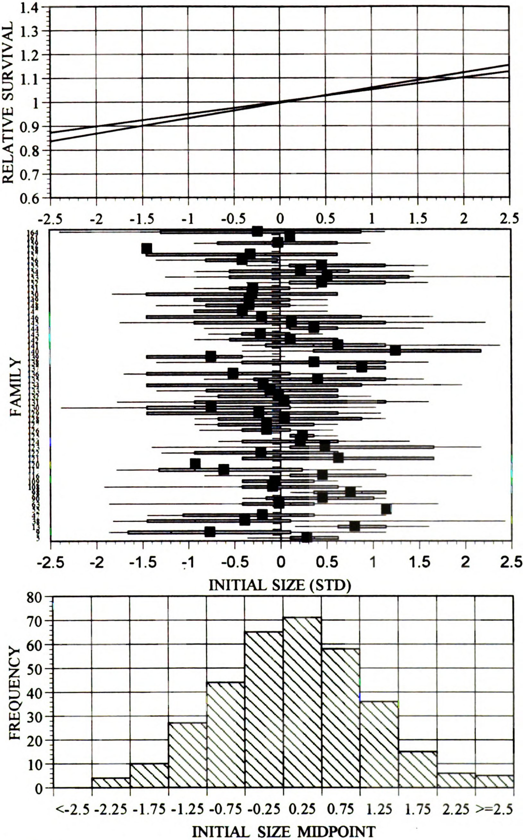

RELATIVE SURVIVAL
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
FAMILY
INITIAL SIZE (STD)
FREQUENCY
80
70
60
50
40
30
20
10
0
<-2.5
-2.25
-1.75
-1.25
-0.75
-0.25
0.25
0.75
1.25
1.75
2.25
>=2.5
INITIAL SIZE MIDPOINT

Figure 28.

Spatial variation in fitness components

In general, there was little significant variation among transects in survivorship or fecundity (Table 17). Transects differed significantly in survivorship only for two episodes in 1992 for both natural and planted seedlings: survival to the onset of winter and survival to spring (natural seedlings only). In contrast, blocks differed significantly in survivorship and fecundity across all episodes for both natural and planted seedlings (Table 17). The one exception to significant spatial variation in survivorship at the scale of blocks were the 1992 planted seedlings, probably due to the limited sample size in that category. The ANOVA models accounted for 3 to 20% of the variation in fitness components when only transect and block were included in the model. For planted seedlings, the inclusion of nested family effects increased the R^2; these models accounted for 20 to 59% of the variance in fitness.

The spatial pattern of variation in survivorship and fecundity among blocks differed across episodes (Figures 29-30BCEF). When contiguous blocks were sampled for all episodes in 1993, survival to establishment was more uniform across adjacent blocks than survivorship in subsequent episodes. Also the variance among blocks increased through subsequent episodes. Coefficients of variation for the grand mean across blocks demonstrated that spring survivorship (CV ranged from 31.4 to 46.0 across years), and fecundity (range=30.5 to 46.2) were much more variable than survivorship through establishment (range=5.5 to 8.0) and survival to the onset of winter (range=12.6 to 29.4). In addition, some blocks showed consistent patterns among years, while others varied across years. For example, spring survival was relatively low in blocks 11 and 12 in both years, however, survival to spring was high in block 1 in 1992, but relatively poor in 1993

Table 17. Nested ANOVA of the spatial variation in survivorship and fecundity for naturally emerging and planted seedlings in 1992 and 1993 at TU Ave for four episodes: 1) survival through establishment, 2) survival to the onset of winter, 3) survival to spring, and 4) final fecundity. Transect, block nested within transect, and family nested within block (planted seedlings only) were treated as random effects and appropriate F-tests were constructed using the Random statement in GLM (SAS Institute, Inc. 1994). Degrees of freedom for the model are for the error term, other degrees of freedom are for the numerator.

Source	Episode									
	Survival to establishment					Survival to onset of winter				
	R^2	df	MS	F	P	R^2	df	MS	F	P
A. Natural 1992										
Model	0.03	1741	0.499	6.24	0.0001	0.07	1593	3.387	15.12	0.0001
Transect		1	1.371	4.69	0.0627		1	20.447	15.49	0.0048
Block (Transect)		7	0.350	4.38	0.0001		7	1.663	7.43	0.0001
B. Planted 1992										
Model	0.21	280	0.131	0.99	0.5166	0.31	225	0.260	1.36	0.0448
Transect		1	0.822	8.03	0.0188		1	2.054	8.69	0.0159
Block (Transect)		7	0.099	0.78	0.6039		7	0.245	1.27	0.2707
Family (Block)		67	0.124	0.93	0.6220		66	0.193	1.01	0.4619
C. Natural 1993										
Model	0.03	2542	0.375	4.31	0.0001	0.04	2289	1.110	5.02	0.0001
Transect		1	0.575	2.07	0.1631		1	0.490	0.56	0.4641
Block (Transect)		18	0.346	3.97	0.0001		18	1.137	5.14	0.0001
D. Planted 1993										
Model	0.20	1104	0.107	1.46	0.0002	0.20	997	0.207	1.35	0.0031
Transect		1	0.000	0.00	0.9700		1	0.975	2.56	0.1260
Block (Transect)		18	0.250	2.58	0.0006		18	0.391	2.44	0.0012
Family(Block)		170	0.102	1.40	0.0011		167	0.161	1.05	0.3338

Table 17 (cont'd).

Source	Episode									
	Survival to spring					Fecundity				
	R^2	df	MS	F	P	R^2	df	MS	F	P
A. Natural 1992										
Model	0.03	954	0.755	3.45	0.0006	0.08	314	104.507	3.38	0.0010
Transect		1	2.009	4.99	0.0448		1	56.792	0.97	0.3395
Block (Transect)		7	0.555	2.54	0.0136		7	101.015	3.27	0.0023
B. Planted 1992										
Model	0.44	140	0.303	1.54	0.0152	0.59	35	32.768	1.23	0.2681
Transect		1	0.022	0.06	0.8165		1	36.344	1.46	0.2456
Block (Transect)		7	0.434	1.63	0.1362		7	22.880	0.66	0.7047
Family (Block)		63	0.291	1.48	0.0287		33	35.569	1.33	0.2013
C. Natural 1993										
Model	0.06	1474	1.105	4.98	0.0001	0.09	533	236.631	2.64	0.0002
Transect		1	1.269	1.62	0.2155		1	105.935	0.64	0.4302
Block (Transect)		18	1.099	4.95	0.0001		18	248.159	2.77	0.0001
D. Planted 1993										
Model	0.26	759	0.283	1.47	0.0003	0.53	156	82.087	1.39	0.0239
Transect		1	0.885	0.99	0.3317		1	5.722	0.04	0.8330
Block (Transect)		18	0.928	4.42	0.0001		18	136.912	1.94	0.0164
Family (Block)		164	0.213	1.10	0.1976		108	73.801	1.25	0.0977

(Figures 29-30BCEF). Although the transects ran parallel to each other, there did not appear to be any association between paired distances along the transects.

Spatial variation in phenotypic traits

Traits did not differ significantly among transects with one exception (Table 18). Only planted seedlings in 1993 displayed significant variation in phenotypic traits among transects, largely due to differences in emergence week. In contrast, phenotypic traits differed significantly among blocks within transect (Table 18). Univariate ANOVA's suggested that fall size varied significantly among blocks for both natural and planted seedlings in both years (Figures 29-30AD), while the significance of other traits varied among years (Table 18).

Spatial variation in phenotypic selection

Both linear selection differentials and gradients, measures of phenotypic selection, showed significant variation among transects (Figures 31-32). Ninety-five percent confidence intervals did not overlap for both the linear differential and gradient for fall size in the natural seedlings in 1993 and for the linear differential for emergence week in the planted seedlings in 1992. Other traits showed little overlap in confidence intervals: 1992 linear differentials for emergence week and fall size and 1993 linear differential on initial size. In this analysis 95% confidence intervals were based on limited resampling (n=250) hindering my ability to detect spatial variation. Despite this limitation, I detected spatial variation in the linear components of phenotypic selection across transects.

Figure 29. The spatial scale of phenotypic and demographic variation for natural (A, B, C) and planted seedlings (D,E,F) in 1992. Block means (± 1 standard error) for phenotypic variation in seed weight (★), emergence week (■), initial size (●), and fall size (▲) (A, D), survivorship through three episodes, survival to establishment (■), survival to the onset of winter (●), survival to spring (▲) (B, E), and fecundity in the final episode (✖) (C, F). The grand mean across blocks is depicted by a line for each episode of survival or reproduction. Blocks are located on forest edge and interior.

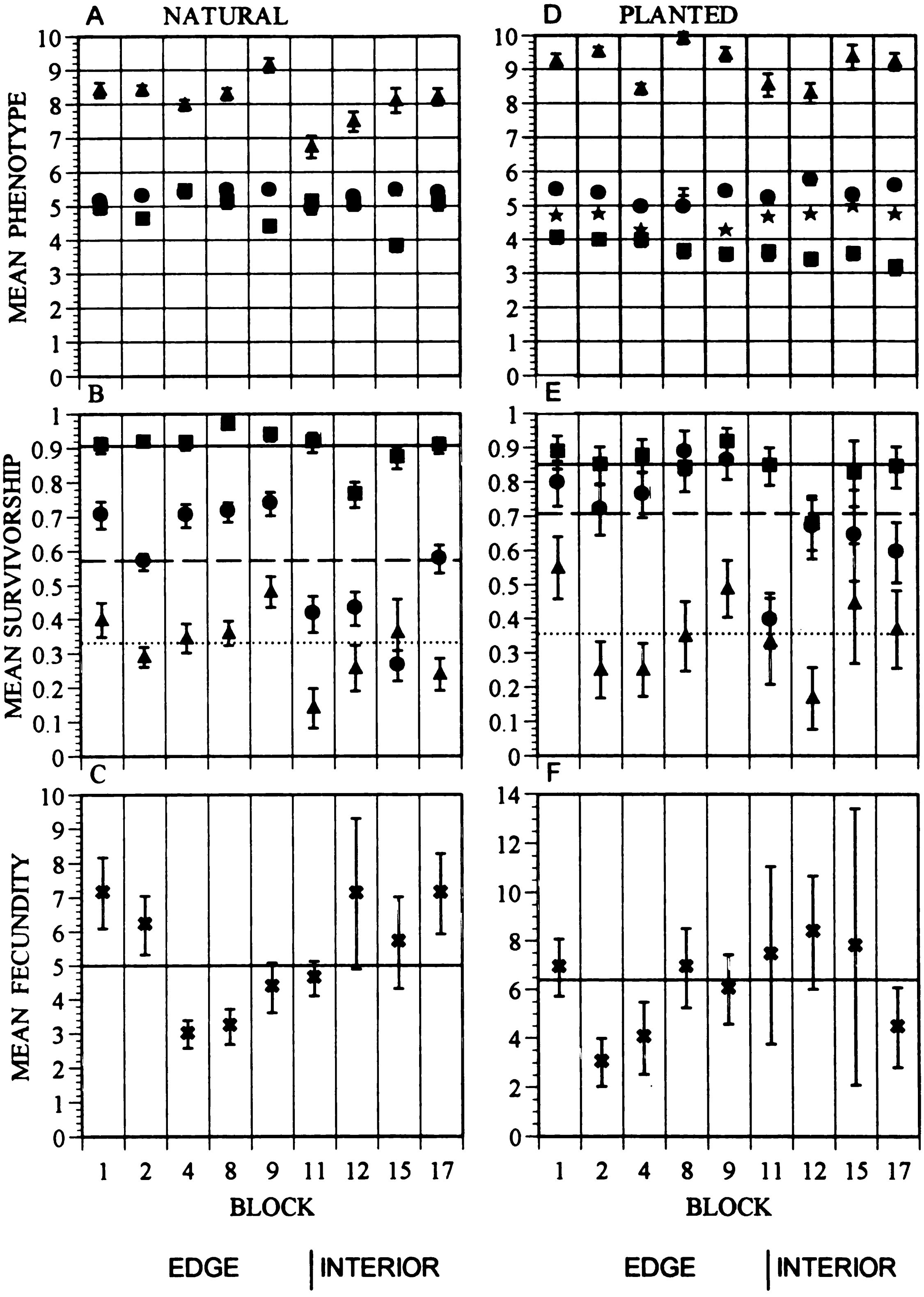

A NATURAL
D PLANTED
B
E
C
F
MEAN PHENOTYPE
MEAN SURVIVORSHIP
MEAN FECUNDITY
0
1
2
3
4
5
6
7
8
9
10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
12
14
1 2 4 8 9 11 12 15 17
BLOCK
EDGE
INTERIOR

Figure 29.

Figure 30. The spatial scale of phenotypic and demographic variation for natural (A, B, C) and planted seedlings (D,E,F) in 1993. Block means (± 1 standard error) for phenotypic variation in seed weight (★), emergence week (■), initial size (●), and fall size (▲) (A, D), survivorship through three episodes, survival to establishment (■), survival to the onset of winter (●), survival to spring (▲) (B, E), and fecundity in the final episode (✖) (C, F). The grand mean across blocks is depicted by a line for each episode of survival or reproduction. Blocks are located on forest edge and interior.

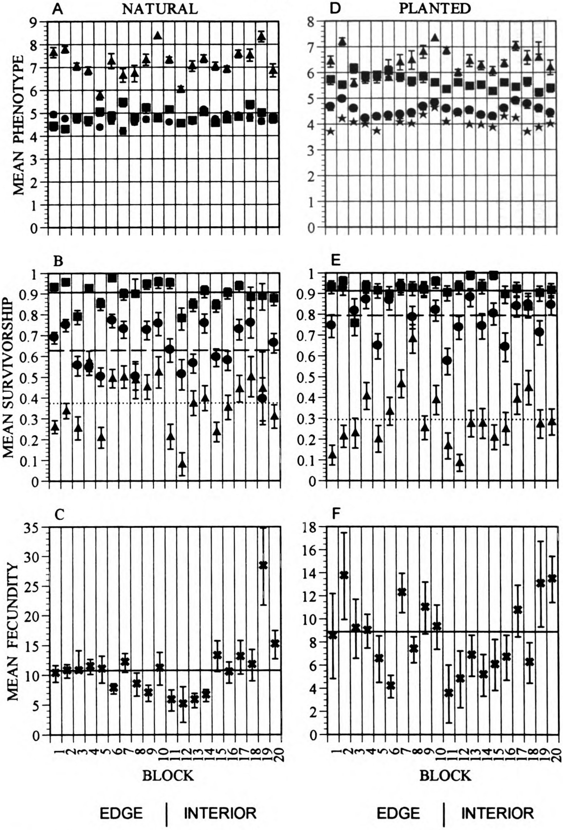

A
NATURAL
D
PLANTED
MEAN PHENOTYPE
B
E
MEAN SURVIVORSHIP
C
F
MEAN FECUNDITY
BLOCK
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
EDGE
INTERIOR

Figure 30.

Table 18. Multivariate nested analysis of variance of the spatial variation in phenotypic traits for naturally emerging and planted seedlings in 1992 and 1993 at TU Ave. The spatial scale of variation was assessed for transects (n=2), blocks within transects (n=4,3 in 1992 and n=10 in 1993), and families within blocks (planted seedlings only, n=77 and 191 in 1192 and 1993, respectvely). All source variables were treated as random effects with appropriate F-tests specified in the MANOVA statement in GLM (SAS Institute, Inc. 1994). Three traits, emergence week, initial size, and fall size were analyzed as dependent variables for naturally emerging seedlings. Four traits including seed weight were treated as dependent variables for planted seedlings. Type III sum of squares were utilized for all analyses. Univariate probabilites are presented for each trait for comparative purposes only.

	MULTIVARIATE					UNIVARIATE			
Source	Wilks' λ	F	Numerator df	Denominator df	P	Seed Weight	Emergence Week	Initial Size	Fall Size
A. Natural 1992									
Transect	0.547	1.38	3	5	0.3504		0.8172	0.0635	0.8181
Block (Transect)	0.930	3.31	21	2734.2	0.0001		0.0001	0.0001	0.0001
B. Planted 1992									
Transect	0.390	1.56	4	4	0.3377	0.2817	0.6559	0.3384	0.6069
Block (Transect)	0.607	1.15	28	217.7	0.2793	0.2490	0.4090	0.5756	0.0378
Family (Block)	0.142	1.37	252	550	0.0012	0.0005	0.0131	0.1777	0.0311
C. Natural 1993									
Transect	0.651	2.85	3	16	0.0699		0.5794	0.4437	0.0343
Block (Transect)	0.829	5.27	54	4386.8	0.0001		0.0015	0.3035	0.0001
D. Planted 1993									
Transect	0.335	7.45	4	15	0.0016	0.8872	0.0010	0.6025	0.7099
Block (Transect)	0.395	2.35	72	635.4	0.0001	0.5087	0.0388	0.0001	0.0001
Family (Block)	0.287	1.69	656	3026.3	0.0001	0.0001	0.0001	0.0560	0.0001

Phenotypic selection also varied across blocks (Figure 33-34). Non-overlapping confidence intervals in the linear differential for initial size in the planted seedlings and for emergence week and initial size in natural seedlings and for the linear gradient for emergence week in the natural seedlings all indicated spatial variability (Figure 34). Other traits showed minimal overlap in confidence intervals i.e. linear differential for fall size (Figure 34E). Despite the very limited resampling effort (n=50), selection varied at a scale of 10 meters for some traits i.e. emergence week and initial size. In contrast, selection on fall size appeared more consistent across larger spatial scales. The spatial scale of variation in phenotypic selection also differed among episodes.

Opportunity for maternal selection

For maternal families planted locally in both years, the variance in relative fitness at the family level indicates the opportunity for maternal selection is greatest in two episodes: spring survival and fecundity (Figure 25). The total variance across episodes is greater for locally planted families than for globally planted families. This difference between families planted at these two spatial scales may indicative of the extent to which spatial variation in biotic and abiotic factors may affect phenotypic differences among families either in traits, in components of fitness, or in phenotypic selection. This comparison is compromised by the different numbers of families considered at these two scales, but the pattern suggests that when families experience local conditions only, the among family variance in relative fitness is greater.

Figure 31. Spatial variation in phenotypic selection between transects along forest edge (1) and interior (2) for natural and experimentally planted seedlings in 1992. The linear selection differentials (A, C) and linear selection gradients (B, D) for three traits, emergence week, initial size, and fall size, in each viability and fecundity episode. Codes for each episode as in Figure 25. Total magnitude of selection across all episodes is depicted by (●) and is based on reconstructed phenotypic variance-covariance matrix (see Chapter 2). 95% confidence intervals for total values are also depicted by (●) and are based on 250 resampled data sets for each transect. Natural seedlings along transect 2 in 1992 have confidence intervals that exceed the upper and lower axis values.

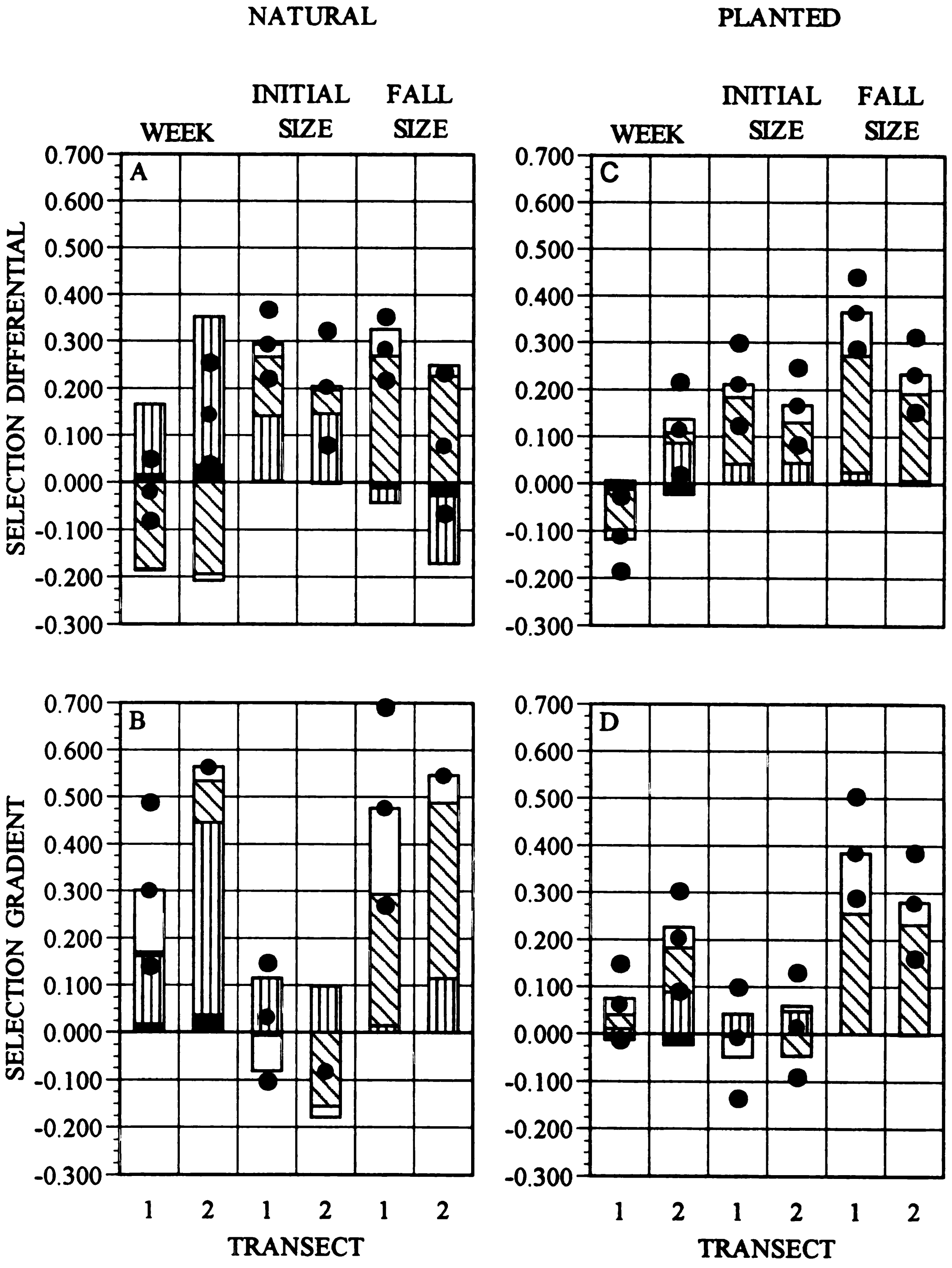
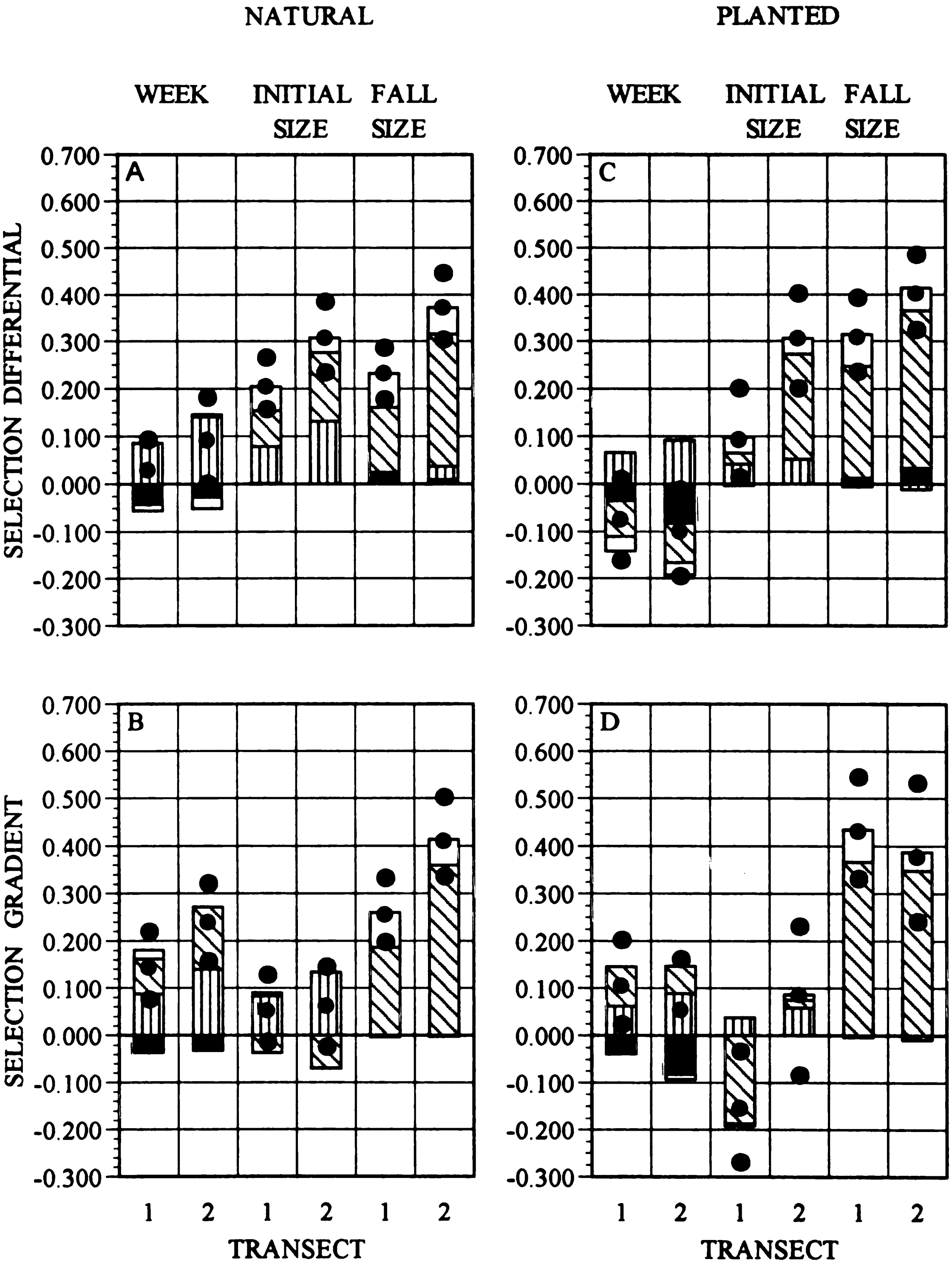

NATURAL
PLANTED
WEEK
INITIAL SIZE
FALL SIZE
SELECTION DIFFERENTIAL
SELECTION GRADIENT
A
B
C
D
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
-0.100
-0.200
-0.300
1
2
TRANSECT

Figure 31.

Figure 32. Spatial variation in phenotypic selection between transects along forest edge (1) and interior (2) for natural and experimentally planted seedlings in 1993. The linear selection differentials (A, C) and linear selection gradients (B, D) for three traits, emergence week, initial size, and fall size, in each viability and fecundity episode. Codes for each episode as in Figure 25. Total magnitude of selection across all episodes is depicted by (●) and is based on reconstructed phenotypic variance-covariance matrix (see Chapter 2). 95% confidence intervals for total values are also depicted by (●) and are based on 250 resampled data sets for each transect.


NATURAL
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WEEK
INITIAL SIZE
FALL SIZE
A
B
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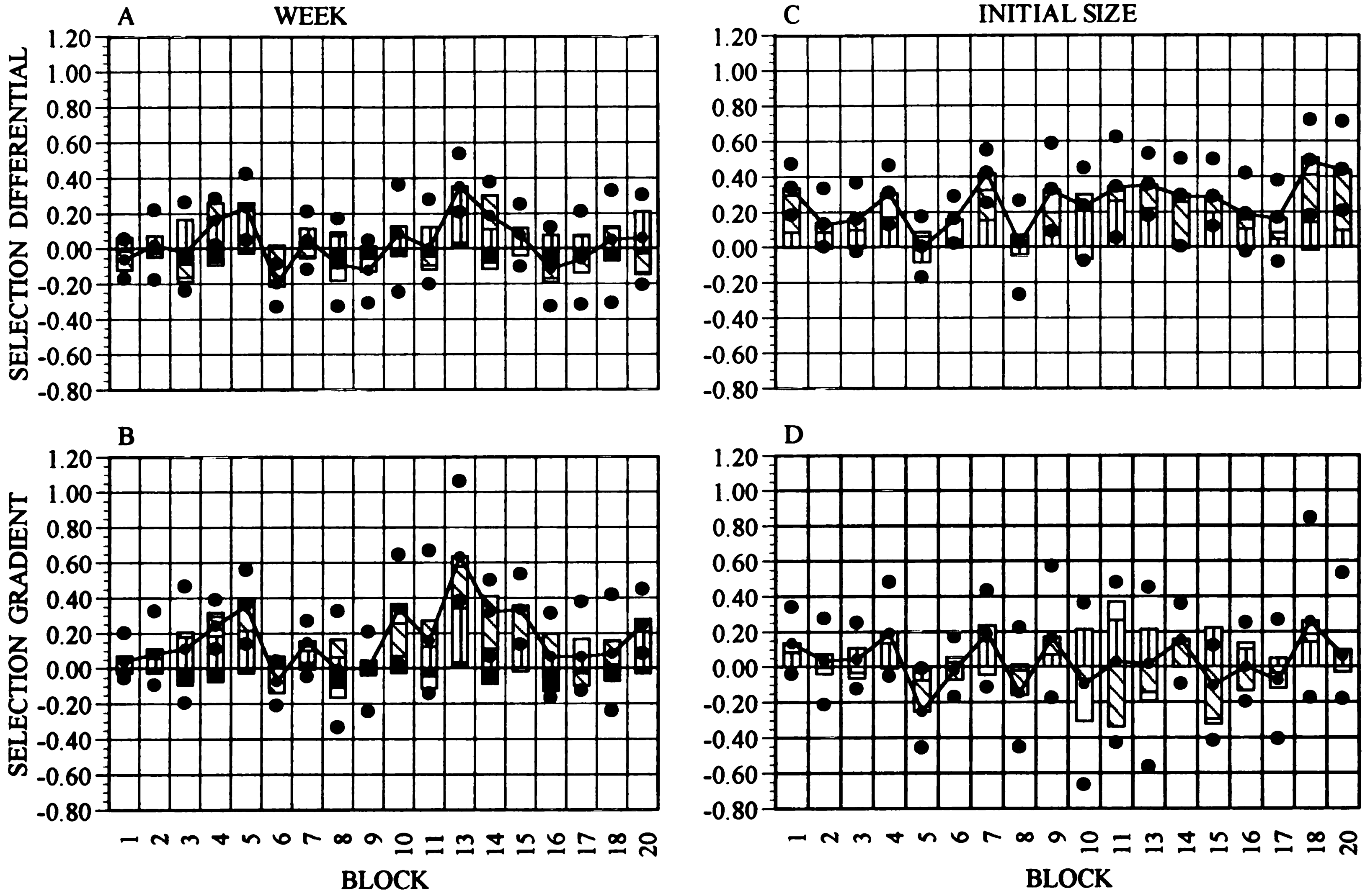
D
SELECTION DIFFERENTIAL
SELECTION GRADIENT
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
-0.100
-0.200
-0.300
1
2
TRANSECT

Figure 32.

Figure 33. Spatial variability in phenotypic selection among blocks along the edge (1-10) and interior (11-20) transects for natural seedlings in 1993. Linear selection differentials (A,B,C) and linear selection gradients (D,E,F) in each viability and fecundity episode for three traits, emergence week, initial size, and fall size are shown. The total magnitude of selection across all episodes is depicted by (●) and is based on reconstructed phenotypic variance-covariance matrix (see Chapter 2). 95% confidence intervals for total values are based on 50 resampled data sets for each block. Total values are connected by a line for visual clarity.


A
WEEK
C
INITIAL SIZE
B
D
SELECTION DIFFERENTIAL
SELECTION GRADIENT
1.20
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.80
1
2
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BLOCK

Figure 33.

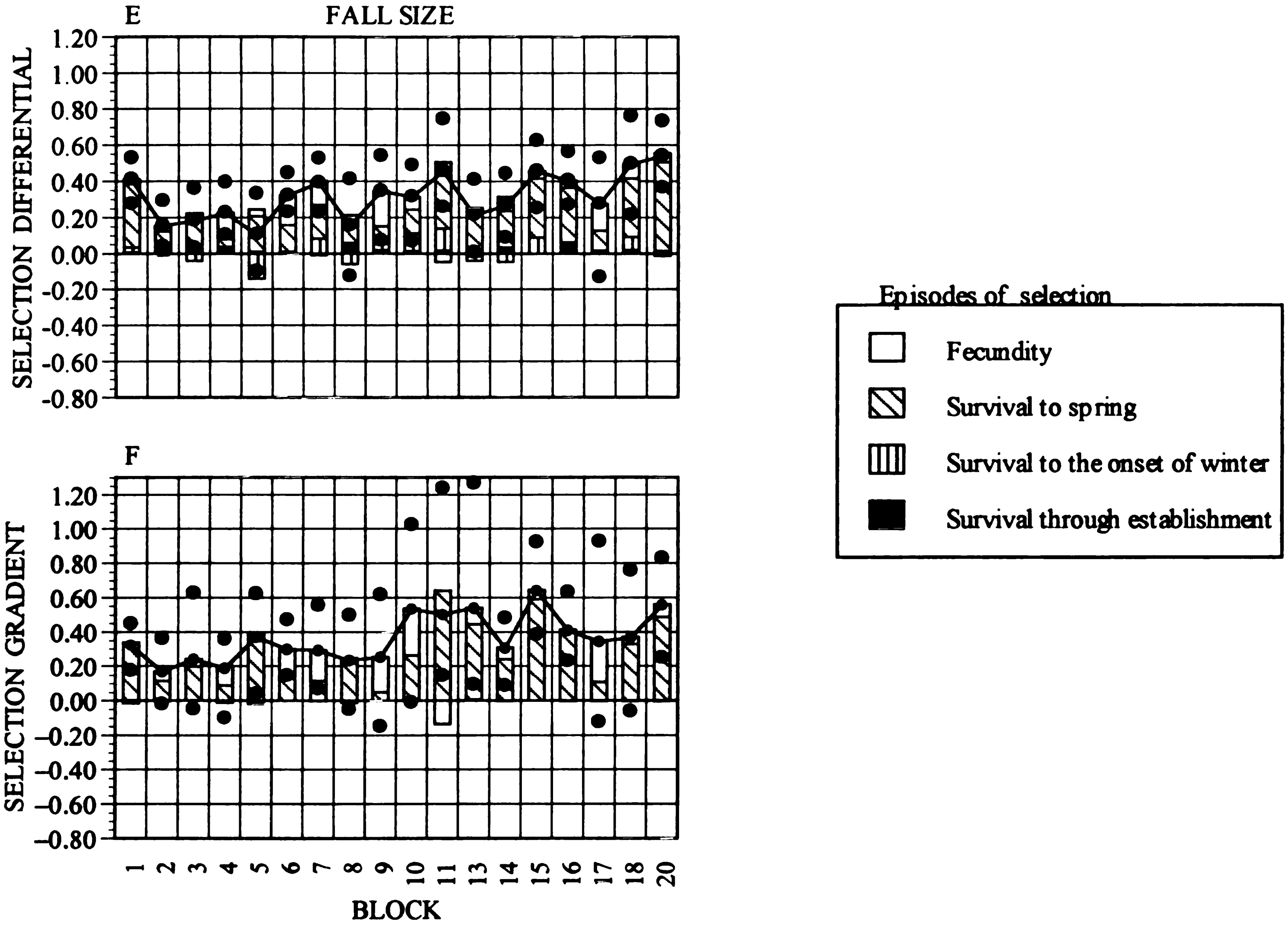

Figure 33 (cont'd).

Figure 34. Spatial variability in phenotypic selection among blocks along the edge (1-10) and interior (11-20) transects for experimental seedlings in 1993. Linear selection differentials (A,B,C) and linear selection gradients (D,E,F) in each viability and fecundity episode for three traits, emergence week, initial size, and fall size are shown. The total magnitude of selection across all episodes is depicted by (●) and is based on reconstructed phenotypic variance-covariance matrix (see Chapter 2). 95% confidence intervals for total values are based on 50 resampled data sets for each block. Total values are connected by a line for visual clarity. Confidence intervals exceed the upper or lower axis values in five cases in blocks 3 and 12.

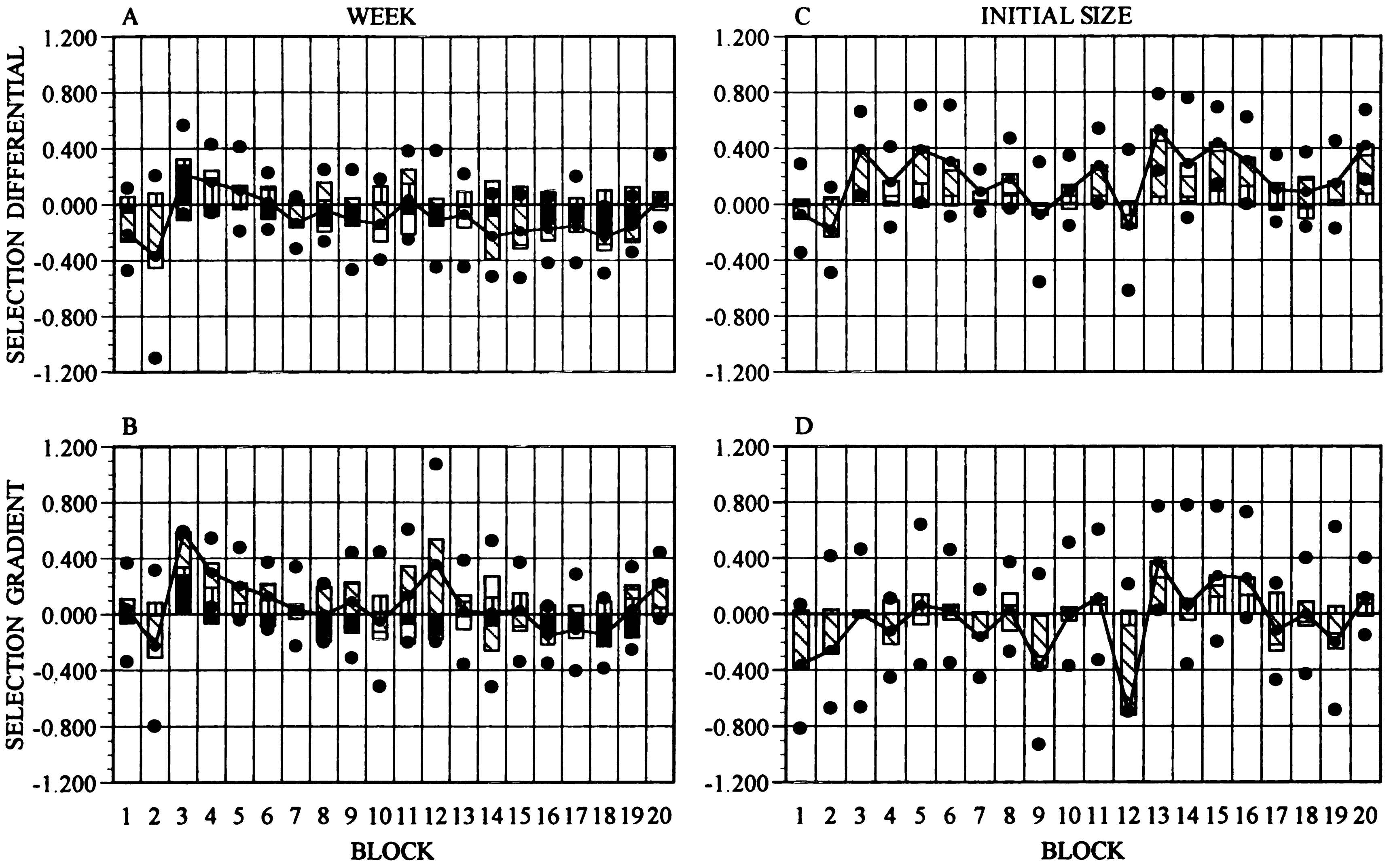
A
WEEK
C
INITIAL SIZE
B
D
SELECTION DIFFERENTIAL
SELECTION GRADIENT
1.200
0.800
0.400
0.000
-0.400
-0.800
-1.200
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
BLOCK

Figure 34.

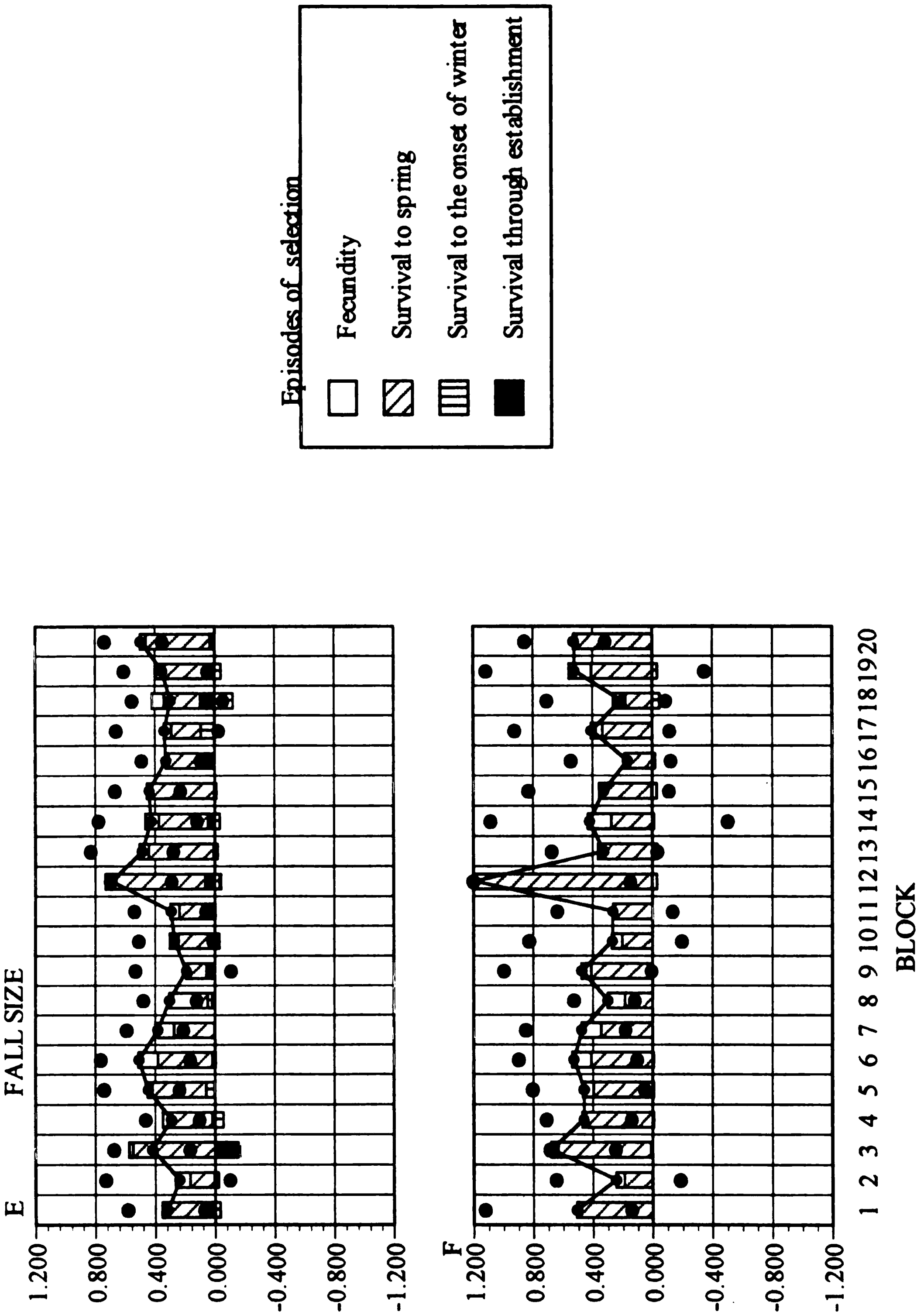

Figure 34 (cont'd).

Local family effects

In most cases spatial variation among blocks within transects accounted for the observed variation in survival and fecundity (Table 17BD). In three cases, however, differences among local families accounted for variation in survival to establishment and in final fecundity (marginally significant) in 1993 and in survival to spring in 1992. In contrast, a multivariate ANOVA demonstrated that in addition to significant variation among blocks in 1993 and no significant spatial variation in 1992, local families differed in their phenotypic attributes in both years (Table 18BD). The univariate analyses suggested that all traits except initial size differed among families.

Does family membership account for any of the observed variation in individual fitness on a local scale? The inclusion of family in an ANCOVA including all phenotypic traits in a given episode demonstrated significant family effects in 10 out of 17 models significantly explaining variation in individual relative fitness in both years (out of a possible number of 106 separate regression models). Limited sample sizes per block were not sufficient for these statistical descriptions of selection. In more complicated contextual models where a family effect was nested within blocks, family contributed significantly to the variance in survival to establishment and final fecundity in 1993 and to spring survivorship in 1992 (Table 19).

Table 19. Nested analysis of covariance to examine maternal selection on individual fitness while accounting for spatial variation in survivorship for planted seedlings in 1992 and 1993 at TU Ave for four episodes: 1) survival through establishment, 2) survival to the onset of winter, 3) survival to spring, and 4) final fecundity. Type III sum of squares were utilized for significance tests.

Source	Episode									
	Survival to establishment					Survival to onset of winter				
	R^2	df	MS	F	P	R^2	df	MS	F	P
A. Planted 1992										
Model	0.22	279	0.190	1.02	0.4375	0.33	223	0.538	1.43	0.0025
Block		8	0.359	1.93	0.0556		8	1.042	2.78	0.0060
Family(Block)		67	0.174	0.94	0.6168		66	0.386	1.03	0.4271
Emergence		1	0.596	3.20	0.0747		1	0.627	1.67	0.1972
Week										
Initial Size							1	1.638	4.37	0.0378
Total		355					299			
B. Planted 1993										
Model	0.22	1103	0.142	1.68	0.0001	0.23	995	0.375	1.61	0.0001
Block		19	0.249	2.94	0.0001		19	0.599	2.57	0.0003
Family (Block)		170	0.117	1.38	0.0018		167	0.240	1.03	0.3900
Emergence		1	2.885	34.14	0.0001		1	8.583	36.80	0.0001
Week										
Initial Size							1	0.779	3.34	0.0678
Total		1293					1183			

Table 19. (cont'd).

Source	Episode									
	Survival to spring					Fecundity				
	R^2	df	MS	F	P	R^2	df	MS	F	P
A. Planted 1992										
Model	0.50	137	2.502	1.84	0.0011	0.63	32	0.929	1.25	0.2554
Block		8	2.376	1.75	0.0933		8	0.775	1.04	0.4246
Family (Block)		63	2.067	1.52	0.0225		33	0.968	1.30	0.2269
Emergence Week		1	1.810	1.33	0.2509		1	1.536	2.07	0.1599
Initial Size		1	0.471	0.35	0.5576		1	1.730	2.33	0.1367
Fall Size		1	5.124	3.76	0.0545		1	1.694	2.28	0.1406
Total		211					76			
B. Planted 1993										
Model	0.32	756	3.772	1.88	0.0001	0.62	153	1.199	1.89	0.0001
Block		19	9.664	4.88	0.0001		19	1.563	2.46	0.0013
Family (Block)		164	2.176	1.10	0.2074		108	0.966	1.52	0.0084
Initial Size		1	3.720	1.88	0.1707		1	1.426	2.25	0.1359
Fall Size		1	1.230	0.62	0.4307		1	1.129	1.78	0.1842
Total		1	83.030	41.96	0.0001		1	7.341	11.57	0.0009

DISCUSSION

In this population of *C. verna* the variance among maternal families in mean relative fitness of individual family members relative to the family mean in the population demonstrates that the prerequisite for maternal selection is met both at the local and global scales. Furthermore, the decline in number of families through the episodes of selection (from 56 to 43 for global families and from 77 to 43 in 1992 and 191 to 128 in 1993 for local families) indicates that there is differential extinction and proliferation of maternal family groups. The examination the relationship between family membership and fitness components at two spatial scales indicates that 1) variation in absolute survivorship, 2) variation in phenotypic traits, 3) variation in the relationship between phenotype and fitness and 4) spatial variation in 1-3 all contribute to among family variance in this natural population.

Maternal selection on a global scale

For globally planted families experiencing the average in environmental conditions, the variance among families does not appear to be influenced by differences in absolute survivorship or fecundity (Table 14). However, significant differences in the multivariate phenotype among global families could contribute to the opportunity for maternal selection. These familial phenotypic differences are consistent with the evidence for maternal inheritance of these traits described in Chapter 1.

Two lines of evidence also suggest that the opportunity for maternal selection is influenced by variation in phenotypic selection among families: 1) families differed in their fitness functions, i.e. slopes were heterogeneous in ANCOVA, or 2) families perceived selection similarly (slopes were homogeneous), but differed in relative fitness for other reasons i.e. significant family effects in ANCOVA excluding non-significant interaction term. In the univariate selection analyses for global families, slopes were significantly heterogeneous for two traits, emergence week on the survival to establishment and seed weight on survival to the onset of winter (Figure 26, $P<0.0548$) (Table 15). In later episodes this ANCOVA approach is compromised by few observations per family. In general, the significance of this interaction would be best evaluated by many observations within families. In the absence of heterogeneity of phenotypic selection among families, one trait, initial size displayed marginally significant family effects on individual survival to the onset of winter (Figure 27, Table 15).

The description of phenotypic selection for global families is limited by the small number of individuals observed. This effect of this small sample size is evident in the comparison of significance of selection coefficients between all planted seedlings (Chapter 2, Table 18) and global seedlings only (Table 15). When only global seedlings are included, the selection coefficients are similar in magnitude but lack significance in a number of episodes. As a result of this statistical limitation, the detection of family effects is also limited. However, in spite of these limitations both types of family effects are evident. These two types of family effects indicate the potential for maternal selection. The amount of variation accounted for by family effects represents the maximum amount of variance that any given maternal family attribute *may* contribute to the model (see Heisler and Damuth 1987). This maternal family attribute is a property of the family group and could include, for example, the family mean phenotype, a specific maternal trait, or an emergent property of the family group. Contextual analysis separates individual from group effects on fitness by including both individual traits and group attributes (Heisler and Damuth, 1987; Goodnight et al. 1992; Stevens et al. 1995). In contextual models, therefore, one could compare selection at the individual and group level. For example, one could evaluate whether group selection favored an attribute that was not favored by individual selection, a common assumption of theoretical models for the evolution of altruism (Breden 1990). Furthermore, one could determine whether individual selection indirectly generates selection at the group level or vice versa (Goodnight et al. 1992). In this study maternal family attributes were not included in univariate selection models, so the nature of selection at different levels can not be evaluated. The significance of this study is that it indicates the *potential* for maternal selection on seed weight, emergence week and initial size in the early viability selection episodes in a natural population. Measures of maternal phenotype and larger sample sizes would allow contextual analysis of phenotypic selection.

Maternal selection on a local scale

Spatial variation in biotic and abiotic factors can also affect the opportunity for maternal selection. The opportunity of maternal selection is greater for locally planted seedlings relative to globally planted seedlings indicating that spatial variation may contribute to among family variance. This comparison is based on different numbers of families between groups which could bias the variance in either direction (Figure 25). However, the evidence for spatial variation in absolute survivorship (Table 17, Figures 29-30BCEF), in the multivariate phenotype (Table 18, Figures 29-30AD), and in phenotypic selection (Figures 31-34) especially at the block scale supports the conclusion that spatial variation contributes to the opportunity for maternal selection. In addition, the spatial pattern of absolute survivorship among the episodes suggests that biotic and abiotic factors acting as agents of selection in different episodes operate at different spatial scales (Figures 31-32).

To evaluate the contribution of family effects on individual relative fitness, I accounted for this spatial variation in two ways. First, I analyzed multivariate phenotypic selection by block including a family effect in an ANCOVA model. This analysis involved a large number of regression models for each episode and each block (n=106 models). As in the analysis of global seedlings, few individuals in each block limited these statistical descriptions of selection. In the 17 significant models, 10 showed significant family effects. This result suggests the potential for maternal selection in these blocks during certain episodes. If I corrected for the large number of regression models tested by adjusting for table-wide significance (Rice 1989), however, this evidence for the potential for maternal selection is no longer significant. Second, in a single ANCOVA I examined differences in individual fitness among blocks and families within blocks by accounting for the average multivariate fitness function across the whole population in each episode (Table 19). In these models the statistical description of selection was more robust because it was based on larger sample sizes. Significant effects of block and family within block indicated the *potential* for selection at two hierarchical scales, among blocks and among families within blocks. In contrast to ANCOVAs by block, these models do not allow for spatial variation in phenotypic selection. Rather, they demonstrate that when phenotypic selection is homogeneous across the population, blocks and families within them differed in relative fitness.

In their contextual analysis of individual size in *Impatiens*, Stevens et al. (1995) found evidence for group selection operating among patches distributed over similar spatial scales as the block in this study. Selection coefficients on individual size and mean size of the group differed in sign indicating opposition across levels of selection. Kelly (1996) experimentally manipulated plant architecture to demonstrate that interactions among near neighbors can have fitness consequences on target individuals in *Impatiens*. His description of this interaction as kin selection depends on his assumption that interacting individuals were relatives. In this study interaction among related offspring was minimized because offspring were separated by a minimum distance of 8 cm when seeds were planted. Therefore, evidence for kin selection is most likely to due to mother-offspring interactions, not sibling interactions after germination.

Studies of spatial variation in individual phenotypic selection have demonstrated significant variation in selection over similar spatial scales (Kalisz 1986; Scheiner 1989; Kelly 1992; Stratton 1992a). The spatial scale of variation in phenotypic selection relative to gene flow and the strength of selection interact to determine the rate and scale of local adaptive evolution. Local adaptation is a common feature in many natural plant populations (e.g. Bennington and McGraw 1995b). Differences in phenotypic selection can produce locally adapted phenotypes over very short spatial scales (Antonovics et al.1971). In this study there is very little evidence for local adaptation because home and away families did not differ significantly in any fitness component. Nevertheless, spatial variation in selection can be a potent force for maintaining genetic variation in populations (Haldane and Jaykar 1963; Barton and Turelli 1987).

Inheritance of group traits

It is possible that response to selection of group attributes may be a function of indirect selection on correlated traits at the individual level that are heritable (Goodnight 1990 a,b). One interesting feature of maternal selection, a type of kin selection , however is the possibility that maternal inheritance (Chapter 1) may provide a mechanistic model for the inheritance of group attributes. While Cheverud (1984) has demonstrated how genetic covariances can affect the evolution of altruistic interactions between mothers and their offspring and produce unusual evolutionary responses, it is also possible that in the absence of pleiotropy the heritability of a maternal attribute with direct effects on offspring fitness could allow response to selection at the maternal family level. For example, genetically based variation among mothers in provisioning could cause differential survival among maternal families. The selection differential on this maternal attribute mediated by offspring survival will determine the mean provisioning value among mothers in the next generation. Genetic covariances among this provisioning trait and offspring traits could constrain or enhance this selection response (see Chapter 1). Thus, maternal inheritance can provide an alternative mechanism for the inheritance of group attributes in a maternal selection model. Understanding the interplay between maternal inheritance and maternal selection and their influence on multivariate evolution would provide a unique view of the role of maternal effects on levels of selection.

Conclusions

In plant populations maternal family groups are spatially structured as a result of limited seed dispersal. Differences in the relative survival or fecundity of individual offspring in these maternal family groups creates the opportunity for selection at two hierarchical levels: among individuals and among maternal families. This study clearly demonstrates the opportunity for maternal selection at two spatial scales. A number of factors contribute to the among maternal family variance in relative fitness. Maternal families vary phenotypically. This phenotypic variation is likely to be due both to maternal inheritance of juvenile traits and to spatial variability in the environment. Furthermore, families vary in survival and fecundity. Variation in relative individual survival and fecundity can be attributed both to phenotypic attributes of the individuals, to family membership, and to spatial location (i.e. block). The magnitude of variation attributed to family or block represents the maximum amount of variance that any specific maternal attribute or group attribute may explain indicating the potential for selection at these hierarchical levels. Furthermore, maternal inheritance of these traits (Chapter 1) provides a mechanism for the inheritance of group level effects.

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