THE EVOLUTION OF PHENOTYPIC PLASTICITY IN A NATURAL WILDFLOWER POPULATION

By

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ABSTRACT

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When the environment is heterogeneous, factors that influence whether genetic specialization or phenotypically plastic generalists are favored include the patterns of natural selection, the amount of genetic variation for trait plasticity, and the degree of genetic interdependence between traits. In this study, I investigated the genetics and fitness consequences of responses to variable light and litter environments in a population of the winter annual wildflower *Collinsia verna*. Over two generations, I measured natural light environments, and manipulated light and litter environments. I used quantitative genetic breeding designs to investigate genetic and environmental effects on traits within generations, and the effects of maternal environment and genotype on offspring performance across generations.

Natural light environments were variable at a scale appropriate to favor plasticity, but correlated across years in a way that might favor the evolution of plastic maternal effects. There were maternal genotype-environment interactions for seed size and dormancy. Maternal effects sometimes improved offspring performance, but also appeared to constrain offspring performance when mothers were stressed.

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length, mean seed mass, and reproductive investment; and strong evidence for genotype-environment interactions (genetic variation for plasticity) for flowering date, specific leaf area, mainstem length, and reproductive investment. There were no strong light environment specialists among the genotypes sampled. However, significant maternal effects on vegetative biomass, seed number, and seed mass without additive genetic variation suggested that maternal genotypes may specialize for different reproductive strategies.

The presence of leaf litter reversed the direction of direct linear selection on emergence date and increased the size of direct linear selection on vegetative biomass. Full sun reversed the direction of direct linear selection on specific leaf area, and increased the size of direct linear selection on reproductive investment. These differences in patterns of selection provide evidence that leaf litter and light are selective agents on these traits.

Together, the parts of this study suggest that genetic variation for emergence date and plastic maternal genetic effects on seed size and dormancy may be maintained by a heterogeneous and unpredictable leaf litter environment. In contrast, the plasticity of the light sensitive traits flowering date and specific leaf area may be at or near optimal levels. Surprisingly, reproductive investment was both heritable and under strong directional selection. Significant genotype-environment interactions appear to maintain genetic variation in this trait.

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I thank all the Kellogg Biological Station graduate students past and present. Their friendship helped make KBS a stimulating and enjoyable place to live and work. Special thanks go to Denise Thiede. Her support, guidance, and encouragement throughout my time at KBS have been invaluable. I especially thank her for advice in experimental design and analysis. I have benefitted greatly from her mastery of SAS programming. In particular, she supplied the files and information necessary to jump-start my genetic analysis, and a SAS bootstrapping macro.

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Sarah Black, Tara Darcy, Becky Fuller, Kari Gorentz, Christy Lynn, Rob Olendorf, and Brent Pav, contributed their time and talents in the field, often in conditions that were miserable at best. Tara Darcy, Christy Lynn, Brent Pav, Carolyn Hornyak, and more volunteers than I can recall provided expert assistance in the lab. Jeff Dudycha, Kay Gross, and Kevin Kosola provided useful comments on earlier versions of these chapters. I thank the Balkema family for permission to conduct this work on their property.

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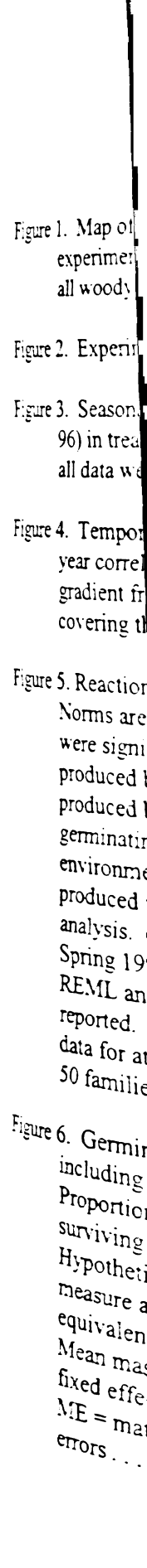

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Chapter 1

INTRODUCTION: MICRO-EVOLUTIONARY CONSEQUENCES OF ENVIRONMENTAL HETEROGENEITY

What are the micro-evolutionary consequences if environmental heterogeneity results in patterns of natural selection that vary in space or time? This question has been a recurring theme in evolutionary biology for at least 50 years, and continues to drive much theoretical and empirical research in the field. By the 1950's, two basic hypotheses were outlined. First, environmental heterogeneity may contribute to the maintenance of genetic variation at multiple scales, including individual level heterozygosity, population level allelic variation, local adaptation within and between populations, and population differentiation (review in Roff 1997, Chapter 9). Second, it was suggested that environmental heterogeneity could lead to the evolution of plastic responses to the environment that would allow individuals to maintain fitness homeostasis (review in Roff 1997, Chapter 6).

Theory developed quickly in the first area, and supported the hypothesis that environmental variation could maintain genetic variation at the individual, population, and metapopulation scales (e.g. Levene 1953, Dempster 1955, Levins 1968, Gillespie 1974, Felsenstein 1976; reviews in Hedrick et al. 1976, Hedrick 1986). In contrast, the theoretical and empirical study of the evolution of phenotypic plasticity progressed slowly until the 1980's (review in Schlichting and Pigliucci 1998). The concept of the reaction norm quantifies plasticity by describing the relationship between phenotype and environment. The idea was proposed by Woltereck in 1909 (discussed in Schlichting and

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Pigliucci 1998), and initially promoted by Schmalhausen (1949). Falconer (1952) developed an alternative approach that viewed a trait expressed in two environments as two genetically correlated traits. Lewontin (1957) argued that variable environments should lead to generalist genotypes that can maintain homeostasis (meaning fitness) across a range of environmental conditions. Lewontin's use of homeostasis is analogous to the term generalist as it has most often been used in more recent discussions of phenotypic plasticity. He argued that adaptive homeostasis implies only the relative constancy of survival and reproduction in variable environments. He pointed out that it is impossible to predict the relationship between homeostasis in this sense and the variability or canalization of physiological and morphological characters. Variability (plasticity) in some morphological and physiological characters can allow others to be canalized. Bradshaw (1965) recognized that phenotypic plasticity is specific for individual traits in relation to particular environmental factors, that the plasticity of a trait is specific in pattern and direction, and that phenotypic plasticity is under genetic control.

MODELS: PLASTICITY EVOLUTION

A variety of terms have been used in discussing phenotypic plasticity. Plasticity has been associated with adaptability, ecological breadth, environmental stability, canalization, homeostasis, environmental resistance, environmental tolerance, environmental sensitivity, specialization, and generalization. The terms generalist and specialist are most common, and usually describe the relative ability to maintain fitness in variable environments through coordinated, plastic responses in underlying traits. Most often, generalists are considered to have adaptive plasticity in physiological and morphological traits that allows them to tolerate unfavorable environments and to boost

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performance when conditions are favorable (e.g. de Jong 1990, van Tienderen 1991, 1997). Generalists have higher average fitness across environments, which is usually manifest in less plastic reaction norms for fitness. Specialists on good environments lack the underlying plasticity in physiological, morphological, and behavioral traits necessary to maintain high relative fitness in variable environments. Consequently, they have more plastic reaction norms for fitness. Specialists on stressful environments often lack the ability to respond when resources are abundant, and so have low, flat reaction norms for fitness. However, there are conflicting usages of the term generalist that can be quite confusing. For example, a recent model by Scheiner (1998) distinguishes three possible evolutionary outcomes in a spatially-structured environment: fixed environmental specialist genotypes, adaptively plastic genotypes, or fixed generalist genotypes. Given these continuing ambiguities, it is important in empirical studies to be explicit about which traits are plastic, in response to which environmental factors with what fitness consequences (Bradshaw 1965).

Types of models

Models of plasticity evolution have used four different approaches (optimality, quantitative genetic, gametic, and genetic algorithms) to address when adaptive plasticity could evolve (reviews: Scheiner 1993, Roff 1997 Chapter 6, Schlichting and Pigliucci 1998). Models differ in their assumptions about the genetic basis of plasticity, the shape of the reaction norm, and the nature of environmental variation. Although most developmental, physiological, morphological, behavioral, and life history traits show some degree of plasticity, the underlying genetic basis of traits, and the genetic, selectional, functional, and developmental constraints on the further evolution of those

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traits can differ. There is empirical evidence that plasticity can result from environmentally dependent expression of alleles and/or the action of regulatory genes (Schlichting and Pigliucci 1995, 1998). However, the relative importance of these two genetic mechanisms is controversial (Via et al. 1995, Roff 1997). It has been argued that for many traits, control of plasticity by regulatory genes should have distinct advantages, including stability of phenotypic expression, the ability to anticipate environmental change by responding to cues, and the relaxation of constraints due to genetic correlations (Schlichting and Pigliucci 1995, 1998). Regulatory control could result in nonlinear reaction norms, a condition that cannot be accommodated by some models.

Optimality models generally do not consider the genetic basis of plastic traits (e.g. Stearns and Koella 1986, Houston and McNamara 1992, Moran 1992, Kawecki and Stearns 1993, Berrigan and Koella 1994, McNamara and Houston 1996). These models are most useful for predicting the fitness function for reaction norms as a compliment to genetic models (Scheiner 1993). The strength of the genetic algorithm models is in their ability to explicitly model the effects of regulatory genes on plasticity evolution (Behera 1997, Behera and Nanjundiah 1997).

The gametic models of de Jong (1988, 1989, 1990a) address how multiple plastic traits interact. These models make no genetic assumptions, and address equilibrium conditions rather than the dynamics of plasticity evolution. De Jong's simplest models examine two plastic traits each governed by a single locus with two alleles with continuous environmental variation. They assume that plasticity is due to variation in the effects of alleles across environments, and not regulatory genes. Each allelic combination produces a different reaction norm. In the environment where reaction norms cross, there

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is no measurable additive genetic variation. The models show that the covariance of traits within particular environments can be positive, zero, or negative. Consequently, depending on the environment, qualitatively different conclusions about the evolutionary independence of traits may be reached (Stearns et al. 1991).

There are two broad classes of quantitative genetic model, the character state approach and the reaction norm approach. The character state approach (Falconer 1952, Via and Lande 1985, 1987, van Tienderen 1991, 1997) treats reaction norm evolution as the evolution of a set of correlated characters. The plasticity of a single trait is modeled as if it were two separate traits, each expressed in a different environment. In character state models, it is the correlation between character states that evolves. Character state models assume discrete spatial variation. Consequently, reaction norms are constrained to be linear between any two environments. Although the character state models do not explicitly consider the underlying genetic basis of plasticity, Via (1993) argues that there is no reason to assume plasticity is due to anything but environmentally sensitive expression of alleles. She argues that this is a reasonable assumption because all selection occurs within environments, and consequently, evolution is constrained to occur only at those loci that control the expression of the trait in that environment. Selection cannot act directly on the slope of the norm, or regulatory genes, if they exist. However, this would not be true if individuals experience selection in more than one environment, or if the genetically related progeny of an individual experience different environments (Schlichting and Pigliucci 1995).

The alternative approach assumes that environmental variation is continuous and defines the reaction norm as a trait that can evolve independent of the mean of the trait.

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The reaction norm is modeled as a function. When the norm evolves, the coefficients of the function change. The simplest reaction norm models (e.g. de Jong 1990a) assume that reaction norms are linear, and so are primarily interested in the evolution of the slope. Others assume that reaction norms can take any shape (Gomulkiewicz and Kirkpatrick 1992, Gavrilets and Scheiner 1993a, b). These models were designed to explicitly allow for the possibility that the genetic basis of plasticity is due to both environmentally sensitive alleles at the loci that govern a trait, and independent regulatory genes (Scheiner 1993). If plasticity is controlled by regulatory switches, then nonlinear reaction norms are likely. There are many examples of threshold traits with nonlinear norms (reviews in Schlichting and Pigliucci 1995, 1998). Gabriel and Lynch (1987, 1992) and Gillespie and Turelli (1989) developed models that assume a Gausian shape to the norm. This shape is appropriate when intermediate levels of an environmental factor lead to maximum trait values. Gavrilets and Scheiner (1993a) and de Jong (1995) have demonstrated that the character state approach is mathematically a special case of the reaction norm approach. However, the two approaches may lead to very different biological interpretations (reviewed in Via et al. 1995)

Predictions

All models predict that plasticity is favored under many common conditions: when spatial and temporal environmental variability is high and fine grained, when differing habitats occur with equal frequency, when the strength of selection is equal in all habitats, and/or when mating is common between individuals in different habitats. As the spatial and temporal scale of environmental heterogeneity becomes coarser, predictions about the evolution of plasticity change (reviewed in Scheiner 1993,

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Schlichting and Pigliucci 1995). Coarse grained spatial variability is more likely to select for specialists (van Tienderen 1991), but temporal variation at a scale longer than the generation time of an organism can still select for plasticity (Lynch and Gabriel 1987, Gabriel and Lynch 1992). Quantitative genetic models show how genetic correlations between traits expressed in different environments affect reaction norm shape and evolution (e.g. Via and Lande 1985, Gavrilets and Scheiner 1993a, b). The presence of regulatory genes can both accelerate the rate of plasticity evolution, and increase the level of adaptation achieved (Behera 1997, Behera and Nanjundiah 1997).

Via and Lande (1985, 1987) and van Tienderen (1991, 1997) have developed models that predict that the dynamics of plasticity evolution are quite different under hard and soft selection. Evolution toward a single plastic, generalist phenotype is more rapid under soft selection. Soft selection may be both frequency and density dependent, but density is not the most useful concept when assessing plant evolution in response to variation in abiotic resources. Because density effects are mediated through resource availability, they may not differ from variation in resource availability due to abiotic causes. The key idea is that the fitness of a genotype is determined locally. Depending on the spatial scale at which local fitness is determined, all genotypes may contribute equally to the next generation. Under hard selection, the fitness of a genotype is determined globally, and is independent of the demographic context. It is likely that soft selection predominates in most natural plant populations (van Tienderen 1997, Schlichting and Pigliucci 1998). Consequently, adaptive plasticity should be common.

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MODELS: PLASTICITY AND GENETIC VARIATION

The relationship between phenotypic plasticity and genetic variation in traits and trait plasticity has caused much speculation in the plasticity literature (reviews in Bradshaw 1965, Sultan 1987, Levin 1988). Sultan (1987) argues that one consequence of plasticity is the reduction of phenotypic selection and genotypic response. If plasticity allows multiple genotypes to produce equally fit phenotypes in the same environment, that is if reaction norms cross (converge) in the most common environments, then genetic variation could persist. This genetic variation would be unmeasurable by quantitative genetic methods in the (most common) environments where reaction norms cross, but could be very important in changing environments. In contrast, some authors have seen plasticity as an alternative to genetic polymorphism (local adaptation) as a strategy to maintain fitness in heterogeneous environments (e.g. Jain 1979, review in Sultan, 1992). A model by Whitlock (1996) suggests that the rate of evolution may be slowed in plastic species with broad niche breadths, a result that supports this second view.

Costs and limits of phenotypic plasticity

Recent attempts to model the effect of phenotypic plasticity on the maintenance of genetic variation have been inconclusive (review in Scheiner 1993). These models draw links to earlier theory on the maintenance of genetic variation. Orzack (1985), de Jong (1988) and Gavrilets and Scheiner (1993a) all found that genetic variation is maintained only under very limited conditions. However, Gillespie and Turelli (1989), and Zhivotovsky and Gavrilets (1992) developed quantitative genetic models based on a different set of assumptions that predict the opposite.

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adaptively plastic generalist genotype can have higher fitness in particular environments than specialists on those environments (Gillespie and Turelli 1989). Models that assume this is possible necessarily assume that there are few limits to plasticity evolution. Consequently, genetic variation is less likely to persist. However, if a "jack of all trades" truly is a master of none, there must be fundamental constraints on plasticity evolution (reviewed in Dewitt et al. 1998) such as strong genetic correlations across environments (Via and Lande 1985), developmental limitations (van Tienderen 1990), or physiological costs (van Tienderen 1991).

If plasticity is sufficiently costly, there should be detectable genotype-environment interactions for fitness that can lead to the maintenance of genetic variation (Bradshaw 1965, Sultan 1987, Gillespie and Turelli 1989, Mitchell-Olds 1992). Van Tienderen (1991, 1997) showed that physiological costs of maintaining the capacity for plastic responses could, in theory constrain plasticity evolution. Under soft selection, costs did not affect evolution toward a single generalist genotype. However, under hard selection costs can result in several adaptive peaks representing either specialists or generalists. Although costs may be an important constraint on plasticity evolution, several studies suggest they may be difficult to detect (van Tienderen 1997, DeWitt et al. 1998). At present, there is little empirical evidence of a cost of plasticity in plants (Sultan 1992, DeWitt et al. 1998, but see Tucic et al. 1998, Callahan et al. 1999).

Predictable environmental variation

Some models have also focused on the importance of predictable variation in the environment for plasticity evolution (e.g. Orzack 1985, Moran 1992, Gavrilets and Scheiner 1993a, Sasaki and Ellner 1997, McNamara 1998, Scheiner 1998, de Jong 1999,

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Sasaki and de Jong 1999). All models agree that if environmental states are temporally unpredictable, plastic responses are less likely to evolve, and genetic variation can be maintained. Efforts to test the prediction that plasticity should be negatively correlated with the predictability of environmental variation have been inconclusive, largely due to the lack of appropriate data (Gavrilets and Scheiner 1993a, Roff 1997).

The value of models

All models have limitations. Except for the quantitative genetic model of Gavrilets and Scheiner (1993b), all models assume stabilizing selection within environments. Consequently, their relevance to the evolution of traits closely related to fitness is uncertain because such traits are assumed to be under directional selection. Gavrilets and Scheiner (1993b) show that under directional selection both the mean value of a trait across environments and the slope of the reaction norm can evolve. The genetic basis of plasticity in a trait is important when considering the validity of predictions. If plasticity in a trait is primarily due to regulatory genes that function as switches in different environments, then the relevance of quantitative genetic models is questionable (but see Via 1993b).

As with all theory in population biology, the predictions of these models should be considered qualitative, not quantitative (Levins 1968). The predictions of models based on a quantitative genetic approach in particular apply only to short term, local projections (Pigliucci and Schlichting 1997). Consequently, long term plasticity evolution is probably less constrained than quantitative genetic theory predicts. In summary, models suggest that most traits should display adaptive plasticity unless this plasticity entails significant costs or environmental variation is unpredictable. Genotype-

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environment interactions should be rare for traits that are highly plastic. Finally, the presence of significant genotype-environment interactions implies strong cross-environment genetic correlations and/or variable, unpredictable natural selection.

The real world is complex, with different abiotic and biotic environmental factors varying at different spatial and temporal scales with differing degrees of predictability. Because many combinations of plasticity and canalization in underlying traits could result in equally fit genotypes, there may be no simple relationship between fitness and plasticity in a trait (Schlichting and Pigliucci, 1995). It is necessary to consider how groups of traits are integrated to fit organisms to their environments. Populations could simultaneously maintain genetic variation in some traits and be adaptively plastic in others. These diverse possibilities underscore the importance of empirical work examining how traits and their plasticity interact to determine fitness in natural populations.

EMPIRICAL STUDIES

Despite the recent profusion of theory addressing plasticity and reaction norm evolution there is still little empirical work bearing directly on these questions (Roff 1997, Lynch and Walsh 1998). There is overwhelming evidence that plants alter their phenotypes in response to the environment (reviews in Bradshaw 1965, Schlichting 1986, Sultan 1987). However, we still lack studies of natural populations where the ecological and evolutionary meaning of phenotypic responses to the environment can best be understood (Scheiner 1993, Schmitt 1995, Via et al. 1995). As mentioned above, there is little evidence for a cost to plasticity, nor are there enough examples to assess the relationship between plasticity and environmental predictability. There is limited support

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for the idea that genotype-environment interactions for fitness combined with spatial variation in the environment can maintain genetic variation (e.g. Bell and Lechowicz 1991, review in Mitchell-Olds 1992). Most empirical studies of reaction norms have been under controlled, laboratory conditions. For example, in a series of greenhouse experiments, Sultan and Bazzaz (1993a,b,c) found that diverse patterns of physiological and morphological plasticity to light, water, and nutrients can produce convergent reaction norms for reproductive performance. Whether fitness differences would emerge under field conditions, and whether these patterns reflect different, adaptive responses and/or tradeoffs in plasticity evolution is unknown.

The best empirical evidence for the potential for plasticity to evolve comes from the applied breeding literature. In a review of the evidence Jinks and Pooni (1988) concluded: "Genetic variation for environmental sensitivity is as ubiquitous as that for mean performance and is at least in part independent of it. As we learn more about the genetic variation for environmental sensitivity and its specificity in respect of character and environmental variable, it becomes clear that it is possible to select a pattern of response to environmental variation to meet almost any requirements."

Contemporary quantitative genetic theory describes the process of multivariate evolution in natural populations using the simple equation $\Delta \mathbf{z} = \mathbf{G}\beta$ (e.g. Lande and Arnold 1983). This equation shows that the predicted evolutionary response across one generation in a vector of trait means ($\Delta \mathbf{z}$), depends on the additive genetic covariance matrix (**G**), and the vector of selection gradients (β). The analytical techniques developed with this theory have been very useful for empirical studies addressing

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questions of selection and genetic variation in natural populations. One result of these studies has been the repeated demonstration that patterns of phenotypic selection are variable (e.g. Kalisz 1986, Stewart and Schoen 1987, Kelly 1992, Stratton 1992, Bennington and McGraw 1995). Further, a small but growing number of field studies have shown that **G** (genetic variances and covariances) can change with the environment (Shaw et al. 1995, Bennington and McGraw 1996, Wulff 1998). Estimates of both selection and genetic parameters across different environments in the field are rare. Consequently, our understanding of the relationships between heterogeneous environments, variable selection, genetic variation and phenotypic plasticity remains largely speculative.

THE EVOLUTION OF PHENOTYPIC PLASTICITY IN A NATURAL POPULATION

Two factors that can be expected to function as strong selective agents on plants are light and leaf litter. Light is a primary plant resource, and has been shown to greatly affect female fitness in many plant populations (reviews in Goldberg 1990, Sultan and Bazzaz 1993a). Light availability for understory plants in forests can be highly variable in time and space (Chapter 2), but in very predictable spatial patterns, and diurnal and seasonal cycles. Thus it is likely that this variation in light availability should favor plastic traits that would allow plants to tolerate low light levels and convert high light availability into increased fitness.

Leaf litter also can affect plant fitness, particularly through reductions in the establishment and survival of seedlings (e.g. Goldberg and Werner 1983, Bergelson 1990, Carson and Peterson 1990, Foster and Gross 1997). In other cases, leaf litter may

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facilitate seedling establishment through the amelioration of abiotic stresses (e.g. desiccation, Fowler 1986, Willms et al. 1986, Hamrick and Lee 1987). Importantly, the incidence, quantity, and persistence of leaf litter at particular locations in forests are unpredictable (Frankland et al. 1963, Sydes and Grime 1981, Facelli and Carson 1991, Molofsky and Augspurger 1992).

In this study I have examined the effects of manipulations to the light and leaf litter environments within a natural population of the winter annual wildflower *Collinsia verna*. I have followed naturally occurring plants and plants of known genetic relationship through two years in the field. This approach has allowed me to investigate several aspects of phenotypic plasticity. Below, I describe the questions addressed in each part of the study.

Environmental heterogeneity

The scale, pattern, and predictability of environmental variability within a population can all effect the evolution of plastic responses. To address these questions about variation in light environments, I measured the spatial variability and temporal correlation across years of light environments within the population. These results are presented in Chapter 2.

Maternal effects in heterogeneous environments

If natural environments are variable, but maternal and offspring environments are correlated, natural selection should favor the evolution of plastic maternal effects on seed traits. The phenotype of seeds should be appropriate for the germination environment they will experience. In Chapter 2, I investigate the effects of maternal genotype, maternal environment, and offspring environment on several measures of performance. I

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address two questions: Are maternal effects genetically variable, and does their expression change with parent and/or offspring environment? Do traits under maternal influence contribute to offspring survival and fecundity, and do these effects depend on the offspring environment?

Quantifying genetic and environmental effects on phenotypes

The environmental sensitivity of traits, genetic variation in the expression of traits, and genotype-environment interactions can all influence plasticity evolution. In Chapter 3, I present the results of two independent genetic studies designed to quantify the genetic basis of light responsive traits. Half-sib breeding designs were completed in each of two years. The resulting seeds were planted in three manipulated and two natural light environments in the field. I present reaction norms, narrow sense heritabilities, and cross-environment additive genetic correlations for nine traits: emergence date, winter size, flowering date, specific leaf area, mainstem length, vegetative biomass, mean seed mass, reproductive investment, and seed number.

Quantifying phenotypic selection

To favor the evolution of plastic responses, environmental heterogeneity must result in variation in the pattern of natural selection. To examine the potential for environment-dependant selection in this population, I manipulated light and leaf litter creating eight different environments. Plants growing in these environments were followed throughout their lives. In Chapter 4, I report the resulting patterns of natural selection on six traits: emergence date, flowering date, specific leaf area, mainstem length, vegetative biomass, and reproductive investment. I explore whether the magnitude or direction of natural selection changes across environments, and investigate

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whether light or leaf litter can be characterized as causes of natural selection.

Together, these chapters attempt to address simultaneously the possible relationships between patterns of environmental variation, patterns of variation in phenotypic selection, and patterns of genetic variation and phenotypic plasticity within a natural plant population. In Chapter 5, I summarize the results, describe ongoing work, and suggest questions for future investigation.

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Chapter 2

PLASTIC MATERNAL EFFECTS: GENETIC VARIATION AND FITNESS CONSEQUENCES IN A NATURAL PLANT POPULATION

INTRODUCTION

Parents generally affect the fitness of their offspring in ways beyond the direct transmission of genes. Evolutionary ecologists have become increasingly interested in understanding how these parental effects evolve (e.g. Rossiter 1996, Lynch and Walsh 1998, Mousseau and Fox 1998a, b, Wolf et al. 1998). In seed plants parental effects are primarily mediated through three avenues: (1) maternal control of seed provisioning with resources essential for early seedling growth and establishment; (2) maternal control of the thickness and permeability of the seed coat that affects dormancy and timing of germination; (3) maternal traits affecting seed dispersal and the subsequent seed germination environment like fleshy fruits, explosive capsules, barbed or winged seed coats, or other dispersal structures directly attached to seeds or fruits (Schaal 1984, Roach and Wulff 1987, Lacey 1991, Schmitt 1995, Wulff 1995, Lacey et al.1997).

Maternal genetic and environmental effects

Maternal environmental effects on seed dormancy and size are well known (reviews in Roach and Wulff 1987, Platenkamp and Shaw 1993). Seed dormancy may be critically important in buffering populations from extinction when the quality of the environment is temporally variable and reproductive success is unpredictable (Brown and Venable 1986, Kalisz and McPeek 1992, 1993, Evans and Cabin 1995). Seed size influences seed dispersal, and seedling size, growth, and competitive ability (e.g. Stanton

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1984, Stratton 1989, Gross and Smith 1991; see also reviews in Haig and Westoby 1988, Schmitt 1995, Sultan 1996).

When the offspring environment is variable, optimal allocation of dormant seeds to the soil seed bank depends on the quality of the germination environment (Brown and Venable 1986). In empirical studies, the rate of seed dormancy varies with environmental quality across populations (reviewed in Philippi 1993, Evans and Cabin 1995). Dormant seeds are most valuable if pre-reproductive survival varies over time. Moreover, the importance of seed size in determining seedling success can depend on the offspring environment (Stratton 1989, reviewed in Haig and Westoby 1988). Under these circumstances, maternal fitness can depend on timing of offspring germination, offspring number, and offspring size; and maternal fitness and the fitness of individual offspring usually conflict (McGinley et al. 1987). Greater seed mass may always benefit individual offspring, but maternal fitness may be maximized by making more, smaller seeds in some environments, and fewer, larger seeds in other environments. Consequently, the optimal allocation of limited maternal resources to dormant seeds, to a few large seeds, or to many small seeds can all depend on the predictability of the offspring environment and how conflicts of interest are resolved (reviews in Shaanker et al. 1988, Haig and Westoby 1988, Forbes 1991).

Although both maternal genotype and maternal environment can contribute to offspring phenotype, most past studies of maternal effects in plants were not designed to distinguish genetic from environmental effects (Lacey 1991). Recently, several researchers have completed studies documenting the genetic basis of maternal effects in plants (Biere 1991a, Platenkamp and Shaw 1993, Shaw et al. 1995, Helenurm and Schaal

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1996, Byers et al. 1997, Husband and Gurney 1998, Thiede 1998). Because maternal effects on progeny traits can change with the environment, they can be viewed as plastic (Lacey 1991, Schmitt et al. 1992, Platenkamp and Shaw 1993, Carriere 1994, Schmitt 1995, Sultan 1996). Major goals for current research are distinguishing the relative magnitudes of maternal genetic and environmental effects in natural populations, and understanding how maternal genotype and maternal environment interact in their effects on offspring fitness. Significant genotype-environment interactions indicate genetic variation for phenotypic plasticity (or reaction norms), and the potential for reaction norm evolution (Schlichting 1986, Sultan 1987). However, genotype-environment interactions for fitness components may also indicate the presence of locally adapted specialist genotypes. Heterogeneous selection is the most common explanation for how such genetic diversity in natural populations is maintained (e.g. Mitchell-Olds 1992), but this idea remains relatively untested (Stratton and Bennington 1998). A few studies have now documented genotype-environment interactions for maternal effects (reviewed in Donohue and Schmitt 1998).

Plastic responses to light

An appropriate pattern and scale of environmental heterogeneity is a necessary condition for plasticity evolution (Bell and Lechowicz 1991, van Tienderen 1991, Pigliucci 1996). Light is a primary plant resource, and is a strong selective agent in plant populations (Goldberg 1990, Sultan and Bazzaz 1993). Because light is critical for seedling establishment and light availability is variable, selection should favor plastic traits that enhance seedling survival and growth in diverse light environments (i.e. tolerance of low light levels and conversion of high light availability into increased

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fitness). In high light environments, seed size may not affect offspring establishment, and increased dormancy or dispersal could decrease offspring fitness by placing offspring in a less favorable environment. However, in low light environments larger seeds may improve offspring establishment, and increased dormancy or dispersal may facilitate movement of offspring to more favorable environments and improve offspring fitness. If light availability within a population varies due to topography or other vegetation, but maternal and offspring environments are correlated, natural selection should favor the evolution of a reaction norm of maternal effects on seed and offspring traits such that offspring are prepared appropriately for the expected environment and maternal fitness is maximized (Schmitt 1995).

Three recent studies have investigated maternal effects on seed and offspring characters in response to variable light environments (Schmitt et al. 1992, Wulff et al. 1994, Sultan 1996). The evidence suggests that plastic responses to light are ubiquitous, but we have little evidence that they are adaptive (but see Schmitt et al. 1995, Dudley and Schmitt 1996). To further our understanding of the interactive effects of maternal genotype and maternal environment on offspring fitness, we must manipulate appropriate environmental factors under natural field conditions, characterize the genetic basis of plastic responses, and measure offspring fitness (Schmitt 1995, Sultan 1995).

In this study, I manipulated light environments across two generations within a natural population of the winter annual wildflower *Collinsia verna*. Using a quantitative genetic breeding design, I investigated the effects of maternal light environment, maternal genotype, and offspring light environment on offspring performance. I measured natural light in the population across two years to learn if light availability was similar across

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years at particular locations (<1 m scale), but varied between different locations (20 m scale). Finally, to determine the consequences of plastic maternal effects, I examined the relationship between maternally influenced traits and offspring fitness in each light environment. I address the following specific questions: Does light availability vary in a way that favors plastic maternal effects? Are maternal effects genetically variable, and does their expression change with parent and/or offspring environment? Do traits under maternal influence contribute to offspring survival and fecundity, and do these effects depend on the offspring environment?

METHODS

Study system

Collinsia verna is a winter annual wildflower found in moist woods in eastern North America. The study population occurs along the south facing edge of a woodlot next to an agricultural field in Kalamazoo County, Southwest Michigan. In each of the last ten years this population has included more than one million reproductive individuals in an area of about one hectare. Fall seed germination is cued by diurnal temperature fluctuations (Baskin and Baskin 1983). Seedlings emerge between September and December, and overwinter with up to one pair of true leaves. Seed and seedling traits of *Collinsia verna* contribute significantly to establishment, overwinter survival, and fecundity (Kalisz 1989, Thiede 1996). The predominantly out-crossing plants flower from late April to mid May. If pollinated, each flower produces a fruit with up to four seeds. Seeds are passively dispersed in early June and remain dormant until fall. Fecundity in the field is variable depending on the micro-environment, ranging from 5-50 seeds. Seeds can remain dormant in the soil for at least three years (Kalisz 1991).

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Mortality in the seed bank is variable but low compared to the pre-reproductive mortality of plants. The soil seed bank can be demographically important for this species, and can buffer populations against extinction (Kalisz and McPeek 1992, 1993, Kalisz et al.1997).

Experimental design

Light treatments-Light levels vary from 25% to 75% of full sun over the one hectare area of the population (Results). To manipulate light levels, I cleared all woody vegetation from a 15 X 20 m area along the southern edge of the population (Figure 1). Fifteen 1 X 1.2 m plots in a 5 X 3 grid with 1 m spacing between plots were established (5 blocks of 3 plots). Because different parts of this area were illuminated as the sun tracked daily from east to west, blocks were oriented perpendicular to the edge of the woodlot. Three light treatments were imposed to bracket the range of natural light variation: 100% (high), 40% (medium), and 10% (low). I assigned light treatments randomly in each block. Light was reduced by placing a wood lattice over the plots that allowed partial light to reach the plants throughout the day (3.5 cm wood slats in a 15 cm (medium) or 7 cm (low) grid). This created a more natural reduction in light levels by retaining sunflecks that can be very important to the total energy balance of plants (Endler 1993). I removed leaf litter from the experimental plots weekly.

Breeding design-To examine the effects of both maternal and offspring light environments on offspring traits, I planted the children and grandchildren of a nested paternal half-sib breeding design in these treatments (Figure 2). In April of 1995 I collected 204 plants from the study population prior to flowering to serve as the first generation. One individual was collected every 5 m along seven 150 m transects spaced 5 m apart. These transects spanned the range of natural variation in light in the

Figure 1. Map of 15 experimental plots with randomly assigned light treatments. The experimental area was placed south of the original fence-row in an area cleared of all woody vegetation.

Figure 1.

Original fence-row
Forest
Block 5
Block 4
Block 3
Block 2
Block 1
Cleared Experimental Area
Southern edge of natural Collinsia population
Edge of successional vegetation
Agricultural Field
1 Meter
Light Treatment
Low
Medium
High
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Figure 1.

Figure 2.

April 1995 204 Individuals collected from the field prior to flowering.

Figure 2. Experimental design: Crossing and planting plan as described in text.

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population. In the greenhouse 50 of these field collected plants were randomly designated as sires and each crossed to three randomly chosen dams in a standard nested design (two sires with small dams were each crossed to one additional dam). Flowers of dams were emasculated in the bud stage to prevent selfing. In July 1995 I weighed the resulting seeds and planted them in 2 cm uniquely numbered plastic straws containing moist soil from the study site. In early August, I transferred the straws containing the seeds to the field. Three seeds from each full-sib family were randomly planted into an 8 X 8 cm grid in each treatment (152 full-sib families X 3 seeds/family X 3 treatments X 5 blocks = 6840 seeds planted). This density is approximately one third of the average in the population at harvest (unpublished data). The entire study area was established within the natural *Collinsia* population, so that the planted seeds grew in a matrix of naturally occurring plants (Figure 1). The experimental area was enclosed with 1.2 m high 5 X 5 cm welded wire fencing.

Mothers (year 1, 1995-96)-Plants emerging from the straws in the field treatments are the mothers in this study (Figure 2, generation 2). I conducted censuses for emergence and survival weekly from September through December. In December, the numbers of cotyledons and true leaves were counted, and the diameters of the largest cotyledon and leaf were measured to the nearest 0.4 mm. Winter size was calculated as cotyledon area + leaf area. In mid-April a census for survival to flowering was done. Plants in all treatments were naturally (open) pollinated. Plants were harvested before seed dispersal in June of 1996 and scored for number of seeds and total seed mass. I call these traits (emergence date, winter size, seed number, and mean seed mass) *individual traits* to differentiate them from the *maternal traits* described below.

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Offspring (year 2, 1996-97)-Seeds collected from the field grown, open pollinated mothers are the offspring in this study (Figure 2, generation 3). Seed production by mothers was quite variable, but I used equal numbers of seeds from medium and high light mothers in the subsequent planting (1325 each). Due to high mortality and low fecundity, only 58 seeds were available from low light mothers. To increase the sample size for the low light maternal environment, I included in the offspring generation an additional 737 seeds produced by naturally emerging plants in the low light plots. All seeds were randomly planted back into the 15 experimental plots (230 seeds X 5 blocks X 3 treatments = 3450) with the constraints that the grandchildren of generation 1 sires were distributed equally in each treatment and block, and one third of the seeds from each generation 2 mother were planted in each of the three environments. Because most of the 795 offspring from the low light maternal environment were of unknown relationship, I excluded these plants from the genetic analyses described below. Separate environmental effects analyses include all three maternal light environments. Planting techniques were as described above. I transferred the numbered straws containing the seeds to the field on September 24, 1996. To increase survival in the low light treatments, I raised light levels in this treatment from 10% to 18% of full sun. Census, harvest, and scoring techniques were the same as in year 1. This species has a well-documented seed bank (Kalisz 1991). Consequently, seeds that did not germinate were considered dormant.

Maternal effects-To address whether mothers alter the phenotype of their offspring in response to the current environment in a way that increases maternal fitness, I scored several measures of maternal performance. Two are traditional measures: the number and size (mean mass) of the seeds produced by each mother. I measured four

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additional traits to quantify maternal performance in terms of offspring traits in each light environment: (1) proportion of offspring germinating; (2) proportion of offspring surviving (both based on number of seeds per mother per light treatment); (3) mean number of seeds produced by surviving offspring; (4) mean mass of seeds produced by offspring. I calculated these later four traits for each light environment by averaging the trait values of each mother's offspring. Because these averages grouped offspring from the same treatment across all 5 blocks, I could not include block in the subsequent analyses. In separate analyses, block effects were small and mostly insignificant (analyses not shown). This approach makes the tests for maternal effects more conservative. Together, I refer to these six traits as *maternal traits* to differentiate them from the traits of individual offspring. Finally, I calculated a cumulative measure of maternal fitness in each light environment as the product of mean number of seeds produced by offspring and proportion surviving divided by proportion germinating. This calculation estimates the mean number of grandseeds per offspring once all offspring germinate. It assumes that all dormant seeds germinate, and that their survival and fecundity would be the same as in the year of this study. Because it does not discount the value of a dormant seed, it is likely an overestimation of cumulative maternal fitness.

Light measurements- I measured photosynthetically active radiation (PAR) to address three questions: Did the light manipulations effectively bracket the natural variation in the light environment? Are parent and offspring light environments correlated? Is light availability spatially variable? All light data were collected using a Decagon AccuPAR light ceptometer (Decagon Devices, Inc. Pullman, WA). The ceptometer has 20 quantum sensors arrayed along a 1 m wand. I measured light in the

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experimental plots weekly at solar noon throughout both years. Each reading consisted of 10 samples taken while moving the wand horizontally across the plot 10 cm above the vegetation surface. Consequently, a reading was the average of at least 200 single point samples (20 sensors X 10 samples). Three readings were taken on each plot at each sampling time.

To determine if light levels were correlated across generations, I measured light levels in the population in fifteen permanent 1 m^2 plots at uniform intervals along three 10 m east-west transects spaced 20 m apart across a light gradient from the southern edge to the forest interior. I made these measurements at solar noon weekly for one month after leaf fall and in May of each year. Light levels for these fall and spring measurements were expressed as percent of full sun. Because ambient light levels differ between spring and fall, I calculated separate spring and fall across year correlations.

To assess spatial variation in light, I made measurements 30 times in the spring of 1997 at 50 points spaced on a 20 X 20 m grid throughout the full spatial extent of the population. I took a reading from a single light sensor at the end of the wand every hour between 1000 and 1500 on five days between May 7 and 24, 1997 prior to canopy leaf out. Each reading was expressed as percent of full sun at each sample interval. I calculated a light score for each point taking the mean of these 30 relative light level values. I plotted the scores for each of the 50 points in a frequency graph.

Data analysis

Environmental effects and genotype-environment interactions-All traits were independently analyzed using the MIXED procedure of SAS (SAS 1992, 1997). Proc. MIXED performs mixed model analysis of variance using a restricted maximum

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likelihood (REML) algorithm to directly compute variance components for random effects. Maximum likelihood methods are generally superior for analysis of unbalanced data (Shaw 1987, Searle et al. 1992, Littell et al. 1996). Fixed effects were tested by computing a Type III F statistic. Denominator degrees of freedom for these fixed effects tests were estimated using the Satterthwaite approximation option (Littell et al. 1996). The significance of variance components for random effects were assessed using one-sided likelihood ratio tests (Littell et al. 1996).

Fixed effects of maternal environment, offspring environment, and their interaction were examined first with the complete data set (including the offspring of naturally emerging mothers from the low light environment). Genetic and environmental effects and their interactions were then analyzed with a mixed, split plot model including only mothers of known relationship from the medium and high light environments. The model for each trait included block (except traits averaged across blocks), maternal environment, sire, dam nested in sire, offspring environment, and their interactions. Sire and dam were split plot factors, while light treatments were whole plot factors. Blocks were treated as fixed because they captured an east-west gradient of diurnal morning to afternoon shading. Environmental effects, and their interaction were also treated as fixed, while sire, dam nested in sire, and their interactions with fixed effects were treated as random. Sire and dam effects both reflect genetic differences among mothers. The full analysis included all main effects and two-way interactions except no dam interactions could be examined for the four maternal traits due to sample size limitations. Highly insignificant three-way and higher interactions with block were omitted from the final model for seed number and mean seed mass of the mothers.

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These analyses assume that genotypes within plots are independent, which is reasonable because the plantings were relatively low density and nearest neighbors were always naturally occurring plants. Light treatment effects may be due to light or variation in the temperature or moisture environment caused by light. They may also be due to competitive interactions with other plants when light levels affected the size and density of competitors. These factors all naturally covary with light.

The germination and survival traits were trimodal and could not be transformed to normality. The offspring of more than one third of the mothers had either complete or no germination or survival, while the remaining families were normally distributed. These distributions were a consequence of several mothers with only a few offspring planted in each environment. Two approaches were used to examine the consequences of this departure from normality. First, by using data from only the largest families, normality assumptions could be met. Second, a balanced subset of the data was analyzed using categorical modeling (SAS CATMOD procedure, SAS 1989). To achieve the necessary sample sizes for categorical modeling, a reduced model with only sire and environment main effects and their interaction was necessary. Both approaches yielded results nearly identical to the proc. MIXED analysis of the full data set with similar models, so only the proc. MIXED results are presented. Seed number (mothers) and mean offspring seed number were log transformed to achieve normality.

When the full mixed model analysis indicated that the effect of offspring environment or the expression of genetic variation depended on the maternal environment (as indicated by significant interaction terms), analyses within maternal environments were done. The approach used for these analyses was as described above, with a model

that included only offspring environment, sire, and dam nested in sire.

Reaction norm figures were constructed for each trait with significant sire or dam effects by plotting family means against environment. Genotypic differences in slope in these reaction norm plots indicate genotype-environment interactions. Statistical and graphical approaches to detecting genotype-environment interactions are complimentary and equally important because the power of statistical methods to detect genotype-environment interaction can be limited (Lewontin 1974, Via and Lande 1985, Wahlsten 1990).

Fitness consequences of maternal effects-The analyses described above revealed effects of maternal genotype and environment on seed mass (see Results). This suggests that maternal effects on offspring fitness may be mediated through seed size. To understand the relationships between seed mass and other traits, phenotypic correlations (Pearson) of mean seed mass with the other five maternal traits were calculated for each environment, and correlations of mean seed mass with individual traits (emergence date, winter size, mean seed mass, and seed number) were calculated for each environment for both years.

I used multivariate episodic selection analysis (Arnold and Wade 1984) to assess the fitness consequences of seed mass and phenotypically correlated traits under maternal influence. Two episodes of selection, survival to flowering and fecundity were analyzed independently for each light environment in both the mother and the offspring generations. The estimates of selection resulting from these analyses address whether a character has fitness consequences in that episode independent of selection in other episodes (Koenig et al. 1991). Traits analyzed in the survival episode were mean seed

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mass, emergence date, and winter size. The traits analyzed in the fecundity episode included mean seed mass, emergence date, winter size, and several phenological and morphological traits of adult plants. Due to snow cover, winter size data was not collected for the offspring generation (Year 2, 1996-97).

Multivariate linear selection gradients (β_i) were calculated as the partial regression coefficients of a trait on relative fitness. Linear selection gradients measure the direct selection on each trait independent of all other traits in the analysis. Relative fitness was calculated by dividing each absolute fitness measure (zero or one for the survival episode, total seeds for the fecundity episode) by the mean for that environment. All phenotypic traits were standardized within environments to a mean of zero and variance of one, and transformed as needed before analysis to improve normality.

While the calculation of regression coefficients on a categorical measure of fitness like survival provides an unbiased estimate of the selection parameter, parametric significance tests are not valid (Mitchell-Olds and Shaw 1987). Significance tests of all selection parameters were performed by bootstrap resampling methods (Dixon 1993). The data set from each environment and year was resampled 1000 times using a SAS macro (SAS 1990). The number of observations in each resampled data set was equal to the number of observations in the original data set. Confidence intervals (95%) for all selection parameters were calculated from the bootstrapped estimates of each parameter by the shift-distribution method (Noreen 1989).

Path analysis was used to examine an *a-priori* model describing hypothesized relationships between seed mass, emergence date, winter size, and survival. Because winter size was not measured in the second year, only the data for the first year (1995-96)

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RESULTS

Light

The light treatments effectively bracketed the average light level over a wide area within the forest over the entire growing season (Figure 3), and natural light levels in 1 m^2 plots were highly correlated across years (Figure 4a). At a 20 m scale, light levels in the natural population were variable, ranging from 25% to 75% of full sun (Figure 4b). The distribution was not normal, with 78% of the sites falling in the lower half of the distribution. Both the mean and median were about 40% of full sun.

Genetic and environmental effects on the 6 maternal traits

These results draw on three separate analyses, the genotype-environment interaction analysis of the genetic data set (medium and high light mothers, Figure 5, Table 1), within-environment genetic analyses of proportion germinating and offspring mean seed mass (Table 2), and the environmental effects analysis of the full data set (mothers from all treatments, Figure 6)

Seed number (mothers)-Seed number increased significantly with light (Figure 5a), and was typical of natural plants growing in the plots. There was a marginally insignificant maternal family effect on seed number (P=0.07) suggesting that maternal genotype or environment could potentially contribute to offspring fitness. Reaction norms show that some families differed both in number of seeds and in plasticity.

Mean seed mass (mothers)-The environment in which seeds were produced effected seed mass (Figure 5b). Seed mass was greater in medium light than either low or high light maternal environments, and was most variable among individuals in low light.

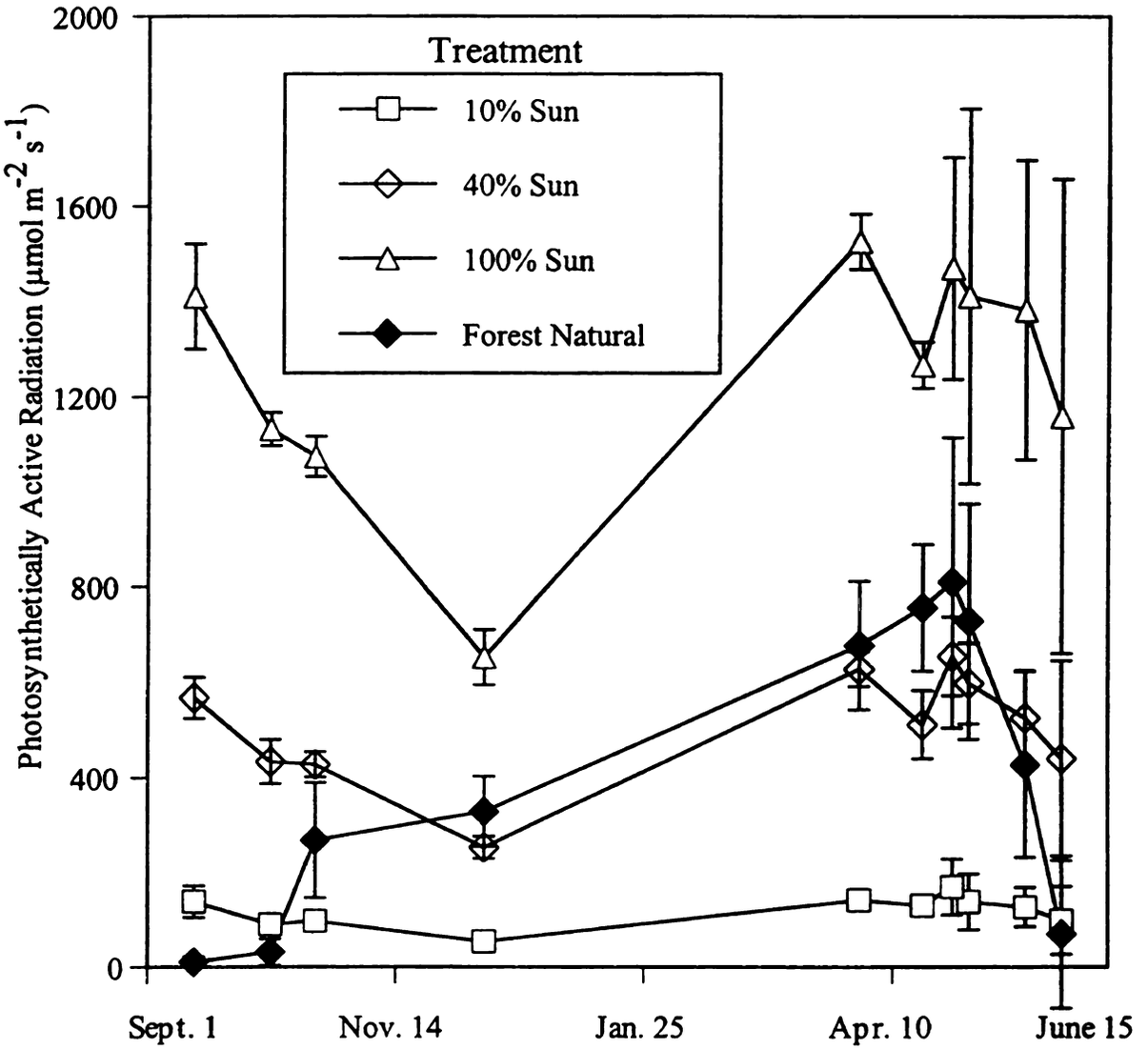


Figure 3. Seasonal variation in photosynthetically active radiation (PAR), Year 1 (1995-96) in treatments and natural forest plots. Each point is the mean of 5 plots, and all data were collected at solar noon on clear days. Bars are standard errors.

Figure 4. Temporal stability and spatial variability of light environments. (a) Between year correlations in light levels for 15 plots spaced uniformly across a light gradient from forest to edge. (b) Variation in light availability at 50 grid points covering the spatial extent of the population.

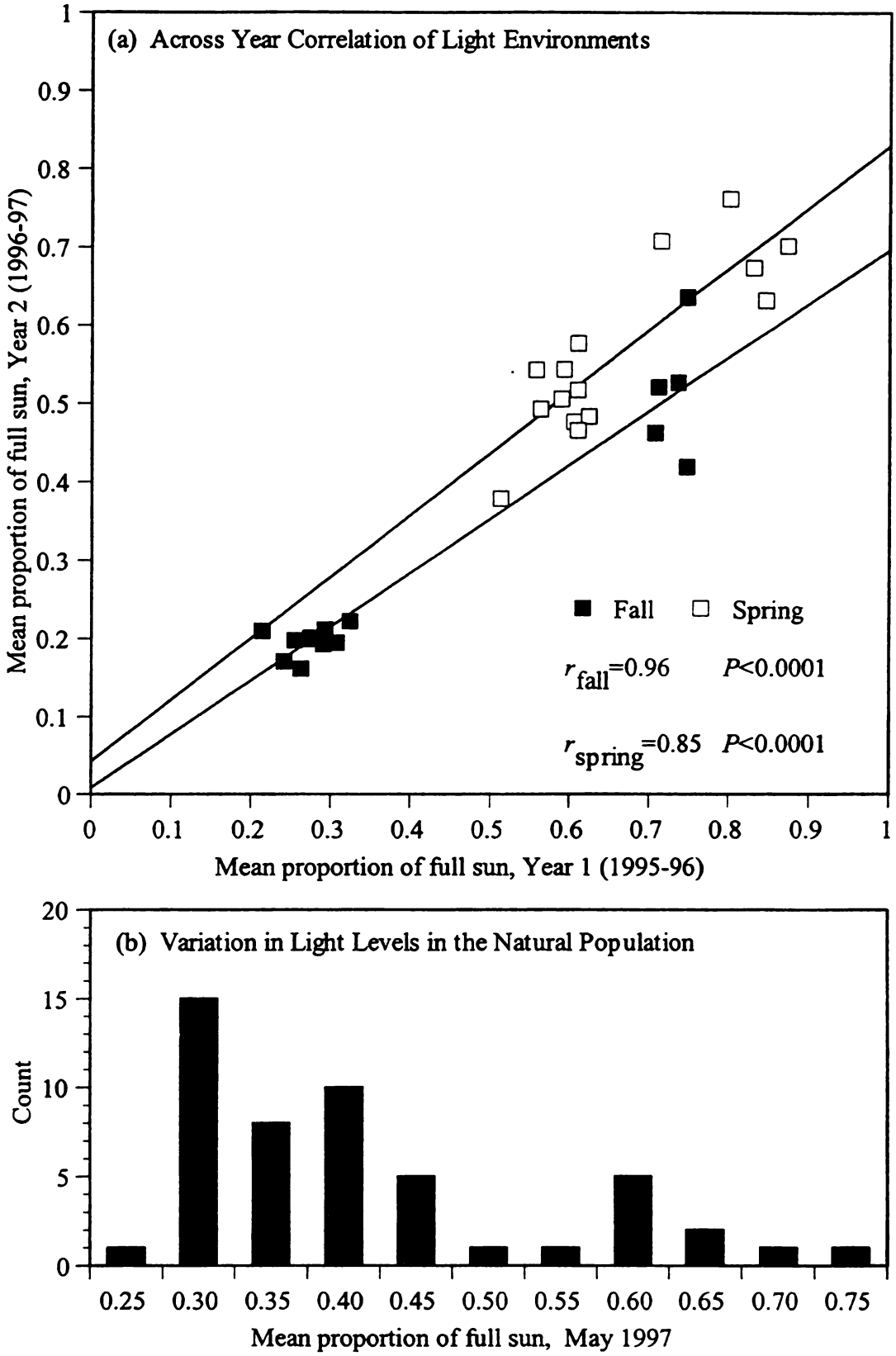
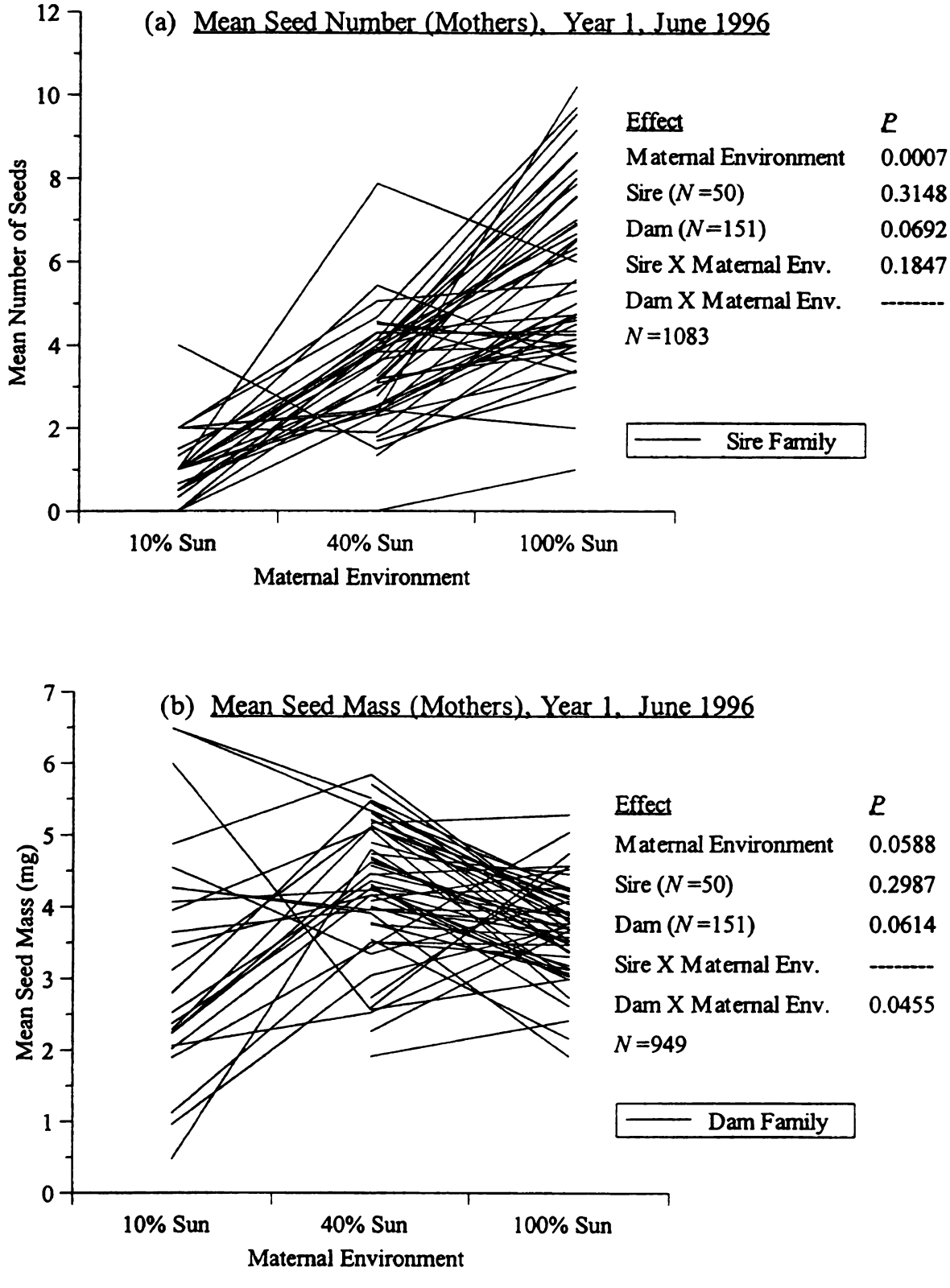

(a) Across Year Correlation of Light Environments
Mean proportion of full sun, Year 2 (1996-97)
Mean proportion of full sun, Year 1 (1995-96)
Fall
Spring
r_{fall}=0.96 P<0.0001
r_{spring}=0.85 P<0.0001
(b) Variation in Light Levels in the Natural Population
Count
Mean proportion of full sun, May 1997

Figure 4.

Figure 5. Reaction norms for traits affected by maternal genotype and/or environment. Norms are for either paternal or maternal families depending on which effects were significant in the mixed model analysis. (a) Mean number of seeds produced by mothers, by paternal family, June 1996. (b) Mean mass of seeds produced by mothers, by maternal family, June 1996. (c) Proportion of offspring germinating, by paternal family, Fall 1996. Note that the x-axis is the maternal environment, not the germination environment, and that there were too few seeds produced in the low maternal light environment for them to be included in this analysis. (d) Proportion of offspring surviving to flowering, by paternal family, Spring 1997. Statistics based on genetic and environmental effects mixed model REML analysis. When a variance component was estimated as zero, no *P* value is reported. Many families did not survive in low light, only those families with data for at least two environments are shown. The number of families varies: (a)-50 families, (b)-58 families, (c) and (d)-20 families.


(a) Mean Seed Number (Mothers), Year 1, June 1996
Mean Number of Seeds
0
2
4
6
8
10
12
10% Sun
40% Sun
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Maternal Environment
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Maternal Environment 0.0007
Sire (N=50) 0.3148
Dam (N=151) 0.0692
Sire X Maternal Env. 0.1847
Dam X Maternal Env. --------
N=1083
Sire Family
(b) Mean Seed Mass (Mothers), Year 1, June 1996
Mean Seed Mass (mg)
0
1
2
3
4
5
6
7
10% Sun
40% Sun
100% Sun
Maternal Environment
Effect
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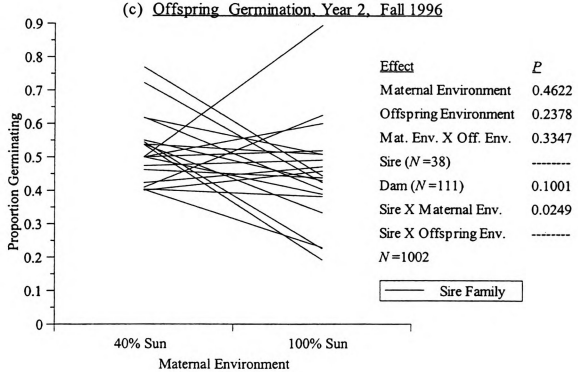
Maternal Environment 0.0588
Sire (N=50) 0.2987
Dam (N=151) 0.0614
Sire X Maternal Env. --------
Dam X Maternal Env. 0.0455
N=949
Dam Family

Figure 5.



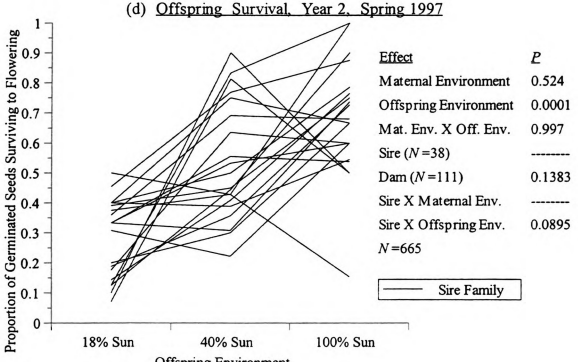


Figure 5 (cont'd).

Table 1. Genetic and environmental effects mixed model REML analysis of the maternal traits offspring mean seed number and offspring mean seed mass. These traits are not presented in Figure 5 because there is no indication of genotype-environment interactions.

	Offspring Mean Seed Number			Offspring Mean Seed Mass		
	N=400			*N*=349		
Fixed Effect or	*F*-Value or Variance Component			*F*-Value or Variance Component		
Source of Variance	Estimate	(Standard Error)	*P*	Estimate	(Standard Error)	*P*
Maternal Environment	$F_{1,391} = 5.21$		0.0229	$F_{1,336} = 3.05$		0.0815
Offspring Environment	$F_{2,379} = 13.17$		0.0001	$F_{2,314} = 7.44$		0.0007
Maternal Env. X Offspring Env.	$F_{2,374} = 0.09$		0.9118	$F_{2,309} = 3.06$		0.0483
Sire	0		------	0.06	(0.0584)	0.1714
Dam(Sire)	0.0016	(0.0047)	0.3574	0.061	(0.0695)	0.1571
Sire X Maternal Environment	0		------	0		------
Sire X Offspring Environment	0		------	0		------
Residual Variance	0.1437	(0.0112)		1.1313	(0.0978)	

Table 2. Within-environment mixed model REML analysis for maternal traits with significant interactions in the full analyses presented in Figure 5c (germination) and Table 1 (seed mass).

Maternal Light Environment (N)	Fixed Effect or Source of Variance	F-Value or Variance Component Est.	(Std. Error)	P
<u>Proportion of Offspring Germinating</u>				
Medium (551)	Offspring Environment	$F_{2,473} = 0.07$		0.9305
	Sire	0		--------
	Dam(Sire)	0.0074	(0.0062)	0.1128
	Residual Variance	0.1777	(0.0118)	
High (451)	Offspring Environment	$F_{2,370} = 2.66$		0.0713
	Sire	0.0085	(0.0081)	0.1283
	Dam(Sire)	0.0156	(0.0095)	0.0161
	Residual Variance	0.1372	(0.0102)	
<u>Offspring Mean Seed Mass</u>				
Medium (192)	Offspring Environment	$F_{2,178} = 10.82$		0.0001
	Sire	0.1053	(0.0774)	0.0429
	Dam(Sire)	0		--------
	Residual Variance	1.0194	(0.1159)	
High (157)	Offspring Environment	$F_{2,126} = 1.32$		0.2705
	Sire	0		--------
	Dam(Sire)	0.203	(0.1441)	0.0564
	Residual Variance	1.207	(0.1735)	

Figure 6. Germination, survival, and maternal fitness measures for the full data set including the offspring of natural mothers in the low light environment. (a) Proportion of offspring germinating, Fall 1996. (b) Proportion of offspring surviving to Spring 1997. (c) Mean seed number for offspring, June 1997. (d) Hypothetical cumulative maternal fitness if all offspring had germinated. This measure assumes no mortality prior to germination in dormant seeds, and equivalent survivorship and fecundity in dormant seeds after germination. (e) Mean mass of seeds produced by offspring. Environmental effects tested with fixed effects REML analysis. No genetic effects were included in the models. ME = maternal environment, OE = offspring environment. Bars are standard errors.

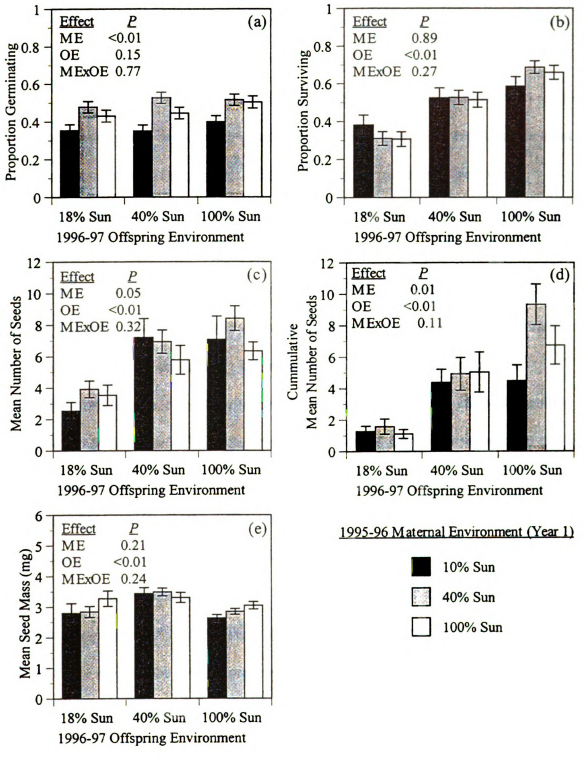

Effect P
ME <0.01
OE 0.15
MExOE 0.77
(a)
Proportion Germinating
18% Sun
40% Sun
100% Sun
1996-97 Offspring Environment
Effect P
ME 0.89
OE <0.01
MExOE 0.27
(b)
Proportion Surviving
18% Sun
40% Sun
100% Sun
1996-97 Offspring Environment
Effect P
ME 0.05
OE <0.01
MExOE 0.32
(c)
Mean Number of Seeds
18% Sun
40% Sun
100% Sun
1996-97 Offspring Environment
Effect P
ME 0.01
OE <0.01
MExOE 0.11
(d)
Cummulative Mean Number of Seeds
18% Sun
40% Sun
100% Sun
1996-97 Offspring Environment
Effect P
ME 0.21
OE <0.01
MExOE 0.24
(e)
Mean Seed Mass (mg)
18% Sun
40% Sun
100% Sun
1996-97 Offspring Environment
1995-96 Maternal Environment (Year 1)
10% Sun
40% Sun
100% Sun

Figure 6.

The significant maternal genotype-environment interaction for mean seed mass, and the considerable crossing of maternal family reaction norms indicate the presence of genetic variation for the plastic provisioning of seeds.

Proportion of offspring germinating-In the genetic analysis, there were no significant main effects on offspring germination: only the sire by maternal environment interaction is significant (Figure 5c). Within maternal environments, there were significant dam effects on offspring germination when seeds were produced in high light, but not in medium light (Table 2). When the low maternal light environment was added to the analysis, the effect of maternal environment became highly significant, while the germination environment continued to have no significant effect (Figure 6a). Overall, seeds produced by mothers growing in the medium and high light environments germinated at higher rates in each offspring environment, but mothers responded quite differently to these environments. Some mothers produced more dormant seeds in the medium light environment, while others did so in high light (Figure 5c). Seeds produced by mothers in low light were least likely to germinate anywhere.

Proportion of offspring surviving-In both the genetic and the environmental effects analyses there were no significant effects of maternal environment on offspring survival. Proportion surviving to flowering simply increased with light (Figures 5d, 6b). In the genetic analysis the sire-offspring environment interaction term was insignificant (P=0.09), but the reaction norm figure shows that mortality patterns for some families differed dramatically across environments (Figure 5d).

Offspring mean seed number-Light had highly significant effects on seed production by offspring in both analyses (Table 1, Figure 6c). Moreover, maternal

environment had a significant effect on offspring fecundity (P=0.02 in the genetic analysis, Table 1; P=0.05 in the environmental effects analysis, Figure 6c). The lowest mean fecundities were for the offspring of low and high light mothers in their home (maternal) environments. The offspring of medium light mothers performed as well as, or better than the offspring of other mothers for this trait (Figure 6c), and in the cumulative fitness calculation (Figure 6d).

Offspring mean seed mass-Light environment also had highly significant effects on offspring seed size in both the genetic (Table 1) and the environmental effects analyses (Figure 6e). Mean seed mass was greatest in the medium light environment, and lowest in the high light environment (Figure 6e). In the genetic analysis there was a significant maternal environment-offspring environment interaction for seed mass (P=0.048, Table 1). A within-maternal-environment genetic analysis was performed to understand this interaction (Table 2). The offspring of medium light mothers altered seed size in response to the environment (P<0.0001), but those from high light mothers did not (P=0.27). These results are consistent with the pattern seen in Figure 6e. Moreover, this analysis revealed genetic effects on seed provisioning expressed in offspring derived from both maternal environments (medium light P=0.04, high light P=0.056).

Fitness consequences of maternal effects

Phenotypic correlations with seed mass-Larger seeds were less dormant (Table 3), achieved a greater winter size (Table 4), and emerged later in the second year (Table 4). Phenotypic correlations between mean seed mass of mothers and proportion of offspring surviving (Table 3), and offspring seed production (Tables 3, 4), were low and insignificant when corrected for multiple tests. This result suggests that if present,

Table 3. Pearson product-moment correlations by light environment for the maternal traits. Offspring traits are the mean values for all the progeny of a single mother planted in each offspring environment. The number in parentheses is the sample size. Traits: Maternal mean seed number (M-Seeds), Proportion of offspring germinating (O-Germ), Proportion of offspring surviving to flower (O-Surv), Offspring mean seed mass (O-Mass), Offspring mean seed number (O-Seeds). Correlations in bold are significant after sequential Bonferroni adjustment at a table-wide level of α=0.05 (Rice, 1989). #P<0.1, *P<0.05, **P<0.01, ***P<0.001.

	M-Seeds	O-Germ	O-Surv	O-Mass	O-Seeds
10% Sun					
Maternal Mean Seed Mass	**-0.16*** **	**0.21*** **	-0.04	0.15#	0.15#
	(449)	(618)	(324)	(144)	(145)
40% Sun					
Maternal Mean Seed Mass	-0.11#	**0.26*** **	-0.02	0.15*	0.03
	(258)	(620)	(338)	(264)	(265)
100% Sun					
Maternal Mean Seed Mass	**0.25*** **	**0.23*** **	0.09	0.10#	0.12*
	(180)	(622)	(350)	(346)	(346)

Table 4. Pearson product-moment correlations with maternal mean seed mass by light environment for traits of individual offspring. Traits: emergence date (Edate), winter size (Wsize), mean seed mass (Smass), seed number (Seeds). Correlations in bold are significant after sequential Bonferroni adjustment at a table-wide level of α=0.05. #P<0.1, *P<0.05, **P<0.01, ***P<0.001.

Year	Environment	Edate	Wsize	Smass	Seeds
1995-96	10% Sun	0.02	**0.25*** **	0.43*	0.31*
	40% Sun	-0.01	**0.27*** **	**0.15*** **	0.11**
	100% Sun	-0.02	**0.25*** **	**0.29*** **	0.06
1996-97	18% Sun	**0.15*** **		0.15#	0.15#
	40% Sun	**0.18*** **		0.15*	0.03
	100% Sun	**0.18*** **		0.10#	0.12*

contributions of seed mass to offspring fitness were most likely mediated through the correlations with timing of emergence and winter size. The phenotypic correlations between maternal seed mass and seed number (Table 3) indicated a significant size-number tradeoff in low light (marginal in medium light), but in high light seed size and number were positively correlated.

Phenotypic selection-Seed mass, emergence date, and winter size had little effect on offspring fecundity (results not shown), so only the results of the survival episode are presented. Seed mass did not contribute directly to survival in any environment in either year (Figure 7a). In high light, later emerging seedlings were more likely to survive in the first year, but early emergence was favored in the second year (Figure 7b). This result is probably due to the absence of winter size in the multiple regression model in the second year, rather than direct selection for early emergence. In the year it was measured, winter size was an important determinant of survival across all environments (Figure 7c).

The path analysis confirmed that winter size was important for survival across all environments (Figure 8), and showed that both seed mass and early emergence were significant determinants of winter size. Interestingly, in the high light environment, the analysis reveals that there was significant direct selection for later emergence, but indirect selection for early emergence through winter size (Figure 8c). In other analyses (Chapter 4), I found that the presence of leaf litter consistently selects for late emergence. These results suggest that increased seed mass may allow seedlings to emerge later, yet still achieve a sufficiently large size to survive the winter. This possibility is consistent with the positive correlation between seed size and emergence date in the second year: larger seeds emerged later (Table 4).

Figure 7. Standardized linear selection gradients for survival episodes in each year. Bars are 95% confidence intervals based on 1000 bootstrap resampled data sets. Winter size data was not collected in Year 2 (1996-97). The y-axis indicates the proportion by which relative fitness would change if trait value changed by one standard deviation. It is scaled the same across all selection figures to facilitate comparisons among years, environments, and traits. Sample sizes: Year 1 (1995-96): low = 1003 medium = 1135 high = 808; Year 2 (1996-97): low = 493, medium = 530, high = 536.

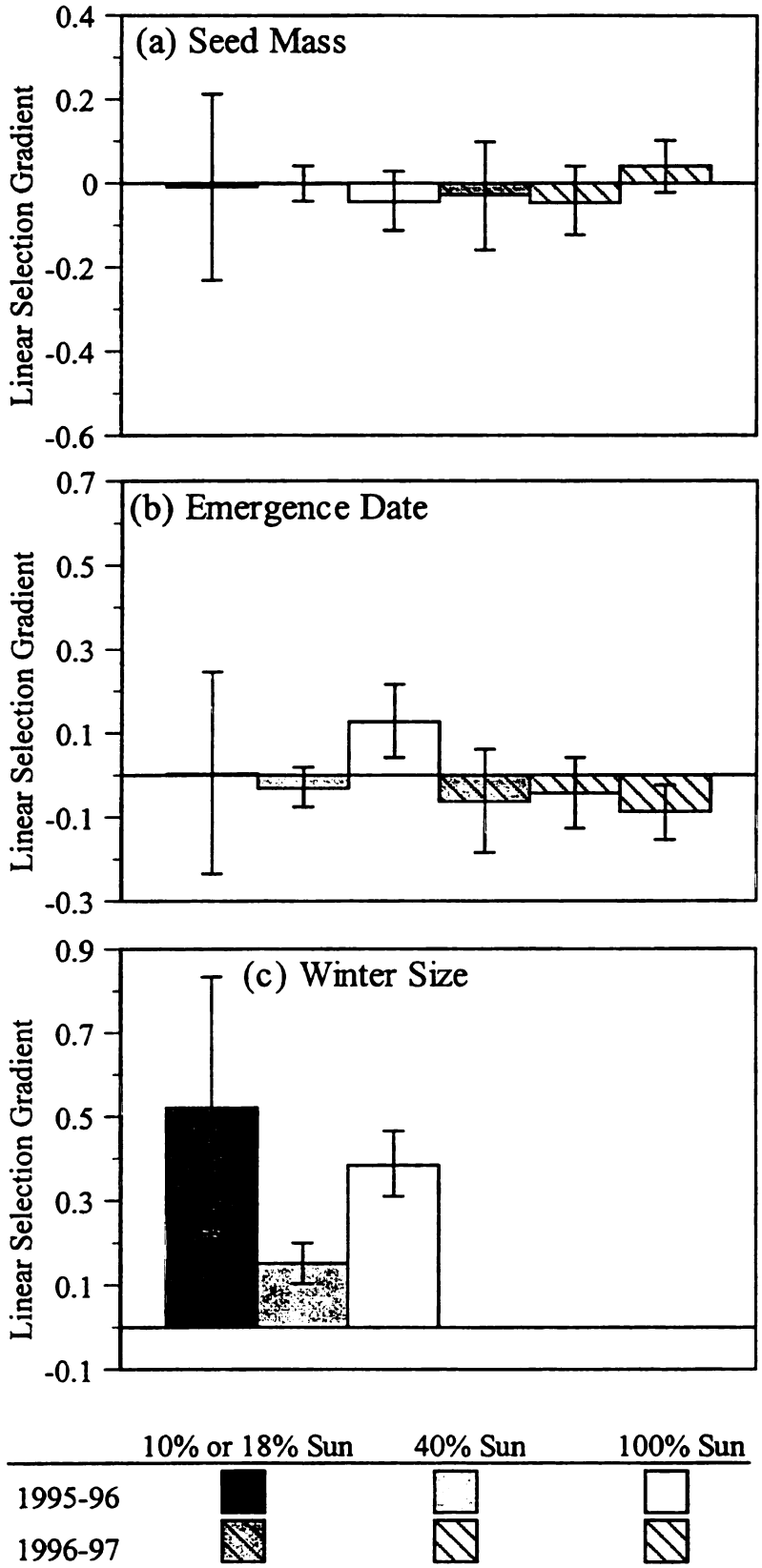

(a) Seed Mass
Linear Selection Gradient
0.4
0.2
0
-0.2
-0.4
-0.6
(b) Emergence Date
Linear Selection Gradient
0.7
0.5
0.3
0.1
-0.1
-0.3
(c) Winter Size
Linear Selection Gradient
0.9
0.7
0.5
0.3
0.1
-0.1
10% or 18% Sun
40% Sun
100% Sun
1995-96
1996-97

Figure 7.

Figure 8. Path diagrams for survival episode in Year 1. Double headed arrows represent correlations among phenotypic traits. Single headed arrows represent hypothesized causal links between traits and fitness. Path coefficients are analogous to standardized linear selection gradients, and were calculated using multiple linear regression on standardized traits. Logistic regression (Janzen and Stern 1998) produced identical results. Dashed arrows represent negative coefficients. The width of arrows is proportional to the magnitude of the standardized path coefficient. U: unexplained variance. Significance values: $*P<0.05$; $**P<0.01$; $***P<0.001$; $****P<0.0001$.

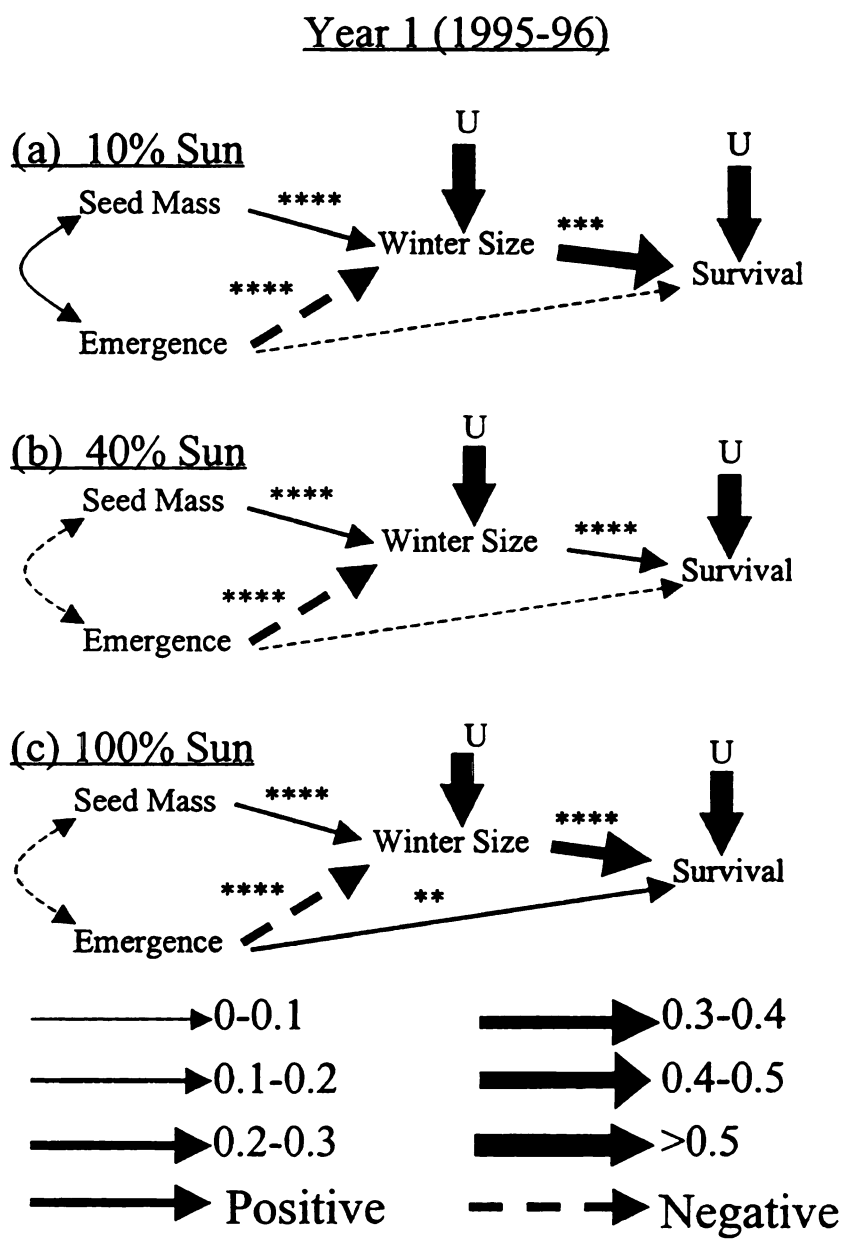

Year 1 (1995-96)
(a) 10% Sun
U
U
Seed Mass
****
Winter Size
***
Survival
****
Emergence
(b) 40% Sun
U
U
Seed Mass
****
Winter Size
****
Survival
****
Emergence
(c) 100% Sun
U
U
Seed Mass
****
Winter Size
****
Survival
****
**
Emergence
0-0.1
0.1-0.2
0.2-0.3
Positive
0.3-0.4
0.4-0.5
>0.5
Negative

Figure 8.

DISCUSSION

The results of this study demonstrate that: (1) light environments in this population of *Collinsia verna* vary in space but are correlated across years in a way likely to favor plastic maternal effects (Figure 4); (2) there are significant maternal environmental effects on seed number (Figures 5, 6), seed size (Figure 5), and dormancy (Figure 6); (3) there are significant genotype-environment interactions in the field for seed size and dormancy (Figure 5); and (4) seed size may have important consequences for offspring fitness through its effect on size at overwintering. Below, I discuss whether these results support the hypotheses that larger seed size increases offspring establishment in reduced light environments and increased seed dormancy benefits mothers in low light environments where offspring survival is unlikely. I conclude with a discussion of what the genotype-environment interactions for seed size and dormancy found in this study suggest about the factors affecting the evolution of plastic maternal effects.

Seed size plasticity and variation in dormancy-In both years of this study, plants growing in the high light environments produced smaller seeds on average than plants growing in the medium light environments (Figures 5b, 6e). In low light in both years mean seed mass was lowest and most variable. Reduced seed mass in the low light environments was probably a consequence of absolute resource limitation. The mean number of seeds produced by mothers in low light (10% sun) was only slightly more than one (Figure 5a), while offspring in the low light environment (18% sun) produced about three seeds (Figure 6e). The significant negative correlation between seed size and number in the low light environment but not in medium or high light (Table 3), suggests

a seed size-number tradeoff in low light.

Increases in seed mass as light resources decline from high to medium levels would be beneficial if offspring experience light environments similar to their mothers (as shown here), and if offspring from large seeds had higher establishment, survival, or fecundity in medium but not high light environments. However, the other results of this study provide limited evidence that larger seeds may be indirectly beneficial to offspring survival and fecundity at all light levels. Although the offspring of medium and high light mothers survived at similar rates across all environments (Figure 6b), and there was no direct selection on seed size in the survival episode (Figure 7), the path analysis suggested that increased seed size benefits offspring indirectly in all environments by contributing to larger overwinter size, and higher survival to flowering (Figure 8). Seed mass was only very weakly correlated with subsequent seed production (Table 4), and was under no direct selection in the fecundity episode (results not shown), but the heavier offspring of medium light mothers produced as many or more seeds than offspring of high or low light mothers by both the direct and cumulative measures (Table 1, Figures 6c-d).

Other studies have consistently shown direct and indirect effects of increased seed size on dormancy, emergence time, early size, early growth rates, and many traits later in the life history (recent reviews in Westoby et al. 1997, Rees 1997). In competitive situations, differences in seedling size can persist throughout the life cycle, and lead to differences in fitness (Gross 1984, Stanton 1984, Fenner 1985, Morse and Schmitt 1985, Stratton 1989, Gross and Smith 1991). Although Thiede's (1996) two-year study of selection on early life history traits in this population of *Collinsia verna* found no direct

selection on seed mass, she did find direct selection for later emergence and larger winter size. In addition, both emergence time and winter size had positive genetic correlations with seed size.

Other univariate (Kalisz 1986, Winn 1988, Biere 1991b), and multivariate (Mitchell-Olds and Bergelson 1990, Stratton 1992, Bennington and McGraw 1995) selection studies also have detected relationships between juvenile traits (seed mass, emergence date, and juvenile size) and survival or fecundity. Donohue and Schmitt (1998) showed that greater seed mass in *Plantago lanceolata* increases individual fitness; however, in contrast to this study, they found that maternal fitness was enhanced in some environments when mothers reduced seed mass and produced more seeds.

The reduced performance of the offspring of low and high light grown mothers (Figures 6c-e) may have many causes. Many of these offspring started from smaller seeds. Resource limitation in the low light treatment likely accounts for the lack of seed set in more than 60% of the plants that survived to harvest. In addition, plants in low and high light environments flowered out of synchrony with the rest of the population (Chapter 4). Consequently, these plants may have received poor pollinator service, and their seeds may have been produced through self pollination (Kalisz et al. 1999). There is modest inbreeding depression in this species (Kalisz 1989), and population (Kalisz et al. unpublished data). As a result, the possible positive maternal effects on survival seen in the home environments of offspring of low and high light mothers may be offset later in the life history by the expression of inbreeding depression.

Other selective factors not addressed in this study may favor small seeds in high light environments. Seed predation has been shown to be higher on forest edges (e.g.

Jules and Rathcke 1999, Manson et al. 1999), and some evidence suggests that seed foraging rodents favor species with larger seeds (e.g. Kelrick et al. 1986, Reader 1993, Hulme 1998). But there is little direct evidence that seed predators select larger seeds within species, and there are many examples of non-size-selective seed predation (e.g. Kerley and Erasmus 1991, Meiners and Stiles 1997). It has been suggested that seed predation on species with a soil seed bank should select for smaller seeds (Thompson 1987).

The significant maternal environment-offspring environment interaction effect on the size of seeds produced by offspring is intriguing (Table 1). Essentially, the size of grandchildren (and possibly their fitness) is in part determined by the environment of their grandmothers. Grandparent environment has been shown to effect grandprogeny phenotype in the lab in both plants (e.g. Case et al. 1996 and references therein) and animals (Fox and Savalli 1998 and references therein), but studies showing such effects in the field are rare. In the present study, the offspring of medium light mothers differentially provisioned seeds depending on their own light environment. In contrast, the offspring of high light mothers were insensitive to their own environment, and always made large seeds. Because in the second year plants in high light produced no more seeds than plants growing in medium light (Figure 6c), the results presented here suggest that high light could also be a stressful environment.

The higher dormancy in offspring of low light mothers (Figure 6a) may allow these mothers to disperse their offspring further in space or time to better environments, or may be beneficial if pre-reproductive mortality in reduced light is variable from generation to generation. However, a strong test of these hypotheses would be very

difficult because individual dormant seeds would have to be followed for many years. Moreover, these more dormant seeds may simply be smaller and less viable because of resource limitation in their mothers, or they may be the less viable products of self fertilization.

One other aspect of the offspring of low light mothers deserves comment. The insignificant trend toward lower mortality in low light and higher mortality in high light for the progeny of low light parents (Figure 6b), may be the result of a beneficial maternal effect, but the result suggests that this effect is mediated through some aspect of seed quality not measured by seed size. First, as mentioned above, each year low light plants on average produced smaller seeds (Figures 5b, 6e). Moreover, the higher rate of dormancy in the offspring of low light mothers suggests that they may have thicker seed coats that would reduce the proportion of total seed mass allocated to resource storage. Differences in nutrient concentration (nitrogen, etc.) in seeds, or the use of more concentrated energy stores (lipids instead of carbohydrates) might affect seed quality but not seed size (Westoby et al. 1992).

Genotype-environment interactions-The most striking results here are the genotype-environment interactions for dormancy and seed size (Figures 5b-c). Nearly all studies that have investigated maternal environment effects on seed mass have found them (reviewed by Roach and Wulff 1987, Platenkamp and Shaw 1993, but see Weiner et al. 1997). More recent studies have demonstrated significant genetic variation in seed mass, germination date, and seedling size (e.g. Shaw et al. 1995, Helenurm and Schaal 1996, Sultan 1996, Byers et al. 1997, Husband and Gurney 1998, Thiede 1998). But studies that show genetic variation for plastic maternal effects are rare (Donohue and

Schmitt 1998). Previous studies in *Collinsia verna* have shown that seed size and seed dormancy are variable (Kalisz 1989, Thiede 1996), and under both additive genetic and maternal genetic control (Thiede 1998, unpublished data). The results of the present study suggest that any main effects of either genotype or environment on these traits should be interpreted with caution.

The genotype-environment interactions for seed size and dormancy can be interpreted as genetic variation for plasticity in these traits. Artificial selection on the plasticity of dormancy and seed size would almost certainly succeed in altering reaction norms. However, the alternate question is why does this genetic variation persist? Other factors may be limiting plasticity evolution and contributing to the maintenance of genetic variation in plasticity of seed size and dormancy (Mitchell-Olds 1992, Schmitt 1995). Future evolution in depends on the patterns of natural selection on these traits, gene flow between environments, and on the genetic correlations among traits. In particular, variable selection can maintain genetic variation in natural populations if there are genotype-environment interactions for components of fitness.

The basic quantitative genetic equation for multivariate evolution, $\Delta \mathbf{z} = \mathbf{G}\beta$, shows that trait evolution depends on both the selection gradients, β, and the genetic covariance matrix, **G**. Two hypotheses stand out as explanations for the persistence of genetic variation in the plasticity of dormancy and seed size. First, direct selection (β) on these traits may be variable within light environments so that optima vary and fluctuate. Second, correlated responses to selection may maintain some traits away from their univariate optima. In both cases, genetic variation would be maintained.

Course grained, between-generation variation in survival to reproduction can select for increased dormancy (reviews in Brown and Venable 1986, Evans and Cabin 1995, Rees 1997). If this selection differs across environments, mothers should produce seeds that are more dormant in some environments than others. However, if variation in survival to reproduction is fine grained (occurring within environments or generations), and there are no good cues to predict offspring survival, then genetic variation in dormancy may be maintained. In a companion study to this one, I have found a twofold difference in survival to reproduction within light environments as a consequence of the presence or absence of leaf litter (Chapter 4). The distribution of leaf litter in deciduous forests during the fall is unpredictably variable at the single leaf scale (Frankland et al. 1963, Sydes and Grime 1981, Facelli and Carson 1991, Molofsky and Augspurger 1992, personal observation). In this population, leaf litter is quite transient, with substantial decomposition occurring before the onset of winter (personal observation). Consequently, leaf litter may be an unpredictable cause of fine grained variable natural selection on dormancy independent of light environment, and may lead to the persistence of genetic variation in dormancy and its plasticity.

There is strong evidence that selection on seed size in this population is indirect through emergence date and winter size (this study, Thiede 1996). Other studies in *Collinsia verna* have found significant positive genetic correlations between seed size and emergence date (Kalisz 1989, Thiede 1998) and seed size and winter size (Thiede 1998); and significant negative genetic correlations between emergence date and winter size (Thiede 1998, Chapter 5). Consequently, selection for larger winter size as is consistently seen produces correlated responses for larger seeds and earlier emergence. However,

since selection for early emergence produces correlated responses for smaller seeds, the overall effects on seed size are unclear. Further, my study of natural selection in this population (Chapter 4) found that the direction of direct selection on emergence date (and consequently selection on seed size) changes depending on the presence or absence of leaf litter across all light environments. Leaf litter selects for later emergence and larger seeds; the absence of leaf litter selects for earlier emergence and smaller seeds.

The path analysis (Figure 8) suggests that early emergence and larger seed size are alternate strategies that both allow seedlings to achieve a size sufficient to survive the winter. Larger seeds may be further advantaged if they emerge later and thereby avoid the negative effects of leaf litter in early fall. More interesting still, seed size appears to be largely controlled by maternal genes (Chapter 3, Thiede 1998), while timing of emergence is an additive genetic trait (Chapter 3, Thiede 1998). Consequently, the evolution of seed size will have very complicated and unpredictable dynamics where selection in parents and offspring is likely to conflict (Westoby et al. 1992).

Conclusion-The spatial and temporal variation in light in this population favors the evolution of plastic maternal effects, and seed size and dormancy are plastic. But genetic variation in plasticity of seed size and dormancy may be maintained by variable direct selection or correlated responses. Environmental factors like leaf litter may contribute to this unpredictable, heterogeneous selective environment resulting in the maintenance of a diversity of specialized genotypes with different seed provisioning and dormancy strategies. Offspring of mothers from the two extreme light environments produced fewer, and often smaller seeds when grown in their home environments. This result suggests that some plasticity of seed size seen in this study may be a maladaptive

consequence of stressful maternal environments.

This study demonstrates the value of performing environmental manipulations in the field to study micro-evolutionary processes. By quantifying maternal effects, plasticity, genetic variation, and fitness components in an ecologically and evolutionarily relevant context, it was possible to assess both the adaptive value of plasticity and the factors that might affect the future evolution of the population. Wider application of this approach will help to answer fundamental questions about maternal effects, plasticity evolution, and the factors that maintain genetic variation in populations.

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Chapter 3

EVOLUTION OF REACTION NORMS: THE QUANTITATIVE GENETICS OF RESPONSES TO VARIABLE LIGHT ENVIRONMENTS IN A NATURAL PLANT POPULATION

INTRODUCTION

The consequences of environmental heterogeneity are an important focus of theoretical and empirical studies in evolutionary ecology. Quantitative traits in all organisms generally exhibit plastic responses to changes in the biotic or abiotic environment (Travis 1994, Roff 1997). Recent studies demonstrate that environmental heterogeneity can cause spatial and temporal variation in natural selection (e.g. Kalisz 1986, Kelly 1992, Stratton 1992, 1995). Depending on patterns of gene flow, the magnitude of fitness differences, and stochastic factors, variable selection can both maintain genetic variation within populations and lead to adaptive differentiation between populations or subpopulations (e.g. Gillespie and Turelli 1989, Mitchell-Olds 1992; see also Hedrick et al. 1976, Hedrick 1986). Other theory predicts that with gene flow between environments, variable selection may lead to phenotypically-plastic generalist genotypes that perform well across a broad range of environments (e.g. Via and Lande 1985, de Jong 1995, van Tienderen 1997, Scheiner 1998; reviews in Scheiner 1993, Roff 1997).

However, many factors may constrain reaction norm evolution and result in the persistence of specialist genotypes and the maintenance of genetic variation. These factors include the scale of environmental heterogeneity (course vs. fine; Lynch and Gabriel 1987, van Tienderen 1991, Gabriel and Lynch 1992), a lack of predictability

about future environmental states (Bradshaw 1965, Moran 1992, Getty 1996), developmental limitations (van Tienderen 1990), physiological costs of plasticity (Via and Lande 1985, van Tienderen 1991, DeWitt et al. 1998), or a variety of genetic constraints (discussed below, Falconer 1952, Via and Lande 1985). Data from natural populations regarding the genetics of responses to variable environments is scarce (recent reviews in Roff 1997, Schlichting and Pigliucci 1998).

Genetic constraints and the evolution of reaction norms

Bradshaw (1965) recognized that the characteristics of plastic responses are specific to particular environmental factors and are under genetic control. In this context, the terms generalist and specialist describe the relative ability to maintain fitness in variable environments through coordinated, plastic responses in underlying traits. Generalists have adaptive plasticity in physiological and/or morphological traits that allows them to maintain performance when resources are scarce, and to boost performance when resources are abundant (de Jong 1990, Thompson 1991). Whether generalists can be as fit in particular environments as specialists on those environments remains controversial, and can affect whether variable selection favors plasticity or the maintenance of genetic variation (Gillespie and Turelli 1989). Specialists for favorable environments lack the underlying plasticity in physiological, morphological, and behavioral traits necessary to maintain high relative fitness in less favorable environments. Specialists for stressful environments may be more fit than generalists in those environments, but may lack the ability to respond when resources are abundant, and so have low, flat reaction norms for fitness.

Because different combinations of plasticity and canalization in underlying

developmental, physiological, or morphological traits can trade off to produce equally fit genotypes, there is no simple relationship between fitness across variable environments and plasticity in a trait (Via 1987, Schlichting and Pigliucci 1995). For example, in a series of greenhouse experiments, Sultan and Bazzaz (1993a, b, c) found that diverse patterns of physiological and morphological plasticity to light, water, and nutrients can produce convergent reaction norms for reproductive performance. Thus, it is necessary to consider how groups of traits are integrated to produce generalists or specialists.

Genetic constraints on the evolution of plasticity include an absolute lack of genetic variation and genetic interdependence between the expression of single traits in different environments. When the same genes determine a trait in different environments, the cross-environment genetic correlation approaches one or negative one. Selection in one environment will change the expression of the trait in all other environments. Values of the cross-environment genetic correlation significantly different from one or negative one indicate the presence of genotype-environment interaction, which is a measure of genetic variation for phenotypic plasticity (Via 1987). As the cross-environment genetic correlation for a trait approaches zero, genetic variation for plasticity of the trait increases. Depending on the relationship between a trait and fitness in different environments, significant negative or positive cross-environment genetic correlations can either slow or accelerate reaction norm evolution. For example, Shaw and coworkers (1995) found a negative genetic correlation for dry mass across manipulated competitive environments in a study of *Nemophila menziesii*. This negative relationship is expected to maintain specialist genotypes in the population and slow the evolution of a competitive generalist. Similarly, genetic correlations between different traits within and across

environments may also affect plasticity evolution.

Plastic responses to light

Light is an important resource for photosynthetic plants, and light availability is a strong selective agent in plant populations (reviews in Goldberg 1990, Sultan and Bazzaz 1993a). Moreover, because light availability varies in space and time, selection should favor generalist genotypes possessing plastic traits that would both allow tolerance of low light levels and the conversion of high light availability into increased growth and reproduction. Genotypic differences in the plastic response of many physiological, morphological, and life history traits to light quantity or quality have been characterized many times in greenhouse or common garden environments (e.g. Clough et al. 1980, Sultan and Bazzaz 1993a, Schmitt 1993, Andersson and Shaw 1994, Pigliucci et al. 1995, Dudley and Schmitt 1995). However, it is uncertain if the genotypic differences seen in these studies would be expressed in the field, or if expressed how they might affect fitness or the dynamics of plasticity evolution under natural conditions.

A better understanding of the evolution of phenotypic plasticity requires the careful manipulation of selectively relevant environmental factors and the characterization of additive genetic variation for phenotypic plasticity within natural populations of plants (Schmitt 1995, Sultan 1995, Pigliucci 1996). In plants, maternal effects are known to be an important source of resemblance between relatives (Roach and Wulff 1987, Donohue and Schmitt 1998). Moreover, it has been shown that maternal effects can obscure genetically based tradeoffs in performance across environments (Shaw et al. 1995). Relatively few studies have characterized additive genetic variance and covariance independent of maternal effects within natural plant populations (e.g.

Mitchell-Olds 1986, Mitchell-Olds and Bergelson 1990, Schwaegerle and Levin 1991, Montalvo and Shaw 1994, Schoen et al. 1994, Campbell 1996, 1997a, b). Fewer still have estimated these parameters across a range of natural field environments (Shaw et al. 1995, Bennington and McGraw 1996, Wulff 1998). Recent empirical comparisons suggest that heritability estimates may be similar across different environments (Roff 1997). However, it is the genetic correlations that are keys to plasticity evolution, and recent comparisons of genetic correlations across environments and between populations have found no consistent patterns (reviews in Roff 1997).

In this study, two generations of half-sib *Collinsia verna* plants were grown in the field in three manipulated and two natural light environments. Here I address the following questions concerning the evolution of phenotypic plasticity: (1) Are there additive genetic and/or maternal effects on traits with plastic responses to light environment? (2) Are there genotype-environment interactions indicative of genetic variation in plasticity to light? (3) Are there fitness differences among genotypes that would suggest specialization for particular light resource environments? By addressing these questions under natural field conditions, this study avoids many of the interpretive limitations of lab, greenhouse or common garden experiments.

METHODS

Study system

Collinsia verna is a winter annual wildflower native to moist woods and floodplain forests in eastern North America. In many *Collinsia* habitats, light is so limiting during the summer months that the forest floor is nearly barren. All growth of *C. verna* occurs during the seasonal light-gap between forest canopy senescence in the fall

and leafout in the spring. The one hectare study population resides along the south facing edge of a woodlot adjacent to an agricultural field in Kalamazoo County, southwest Michigan. Seed germination in the fall is cued by diurnal temperature fluctuations (Baskin and Baskin 1983). Seedlings emerge between September and December, and overwinter with only cotyledons or a single pair of leaves. Flowering begins in late April and ceases as the forest canopy closes in May. By mid June fruits ripen, seeds are passively dispersed, and plants die. Fecundity depends on the micro-environment, ranging from five to fifty seeds.

Experimental design

Light treatments-Light in this population is spatially and temporally variable. Peak irradiance in full sun at solar noon varies between 500 and 2000 μmoles m^{-2} sec^{-1} over the October to May growing season. Depending on adjacent woody vegetation and proximity to the southern edge, small patches within the forest receive from 25% to 75% of this maximum (Figure 4 of Chapter 2). To establish different light levels in the field, in July 1995 I cleared all trees and shrubs from a 15 X 20 m area along the southern edge of the woodlot in the densest area of the *Collinsia* population. I then established 15 1.2 m^2 plots in a 5 X 3 grid with 1 m spacing between plots (5 blocks of 3 plots each). I randomly assigned one of three light treatments to the plots of each block: 100% sun (high), 40% sun (medium), and 10% sun (low) (Figure 1 of Chaper 2).

Light treatments were constructed using a wood lattice that continued to allow sun-flecks to reach the plots (Chapter 2). Mortality in low light during the first year (1995-96) was greater than 95%. To increase survival for the second year (1996-97), light levels in this treatment were increased from 10% to 18% of full sun in September

1996. For the second year, 10 additional natural plots were established. These plots were chosen to represent the range of natural light experienced by the population. Five plots were located in the forest interior (45% Sun) and five were located along the southern edge of the woodlot (70% Sun).

Breeding design-I used two independent breeding designs to generate the families used in this study. Plants were collected prior to flowering at 5 to 20 m intervals from the full area of the population (204 in year 1, 50 in year 2). Crosses were performed in the greenhouse at Kellogg Biological Station. Sires were randomly chosen (year 1: 50; year 2: 12). Each sire was mated to three unique dams in standard nested half-sib designs (North Carolina Design I, Lynch and Walsh 1998, Chapter 18). In the first year, two sires with small initial mates were each mated to one additional dam. Flowers of dams used in crosses were emasculated in the bud stage to prevent self pollination. The crosses produced 9338 and 1776 seeds in each year respectively. In year one, an average of 45 seeds from each full-sib family were randomly planted into the field plots (6840 seeds total: 152 full-sib families X 3 seeds/family X 3 light treatments X 5 blocks). In year two, an average of 50 seeds from each full sib family were randomly planted into each field plot (1776 seeds total: 36 full-sib families X 2 seeds/family X 5 light treatments X 5 replicates). Seeds were individually planted in 2 cm long uniquely numbered plastic straws inserted in the ground.

In each year emergence and survival censuses were conducted weekly from September through December. When emergence ceased the number of cotyledons and true leaves on each seedling were counted, and the diameter of the largest cotyledon and leaf were measured. These data were used to calculate winter size (cotyledon area + leaf

area). I conducted a census for survival to flowering in mid-April. Starting in late April, the date of first flowering was recorded daily. After a plant flowered, a uniform sized piece of leaf tissue was collected with a hole punch from the youngest fully expanded leaf. Samples were dried and weighed to determine specific leaf area. This trait quantifies the morphological changes made in leaf characters to optimize photosynthetic ability in different light environments. Plants surviving to the end of the experiment were harvested prior to seed dispersal in June of each year and scored for mainstem length, above ground vegetative biomass, number of seeds, and total seed mass. Reproductive investment was calculated as the proportion of total above ground biomass allocated to seeds. Because plants were dead or dying, this trait measures the efficiency with which they were able to convert vegetative biomass to seed mass.

The light manipulations used in this study may simultaneously change many aspects of the biotic and abiotic environment. To better understand these affects, I measured light (Chapter 2) and several other parameters in the study plots. I counted number of conspecifics at overwintering and harvest; I measured soil temperature throughout the season using Onset Hobo data loggers; and I measured volumetric water content of the soil weekly in the fall using time-domain reflectometry.

Data analysis

Heritability-Within environment heritabilities (h^2) were estimated using MTDFREML (Multiple Trait Derivative Free Restricted Maximum Likelihood, Boldman et al.1995). MTDFREML is a set of programs developed to apply the animal model (description in Lynch and Walsh 1998, pp. 755-758) to the estimation of genetic variance components and the prediction of breeding values. Unlike ANOVA methods, the animal

model uses all available pedigree information to produce restricted maximum likelihood (REML) estimates of genetic variance components. From these variance components the BLUP (best linear unbiased prediction) breeding values were calculated. Likelihood ratio Chi-square tests were used to assess the significance of the additive genetic variance components. Vegetative biomass and seed number were log transformed to improve normality prior to all analyses.

All assumptions of the nested design for estimation of quantitative genetic parameters were met in this study (Mitchell-Olds 1986, Mitchell-Olds and Rutledge 1986). Although *Collinsia verna* is self-compatible, outcrossing rates in this population were above 0.9 in each year of a three-year study (Kalisz et al. unpublished data), so upward bias in heritabilities due to inbreeding should be negligible. The high pre-flowering mortality in some environments in each year could bias the estimation of genetic parameters for subsequent traits if mortality was associated with some sibships. However, there were no significant effects of sire or sire-environment interaction on survival, indicating that no measurable selection on half-sib families occurred at this stage (see Results).

Environmental effects and interactions-Main effects of environment and genotype, and genotype-environment interactions were analyzed statistically using maximum likelihood methods and graphically by examining reaction norm plots for genotypic differences in slope. The approaches are complimentary and equally valuable because the power of statistical methods to detect genotype-environment interaction can be limited (Lewontin 1974, Via and Lande 1985, Wahlsten 1990). Reaction norm figures were constructed for each year by plotting paternal half-sib family trait means

against light environments. Genetic variation for trait plasticity is suggested when trait values for families change rank across environments. Changes in rank for fitness suggest that negative cross-environment genetic correlations may retard the evolution of generalists (Via 1984).

Mixed model REML analysis was used to examine the main and interactive effects of light environment, block, sire, and dam on each trait in each year. A split-plot model was used, with light environment as the whole plot factor, and sire and dam as subplot factors. Maximum likelihood methods are generally superior for analysis of unbalanced data (Searle et al. 1992, Littell et al. 1996). All analyses were completed using the MIXED procedure of SAS (SAS 1992, 1997). Blocks were treated as fixed because they were designed to capture an east-west gradient of diurnal morning-afternoon shading. Environmental effects were also treated as fixed, while sire, dam nested within sire, and their interactions with fixed effects were treated as random. Because the new natural plots in the second year could not be included in blocks with the existing experimental treatments, no block effect was included in the full analysis this year. Block effects in the first year and in an analysis of the three treatments in the second year were all insignificant.

The final analysis included all main effects and two way interactions, except for the dam by block interactions. When examined in reduced models, the dam by block interactions and all three way interactions were highly insignificant. The significance of random effects were assessed using likelihood ratio tests, while fixed effects were tested by computing a Type III F statistic using the Satterthwaite option to estimate denominator degrees of freedom (Littell et al. 1996). No sequential Bonferroni

corrections (Rice 1989) have been applied to the results of these analyses because correction for multiple tests is not appropriate when correlations are expected between traits (Manly 1991, Simons and Roff 1996, Lynch and Walsh 1998 page 641). It is the general patterns that are of interest here, rather than specific comparisons among traits or environments.

Because germination and survival are discrete variables they were analyzed using maximum likelihood logistic regression (SAS LOGISTIC procedure, SAS 1989). This is the most appropriate analysis for data of this type, but the procedure cannot accommodate the nested structure of the data (dams nested within sires). The fit of models with sire effects were compared to the fit of models with dam effects using likelihood ratio chi-square tests. Models with dam effects generally provided a poorer fit to the data, and never significantly improved the likelihood of sire based models. Consequently, the results presented are for models including terms for sire, light environment, and sire by light environment interaction.

Genetic correlations across environments-The genetic correlations (r_g) between the same trait expressed in different environments indicate the degree to which traits are free to evolve independently in those environments. They are directly interpretable in terms of evolutionary quantitative genetic theory and they provide a dimensionless measure for comparisons between traits and across environments (Via 1984, 1987, Via and Lande 1985). The best way to estimate genetic correlations across environments is the subject of much discussion and research (Via 1984, Dutilleul and Potvin 1995, Windig 1997, Dutilleul and Carriere 1998). The half-sib breeding designs used in this study allow the calculation of the narrow sense additive genetic correlations (unbiased by

any dominance or maternal effects) by two complementary methods.

First, the cross environment additive genetic correlations were calculated as the Pearson product-moment correlations of the within environment predicted sire breeding values from the MTDFREML analyses described above. This method is similar to the family means approach (e.g. Via 1984, Simons and Roff 1996) but it eliminates bias due to unbalanced data and fractional contributions of dominance and environmental effects (Shaw et al. 1995, Lynch and Walsh 1998). Limitations of this approach are that genetic correlations cannot be calculated when additive genetic variance components are estimated as zero, and as in the case of family mean correlations, sampling error may cause the calculated genetic correlations to underestimate the true values (Cameron 1993, Notter and Diaz 1993, Mathur and Horst, 1994). This bias means that traits may not be as genetically independent across environments as it appears based on the correlations among breeding values.

For comparison the cross environment genetic correlation was also estimated from the variance components of mixed-model REML analyses (SAS proc. MIXED) according to the formula:

$$r_g = \frac{\sigma^2_{sire}}{\sqrt{\left(\sigma^2_{sireE1}\right)\left(\sigma^2_{sireE2}\right)}}$$

where σ^2_{sire} is estimated by the sire variance component from a two-way analysis, and σ^2_{sireE1} and σ^2_{sireE2} are estimated by the sire variance components from separate one-way analyses in each environment (Yamada 1962, Fry 1992, Windig 1997). As with estimating the genetic correlation from breeding values, the genetic correlation estimated by this technique is undefined whenever σ^2_{sireE1} or σ^2_{sireE2} are zero. When σ^2_{sireE1} or σ^2_{sireE2}

are near zero, sampling error can produce correlations that are outside of theoretical bounds, sometimes resulting in estimates of r_g that can be much in excess of one (Fry 1992).

The advantage of calculating product-moment correlations from predicted breeding values is that they produce estimates that are within the theoretical bounds for correlations and the usual *t* test of the null hypothesis $r_g = 0$ is easily applied. However, the use of Fisher's *z* transformation (Sokal and Rohlf 1981) to test the alternative null hypothesis for genetic data ($r_g = 1$) has been shown to produce biased (Roff and Preziosi 1994) and highly unreliable results (Windig 1997). Fortunately, for the cross environment genetic correlations calculated from mixed model REML analyses, the test of the sire-environment interaction term is a test of the null hypothesis $r_g = 1$, and the test of the sire effect is a test of the null hypothesis $r_g = 0$ (Fry 1992). An insignificant sire-environment interaction component suggests that the genetic basis of a trait is the same in each environment. Other recent studies have applied jackknife or bootstrap resampling methods to directly calculate standard errors of genetic correlations (e.g. Roff and Preziosi 1994, Windig 1994, Reusch and Blanckenhorn 1998, Phillips 1998). However, the appropriate resampling level in complex designs such as this one is unclear (Shaw 1992). The available tests are sufficient to allow full interpretation of the results.

RESULTS

Light environment effects

All traits were significantly plastic in at least one year (germination, emergence date, winter size, survival to flowering, flowering date, specific leaf area, mainstem length, vegetative biomass, seed number, mean seed mass, and reproductive investment;

Figure 9a-v). The primary difference between years occurred in traits expressed early in the life cycle. The autumn of the first year was warm and dry. As a result, germination rates were more variable, and emergence was delayed. This resulted in less variation in emergence date and winter size. A more favorable germination environment in the second year resulted in a higher fraction germinating, and earlier, more variable emergence dates. Earlier emergence gave plants more time to respond to variation in resources and resulted in greater size variation in December.

The light manipulations affected other aspects of the environment. There was a higher incidence of grazing on *C. verna* by small herbivores in the low and medium light treatments. Under winter snow cover, the temperature of the top 1 cm of soil remained constant near freezing in the natural and full sun treatments, but the lattice covers prevented snow accumulation, and temperatures in these treatments plunged to as much as -15 degrees C with wide diurnal fluctuations. In spring, the top 1 cm of soil in the high light treatments was warmer and had more variable temperatures. The daily maximum temperature in these plots in May 1997 often exceeded 35 degrees C compared to less than 20 degrees C in shaded plots. The soil was drier in the high light treatments. The mean weekly volumetric water content of top 5 cm of soil during October 1996 in high light was 0.12 m^3 water / m^3 soil compared to 0.15 m^3 water / m^3 soil in each of the other treatments (0.01 s.e. in all cases).

In contrast to these patterns, there were no clear relationships between the light treatments and the density of naturally occurring conspecifics at harvest in either year. The range was broad (from 75 plants/m^2 in high light in year two, to 879 plants/m^2 in high light in year one), but most plots in each year had between 100 and 200 plants/m^2 at

Figure 9. Paternal half-sib family reaction norms for Years 1 (50 sires and 3 light treatments), and 2 (12 sires and 5 light treatments (3 manipulated and 2 natural)). The significance values for all traits except germination and survival are based on univariate mixed model REML analysis. Year 1 traits were analyzed with a model that included sire, dam(sire), light, block, and all two way interactions, except the dam-by-block interaction. The model for the second year omitted all block terms. Germination and survival were analyzed by logistic analysis with a simplified model including only sire, light, and their interaction. See text for details. The bars on the right side of the figures represent one average standard error for all sires in the most variable environment. No error bars are presented on the vegetative biomass and seed number graphs because this data is plotted on a logarithmic scale. The same symbol is used for the same sire throughout all figures. The light level in the forest interior plots averaged 45% of full sun and on the edge it averaged 70% of full sun.

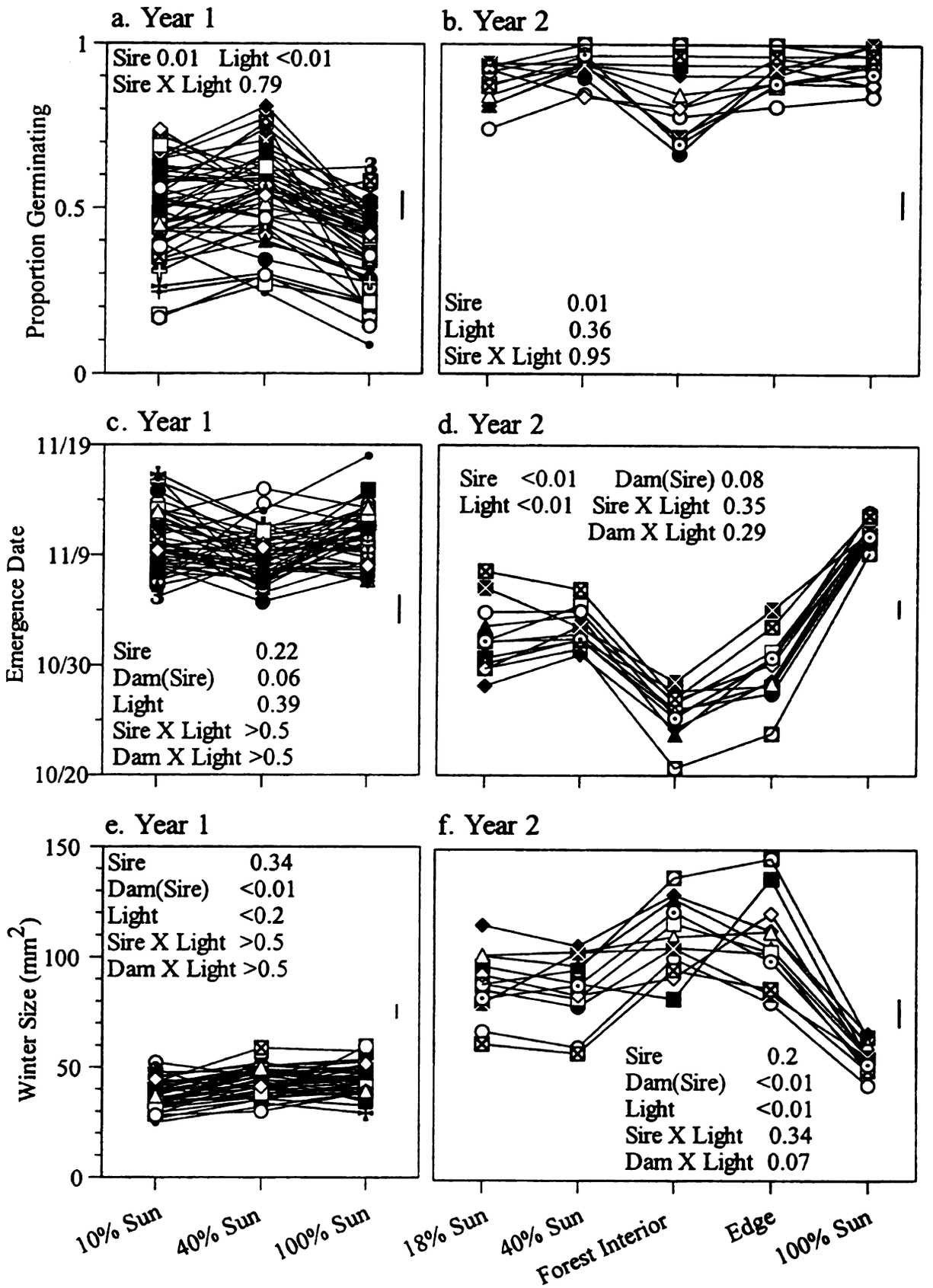
a. Year 1
Sire 0.01 Light <0.01
Sire X Light 0.79
Proportion Germinating
1
0.5
0
b. Year 2
Sire 0.01
Light 0.36
Sire X Light 0.95
c. Year 1
Sire 0.22
Dam(Sire) 0.06
Light 0.39
Sire X Light >0.5
Dam X Light >0.5
Emergence Date
11/19
11/9
10/30
10/20
d. Year 2
Sire <0.01 Dam(Sire) 0.08
Light <0.01 Sire X Light 0.35
Dam X Light 0.29
e. Year 1
Sire 0.34
Dam(Sire) <0.01
Light <0.2
Sire X Light >0.5
Dam X Light >0.5
Winter Size (mm2)
150
100
50
0
f. Year 2
Sire 0.2
Dam(Sire) <0.01
Light <0.01
Sire X Light 0.34
Dam X Light 0.07
10% Sun
40% Sun
100% Sun
18% Sun
40% Sun
Forest Interior
Edge
100% Sun
Light Environment

Figure 9.

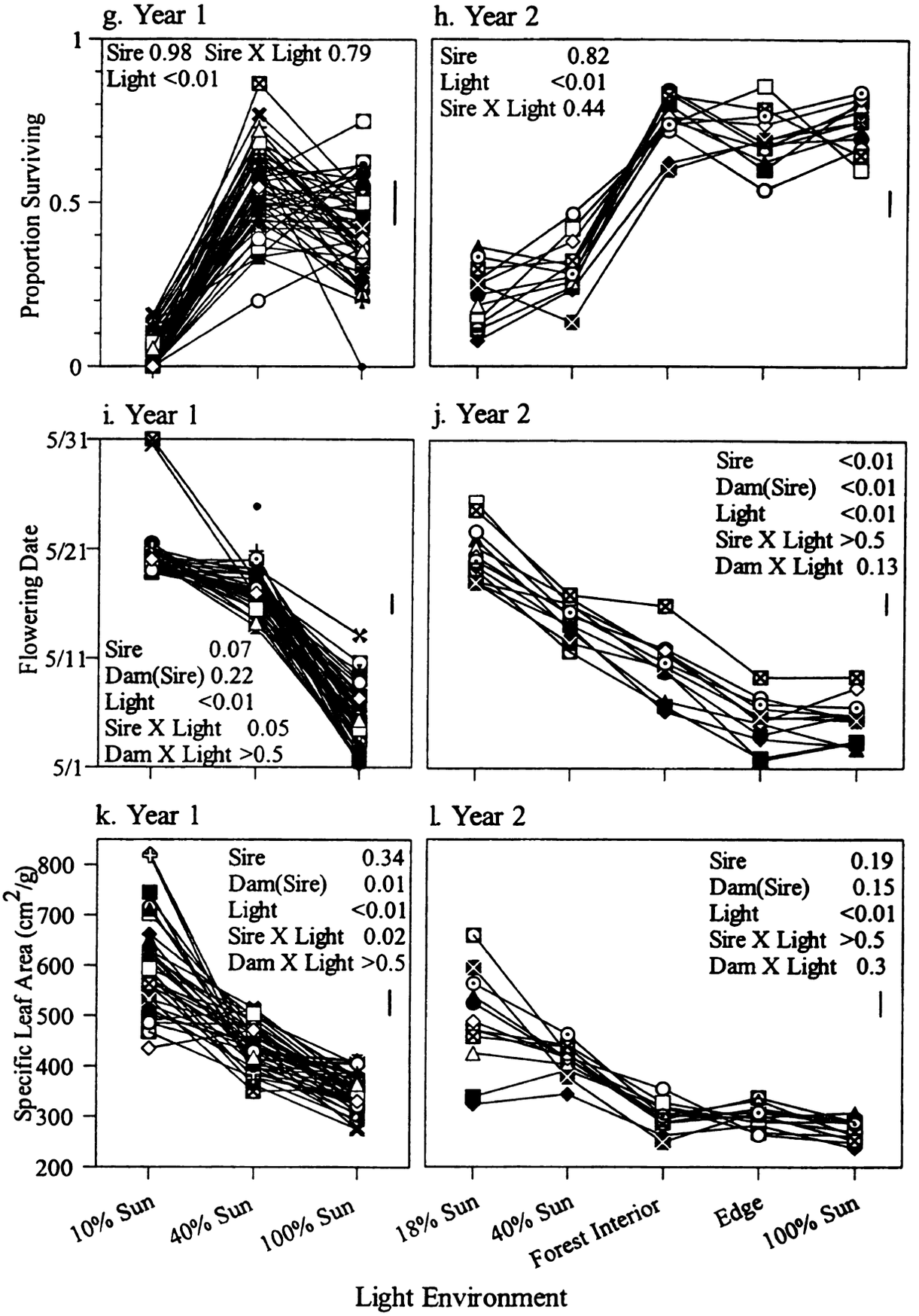

Figure 9 (cont'd).

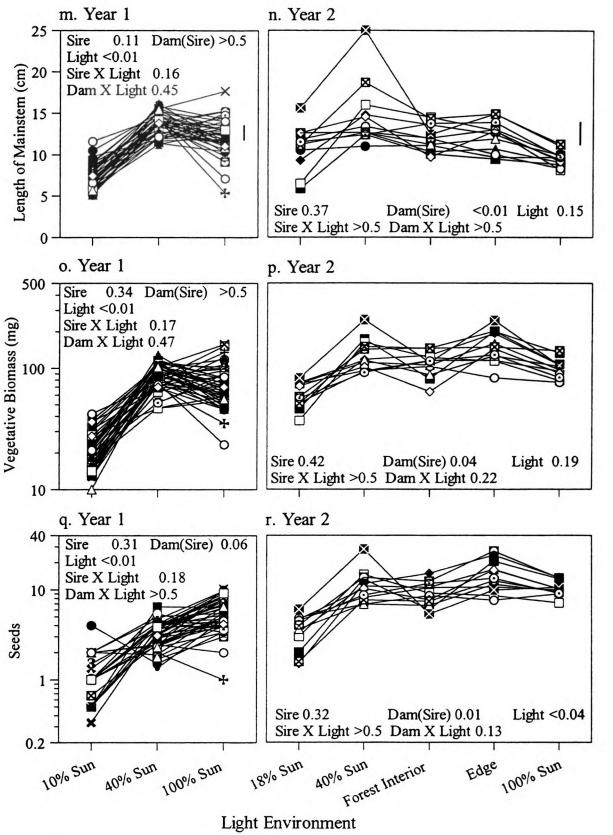

Figure 9 (cont'd).

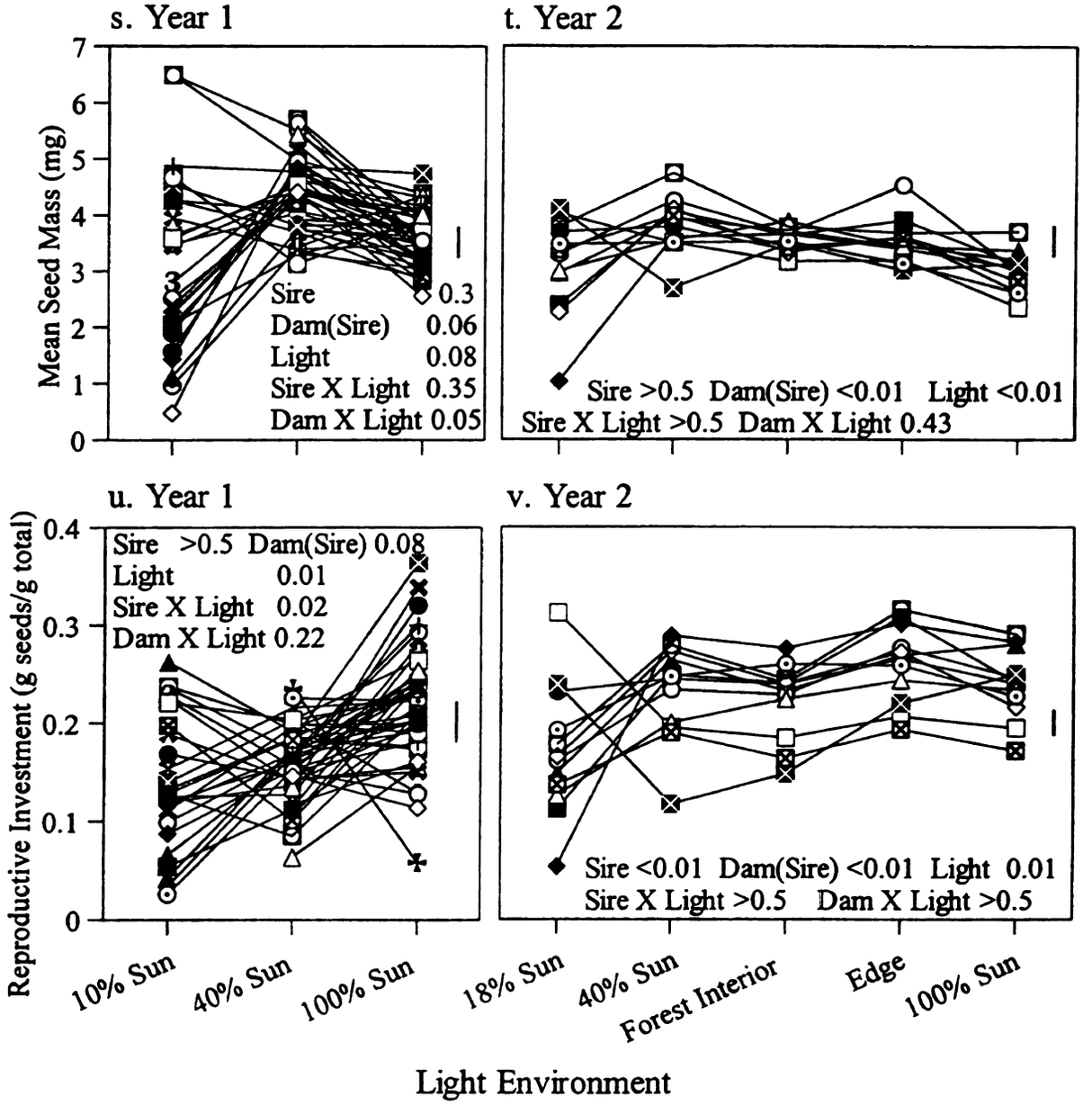

Figure 9 (cont'd).

harvest. Densities were reduced in the second year most likely because all plants in the first year were harvested prior to seed dispersal. Consequently, most of the plants in year two were recruited from the seed bank.

Additive genetic effects and narrow sense heritabilities

Overall, there were significant narrow sense heritabilities in at least one environment for six of nine traits (Table 5). Surprisingly, the reduced number of sires in year two does not appear to have limited the ability of these analyses to detect significant genotypic effects and heritabilities. Paternal families differed significantly in the proportion of seeds that germinated in both years (Figures 9a-b). There were also significant sire effects on emergence date, flowering date, and reproductive investment in at least one year (Figures 9c-d, i-j, u-v), and these traits were frequently heritable within environments (Table 5). Although there were no significant sire effects on specific leaf area, mainstem length, and mean seed mass (Figures 9k-n, s-t), all were significantly heritable in at least one environment (Table 5). Significant within environment heritabilities in the absence of significant overall sire effects are evidence for genotype-environment interactions (Feldman and Lewontin 1975). Winter size, survival to flowering, vegetative biomass, and seed number had no significant sire effects (Figures 9e-h, o-r), and were not significantly heritable in any environment (Table 5). This result is expected of traits closely linked to fitness because they are likely under strong selection.

There were more non-zero and significant heritability estimates in both years in the higher light environments (full sun and natural edge, Table 5). In particular, flowering date, specific leaf area, and mean seed mass were most heritable in high light.

Table 5. Narrow sense heritabilities of continuous traits based on the additive genetic variance components estimated using MTDFREML (Boldman et al. 1995) in univariate, within environment analyses. The numbers in parentheses are sample sizes. Year 1 (1995-96): 50 sires and 152 dams, Year 2 (1996-97): 12 sires and 36 dams. Significance values are based on likelihood ratio tests of the additive genetic variance components. Corrections of significance levels for multiple tests are not appropriate due to correlations between traits and across environments (see Methods). $\$P<0.15$, $\#P<0.1$, $*P<0.05$, $**P<0.01$.

		Narrow sense heritability (sample size)									
		Environment									
Trait	Year	10% Sun		40% Sun		100% Sun		Forest Interior		Edge	
Emergence Date	1	0	(1104)	0	(1199)	0	(876)				
	2	0.34*	(316)	0.1*	(331)	0	(331)	0.21*	(286)	0.23**	(317)
Winter Size	1	0	(1003)	0.09	(1135)	0	(813)				
	2	0.13	(298)	0.18	(298)	0.03	(321)	0	(256)	0.12	(282)
Flowering Date	1	0.26	(79)	0.09#	(765)	0.41**	(424)				
	2	0.1	(64)	0	(99)	0.42#	(237)	0.54#	(196)	0.49	(212)
Specific Leaf Area	1	0	(81)	0.19**	(783)	0.22**	(368)				
	2	0	(53)	0	(87)	0.23*	(155)	0	(172)	0	(164)
Mainstem Length	1	0	(86)	0.13#	(798)	0.01	(425)				
	2	0	(70)	0	(103)	0	(242)	0.15$	(217)	0.23*	(221)
Log Plant Mass	1	0	(92)	0.03	(811)	0.04	(424)				
	2	0	(71)	0	(102)	0	(242)	0	(216)	0	(221)
Log Seeds	1	0	(53)	0.09$	(661)	0.11$	(369)				
	2	0	(67)	0	(103)	0	(240)	0	(217)	0.08	(219)
Mean Seed Mass	1	0	(35)	0	(574)	0.11	(340)				
	2	0	(51)	0	(90)	0.24*	(229)	0	(205)	0.04	(201)
Repro. Investment	1	0.82	(35)	0.22*	(573)	0.13	(340)				
	2	0	(51)	0.27	(90)	0.24#	(229)	0.14	(205)	0.23*	(201)

Emergence date was more heritable in the second year when this trait was more phenotypically variable. Only reproductive investment was consistently heritable across all environments in each year.

Dam effects

Significant dam effects on a trait in the absence of significant additive genetic variation (sire effects) may be attributable to dominance, maternal genotype, or maternal environment. There were highly significant dam effects on winter size in both years (Figures 9e-f). Interestingly, in the second year there were significant dam effects on all late life-cycle traits except specific leaf area (flowering date, mainstem length, vegetative biomass, seed number, mean seed mass, and reproductive investment, Figures 9i-v).

Genotype-environment interactions

Statistical tests provide evidence for significant genotype-environment interactions in the first year for flowering date (Figure 9i), specific leaf area (Figure 9k) and reproductive investment (Figure 9u). As mentioned in the previous section, the contrasting results of significant within environment heritability estimates (Table 5) but insignificant sire effects for specific leaf area (Figures 9k-l), mainstem length (Figures 9m-n), and mean seed mass (Figures 9s-t) also suggest genotype-environment interactions. In the second year, no statistical tests for additive genetic variation in reaction norms approached significance. The reduced number of sires in this year limits the power of these tests.

The reaction norm plots show considerable diversity in reaction norm shape among sires, indicative of genotype-environment interactions and genetic variation for plasticity (Figure 9). Reaction norms cross between environments for all traits, but often

it is just a few genotypes that are responsible for most of the diversity in their shape. This diversity is particularly important for evolution, but is not likely to be detected in statistical tests of genotype-environment interaction (Lewontin 1974). Although mortality in some environments was quite high, there was no evidence of differential mortality or cross environment tradeoffs among paternal families in either year (Figures 9g-h).

Genetic correlations across environments

The cross-environment genetic correlations are generally consistent with the genotype-environment interaction results. In year 1, very few cross-environment genetic correlations were significantly different from zero by either method, indicating considerable cross-environment independence of traits (Table 6). Correlations for flowering date and specific leaf area across the medium and high light environments were significantly greater than zero by the breeding value method and significantly less than one by the variance component method. In year 2 most genetic correlations were significantly greater than zero by both methods, and none were significantly less than one by the variance component method (Table 7). All cross-environment correlations in year 2 were larger in magnitude than their comparable values in year 1. These results suggest that in contrast to year 1, the genetic basis of traits in year 2 was very similar across environments. There were no significant negative genetic correlations in either year that would indicate the presence of cross environment tradeoffs or genetic constraints on plasticity evolution.

Table 6. Cross environment additive genetic correlations. Data from 50 sires from Year 1 (1995-96). Methods: *bv*-from predicted breeding values. σ^2_{sire}-from variance components. Empty cells occur when there was no additive genetic variance for the trait in at least one of the environments. Significance testing for the *bv* method done with individual *t* tests. Significance testing for the variance component method is based on the results of mixed model REML analyses. Because estimated variance components were not greater than zero in at least two environments, cross-environment genetic correlations could not be estimated for emergence date, winter size, and mean seed mass in Year 1, and specific leaf area, vegetative biomass, and seed number in Year 2. Significance values: ***bv* method-** H_o: r_g=0: \$$P$<0.15; #$P$<0.1; *$P$<0.05; **$P$<0.01; ***$P$<0.001. Correlations in bold are significantly different from zero after a sequential Bonferroni adjustment within traits (α=0.05) (Rice, 1989). **σ^2_{sire} method-** H_o: r_g=0: \$$P$<0.15; #$P$<0.1; **$P$<0.01; H_o: r_g=1: $\underline{P<0.2}$; $\underline{\underline{P<0.05}}$.

Trait	Method	Year 1: 1995-96 Environment Pairs		
		10% Sun-40% Sun	10% Sun-100% Sun	40% Sun-100% Sun
Flowering Date	*bv*	0.05	0.15	0.31*
	σ^2_{sire}	$\underline{\underline{0.45}}$#	$\underline{\underline{0}}$	$\underline{\underline{0.57}}$#
Specific Leaf Area	*bv*			**0.28***
	σ^2_{sire}			$\underline{\underline{0.4}}$
Mainstem Length	*bv*			
	σ^2_{sire}			$\underline{\underline{0.57}}$\$
Log Plant Mass	*bv*			0.01
	σ^2_{sire}			$\underline{\underline{0}}$
Log Seeds	*bv*			0.25#
	σ^2_{sire}			$\underline{\underline{0.54}}$
Reproductive Investment	*bv*	0.09	-0.01	0.11
	σ^2_{sire}	$\underline{\underline{0.07}}$	$\underline{\underline{0}}$	$\underline{\underline{0}}$

Table 7. Cross environment additive genetic correlations from data for 12 sires from Year 2 (1996-97). Details as in Table 6

		Year two: 1996-97 Environment Pairs									
Trait	Method	Low-Medium	Low-High	Medium-High	Forest-Low	Forest-Medium	Forest-High	Edge-Low	Edge-Medium	Edge-High	Forest-Edge
E. Date	*bv*	**0.77****			0.39	0.44		**0.71****	0.66*		0.66*
	σ^2_{sire}	1.92**			0.45**	1.19**		0.87**	1.63**		0.92**
Winter Size	*bv*	**0.85*****	**0.77****	**0.83*****				0.56#	0.54#	0.64*	
	σ^2_{sire}	1.13	1.43	1.67				0.71	1.22	1.5	
F. Date	*bv*		0.3		0.53*		**0.86*****	**0.75****		0.64*	**0.73****
	σ^2_{sire}			2.29**		2.39**	1.39**		1.66**	1.10**	1.17**
Mainstem	*bv*										**0.75****
	σ^2_{sire}										
Seed Mass	*bv*										0.37
	σ^2_{sire}			0.67					1.63	0	
Repro. Inv.	*bv*			0.63*		**0.83*****	0.64*		**0.90*****	**0.80****	**0.85*****
	σ^2_{sire}			1.26**		1.91**	1.09**		9.91**	1.08**	1.57**

DISCUSSION

This study of a natural population of *Collinsia verna* has found a surprising diversity of genetic effects expressed in diverse field environments. There was additive genetic variation in at least some environments for germination, timing of emergence, flowering date, specific leaf area, mainstem length, mean seed mass, and reproductive investment. There was strong evidence for genotype-environment interactions (genetic variation for plasticity) for flowering date, specific leaf area, mainstem length, and reproductive investment. The lack of evidence for genotype-environment interactions in the fitness components survival, vegetative biomass, and seed number suggest that there were no strong light environment specialists among the genotypes sampled. However, significant maternal effects on vegetative biomass, seed number, and seed mass in the absence of additive genetic variation suggest that maternal genotypes specialize for different reproductive strategies.

Patterns of genetic variation

Heritability-The magnitudes of the heritabilities estimated in this study are low but compare favorably with other narrow sense, field-based heritability estimates for non-floral traits (e.g. Mitchell-Olds 1986, Bennington and McGraw 1996, Campbell 1997a, Thiede 1998). As in numerous other studies, traits closely linked to fitness (vegetative biomass, seed number) display little heritability. These traits are under strong selection (see Chapter 4) Two traits in this study, emergence date and winter size, were previously studied in this population (Thiede 1998). The significant heritability estimates for emergence date found in all but the high light environment (Table 5) are similar to previous estimates for this trait in the greenhouse ($h^2 = 0.14$) and field ($h^2 = 0.25$) (Thiede

1998). Winter size was most strongly influenced by maternal effects in this study and that of Thiede (1998), but she also found a significant narrow sense heritability in the greenhouse ($h^2 = 0.27$). In this study the narrow sense heritability of winter size was non-zero in all but the forest interior in at least one year, but was never significant.

Maintenance of genetic variation-In Chapter 4, I found that patterns of survival and selection on emergence date depend on the presence or absence of leaf litter. Survival to flowering is generally higher in the absence of leaf litter. Since germinating seedlings are unable to predict if they will be trapped under a fallen leaf, this variable selection could maintain the genetic variation for dormancy found in this study. The absence of leaf litter also selects for early emergence, while the presence of litter selects for late emergence. The unpredictability of these litter effects again may maintain genetic variation in emergence date.

Environmental effects on the expression of genetic variation-There are many ideas about how the expression of genetic variation might change along resource gradients or in novel or stressful environments. For example, novel or stressful environments have been predicted to increase genetic variance (Holloway et al. 1990). Alternatively, if stressful conditions are common and result in strong selection, genetic variation would be lost faster. Similarly, relaxed selection in benign and high resource environments may allow more genetic variation to persist. Another possibility is that genetic variation may be lowest in the most common natural environment where selection occurs most frequently.

All these patterns have been found in nature. Novel or stressful environments have been shown to increase heritability for many animal traits, but no patterns are seen in data from plants (review in Hoffmann and Parsons 1991). More recent work in plants

has found a decrease in genetic variance under stressful conditions (Sultan and Bazzaz 1993b, Bennington and McGraw 1996, J. Conner unpublished data). Increased genetic variance under conditions of resource abundance has been demonstrated for other plant species across resource gradients (Clough et al. 1980, Schwaegerle and Bazzaz 1987), but the patterns are not always consistent, even within a species. For example, *Polygonum* genotypes were found to display increased genetic variance along a soil moisture gradient (Sultan and Bazzaz 1993b), but not with respect to a light gradient (Sultan and Bazzaz, 1993a).

In this study both the full sun and the low light environments are novel, and consequently may be stressful. Natural variation in light availability in this population ranges from 25% to 75% of full sun (Chapter 2). The results show that heritability estimates along a light gradient vary, depending on the trait. Heritability estimates for flowering date, specific leaf area, and mean seed mass were high or highest in full sun, while estimates for emergence date and reproductive investment were highest in low light. Winter size and mainstem length were most heritable in the intermediate or natural light environments. Overall, trait heritability was lowest in the low resource environments (Table 5: 10% sun, 40% sun, and forest interior). It is likely that genetic variation is absent in low light simply because all genotypes performed poorly.

Maternal effects

In a half-sib design, the maternal variance component a fraction of the additive genetic variance plus a portion of dominance variation and maternal environmental and genetic effects (Falconer and Mackay 1996). In this study, winter size and mean seed mass in both years, and length of mainstem, vegetative biomass, and seed number in the

second year had no additive genetic variance, but substantial among dam variance. It is quite possible that these effects indicate genetic differences among mothers in their effects on offspring phenotype (rather than dominance or maternal environment effects). First, the relatively uniform greenhouse conditions under which the dams produced seeds should minimize maternal environment effects. Second, other studies of this population have found substantial maternal genetic effects on seed mass and winter size (Chapter 2, Thiede 1998). Finally, there is no evidence for dominance variation in seed mass or winter size in this population (Thiede 1998).

The persistence of maternal effects in the absence of additive genetic variation late in the life cycle in the second year is a striking result. The significant differences among maternal families for seed number in the second year are clear evidence that selection differentiated among maternal families. Further, the marginal dam-by-light interaction term (Figure 9r) raises the possibility that different maternal genotypes may be favored in different environments, and suggests that maternal plants specialize for different seed provisioning strategies. A greenhouse study of this *Collinsia* population also found that maternal effects persisted up until flowering for two size related traits (Thiede 1998). In contrast, most other studies have found that maternal effects decline through the life cycle (e.g. Biere 1991, Montalvo and Shaw 1994, Schmid and Dolt 1994).

The between year differences in dam effects late in the life cycle may be due to differences between years in the timing of emergence. Although there are strong dam effects on winter size in both years, late emergence in the first year resulted in little phenotypic variation in winter size (Figures 9e-f). Without a strongly established size

hierarchy in the fall, over winter mortality and spring growth could not differentiate among maternal families. Consequently, maternal effects could not persist into later life-history stages. Earlier emergence in the second year resulted in much more variation in overwinter size (Figures 9e-f), and possibly more variation among maternal families in survival and spring growth (Figures 9m-v). Several other studies have found that the expression of maternal effects may depend on the offspring environment (Stratton 1989, Schmitt et al. 1992, Schmid and Dolt 1994, Thiede 1998).

Genetic variation for plasticity

The integration of plastic traits in coordinated responses to environmental heterogeneity remains one of the most complex and poorly understood aspects of the general phenomenon of phenotypic plasticity (Schlichting and Pigliucci 1998). The genetic and environmental effects on the suite of traits studied here were diverse and complex. Consequently, generalizations about the effects of light gradients on the expression of genetic variation, or plasticity evolution would be foolhardy. However, the evidence found here for genetic variation for plasticity in several traits sheds light on several issues.

Antagonistic pleiotropy-Mainstem length and reproductive investment are under consistent directional selection (Chapter 4). Moreover, estimates of genetic correlations found strong and significant positive genetic correlations between mainstem length and vegetative biomass (Chapter 5, Table 15), and vegetative biomass is also under very strong directional selection (Chapter 4). Under these circumstances, little genetic variation would be expected to remain for these traits or their plasticity. Yet there is strong evidence for genetic variation for plasticity for reproductive investment, and

modest evidence for mainstem length. A possible explanation for this pattern is that there are fundamental genetic tradeoffs between the ability to efficiently utilize resources and convert them to seeds (the reproductive investment trait), and the ability to rapidly acquire resources and convert them to biomass (the mainstem length and vegetative biomass traits). If there are negative genetic correlations between reproductive investment and the size traits, selection for greater opportunistic growth ability and biomass accumulation may also select for reduced efficiency in reproductive investment and vice versa. This antagonistic pleiotropy may maintain genetic variation. Genetic correlations of reproductive investment with mainstem length and vegetative biomass were indeed negative, but they were insignificant (Chapter 5, Table 15).

Genotype-environment interactions for fitness-There is a simpler explanation for genetic variation in plasticity of reproductive investment. The strong selection on this trait across all environments simply favors different genotypes in different environments. Consequently, genotype-environment interactions for fitness maintain genetic variation for plasticity in this trait.

Control of plasticity via a genetic switch-Some have suggested that plants should be generalists for resource utilization regardless of the pattern of genetic correlations between underlying traits (Chapin 1991, Chapin et al. 1993). Ideally, plants would be able to both tolerate stressful low resource conditions and grow rapidly when resources are abundant. Interspecific comparisons show that compared to sun plants, shade tolerant plants have low relative growth rates, low photosynthetic rates, low transpiration and stomatal conductance, low leaf turnover, and high ability to use sun flecks (e.g. Grime 1979, Chapin 1980, Chapin et al. 1993). Interestingly, when high light plants are grown

under shady conditions, they show many of these shade plant characteristics, which may represent an adaptive "stress resistance syndrome" (Chapin 1991, Chapin et al. 1993).

Chapin argues that conversion of a high resource genotype to a stress tolerant one may involve a simple genetic switch. Hormonal regulators of plant growth, development, and stress responses are known which meet the criteria of a genetic switch (Voesenek and Blom 1996, Schlichting and Pigliucci 1998). If plastic responses are under single gene control, quantitative genetics is an inappropriate model for understanding the evolution of plasticity in these traits. It should be relatively easy for adaptively plastic responses to resource limitation to evolve regardless of the genetic correlations among traits, and consequently, we would expect most genotypes to be generalists with respect to light. It is notable that there was no evidence in this study for differences among paternal families in survival or fecundity. At this level, all genotypes appeared to be generalists.

Adaptive plasticity relaxes natural selection?-Flowering date and specific leaf area also show strong evidence for genetic variation for plasticity. But in contrast to most other traits, they are under little direct selection (Chapter 4). In this case, the absence of selection may allow genetic variation to persist. Intriguingly, adaptive plasticity may also be responsible for the absence of selection: By being plastic, all genotypes produce phenotypes appropriate for their environment.

Conclusion

The results of this study of *Collinsia verna* suggest that the patterns of genetic and environmental effects on each trait are unique. Genotypes appear to specialize for particular patterns of germination, emergence, and reproductive investment, while at the same time they may be adaptively plastic for flowering date and specific leaf area.

Persistent maternal effects suggest that maternal genotypes may specialize for different reproductive strategies.

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Chapter 4

EVOLUTION OF REACTION NORMS: ENVIRONMENT-DEPENDENT SELECTION IN A NATURAL POPULATION OF *COLLINSIA VERNA*

INTRODUCTION

Many recent studies have documented spatial and temporal variation in natural selection within natural plant populations (e.g. Kalisz 1986, Kelly 1992, Stratton 1992, 1995, Gross et al. 1998). Depending on a variety of ecological and genetic factors, theory suggests that the variable selection seen in these studies may lead to phenotypically plastic generalist genotypes or to the persistence of specialist genotypes and the maintenance of genetic variation (reviews in Hedrick 1986, Scheiner 1993, Roff 1997, Schlichting and Pigliucci 1998). To date, we have little ability to predict which of these alternatives might prevail in particular populations. Moreover, a particular selective regime may simultaneously result in plasticity in one suite of traits, and the maintenance of genetic variation in another.

A better understanding of the evolutionary consequences of variable selection requires detailed studies of how variation in environmental factors affect phenotypic traits, plant fitness, and the expression of genetic variation in natural populations. In particular, manipulation of potential selective agents is necessary to identify which environmental factors are the causes of natural selection (Mitchell-Olds and Shaw 1987, Wade and Kalisz 1990, Dudley and Schmitt 1996). Once a cause is identified, information about the magnitude and predictability of variation in the selective agent is necessary to predict whether this environmental variation would favor the evolution of

adaptive plasticity or the maintenance of genetic variation in particular traits (e.g. Bradshaw 1965, Via and Lande 1985).

Effects of light and leaf litter

Two factors that can be expected to function as strong selective agents on plants are light and leaf litter. Light is a primary plant resource, and has been shown to have a strong effect on female fitness in many plant populations (reviews in Goldberg 1990, Sultan and Bazzaz 1993). Light availability for understory plants in forests can be highly variable in time and space (Chapter 2), but in very predictable spatial patterns, and diurnal and seasonal cycles. Thus it is likely that this variation in light availability should favor plastic traits that would allow plants to tolerate low light levels and convert high light availability into increased fitness. If this selective regime has allowed the plasticity of light responsive traits to evolve close to their optima, we would expect to see relatively little direct selection on these traits in all but the most extreme light environments.

Leaf litter also can have a major impact on plant fitness, particularly through reductions in the establishment and survival of seedlings (e.g. Goldberg and Werner 1983, Bergelson 1990, Carson and Peterson 1990, Foster and Gross 1997). Litter inhibits establishment through physical interference with the growth of emerging seedlings and the reduction of light quantity and quality below the compensation point (Facelli and Pickett 1991b, Foster and Gross 1998). Indirect negative effects of leaf litter on plant fitness may include promotion of fungal pathogens, and creation of habitat for litter dwelling seed predators and herbivores (Facelli 1994). However, in some habitats leaf litter may facilitate seedling establishment through the amelioration of abiotic stresses (e.g. desiccation, Fowler 1986, Willms et al. 1986, Hamrick and Lee 1987). An

important abiotic stress affecting establishment of winter annuals in temperate deciduous forests may be the diurnal freeze-thaw cycle that occurs at the soil surface during winter months. Leaf litter may reduce the amplitude and frequency of these thermal cycles (Facelli and Pickett 1991a). Thus, the effects of litter may be negative at germination, but positive at later stages of growth. Importantly, the incidence, quantity, and persistence of leaf litter at particular locations in forests are unpredictable (Frankland et al. 1963, Sydes and Grime 1981, Facelli and Carson 1991, Molofsky and Augspurger 1992).

Direct and indirect selection

Since the development of multiple regression methods for the study of phenotypic selection (Lande and Arnold 1983, Arnold and Wade 1984a, b), selection gradients have received the most attention in the evolutionary ecology literature because they measure only direct selection on a trait independent of variation in other traits, and they are easily interpretable in terms of quantitative genetic equations for multivariate evolution (Brodie et al. 1995). Selection differentials are problematic because although they measure total selection on a trait. The indirect selection that is part of the differential is evolutionarily important only if the phenotypic correlations among traits are representative of the genetic correlations. Often, information about genetic correlations is unavailable or would be prohibitively difficult to obtain. However, if traits are genetically correlated, then correlated responses to selection can be a primary cause of evolutionary change in a trait, and failure to consider correlated responses may lead to erroneous conclusions. A great challenge for ecological genetic studies is to assess when indirect selection may have important evolutionary consequences, especially in cases where selection gradients and selection differentials suggest very different relationships between a trait and fitness

(Brodie et al. 1995). Path analysis (structural equation modeling) is an underutilized tool that can aid in understanding the potential sources of indirect selection on traits (Kingsolver and Schemske 1991, Mitchell 1992, Conner 1996, Conner et al 1996).

In this study, I manipulated light and leaf litter within a natural population of the forest winter annual *Collinsia verna* to quantify their effects on six phenotypic traits: emergence date, date of first flowering, specific leaf area, plant height, above ground vegetative biomass, and reproductive investment. The relationship of these traits to survival and three multiplicative components of lifetime fitness in each environment was estimated. I ask: Are traits plastic in response to these factors? Does the magnitude or direction of selection on traits change across light and/or leaf litter environments? If selection changes between particular environments, can light or leaf litter be characterized as causes of natural selection? Do patterns of indirect selection differ from those of direct selection in a way suggestive of tradeoffs between traits? If traits are plastic, is the direction of phenotypic change consistent with the observed pattern of selection in such a way that the plasticity could be characterized as adaptive?

METHODS

Study system

Collinsia verna is a winter annual wildflower of moist woods in eastern North America. The winter annual life history allows plants to grow in a window of light availability between canopy leaf senescence in the fall and leafout in the spring. Seed germination is cued by diurnal temperature fluctuations in the fall (Baskin and Baskin 1983). Flowering in this mostly out-crossing species begins in late April and ceases as the canopy closes in May. Fruits ripen, seeds fall passively to the ground and plants die

by mid-June. Biotic and abiotic conditions during the growing season are inherently unpredictable, and can result in variable emergence, survival, and seed set (Chapter 2 and this Chapter). Reproductive assurance in this mostly outcrossing species is achieved through backup selfing (Kalisz et al. 1999). Long term persistence is ensured through the production of a significant fraction of dormant seeds (Kalisz 1991).

Experimental design

The study population (described in Thiede 1998), occurs along the south-facing edge of a woodlot adjacent to an agricultural field in Kalamazoo County, southwest Michigan. Eight different light and leaf litter environments were created at the field site. Each light and litter combination was replicated five times for a total of 40 1.2 m^2 plots. To control light levels, the entire woody canopy along the southern edge of the population was cleared, and three light treatments were assigned randomly to the 15 plots in this area: full sun, 40% of full sun, and 18% of full sun (Figure 1 of Chapter 2). Reduced light was achieved by placing a wood lattice (3.5 cm wood strips in a 15 cm or 7 cm grid) over the plots that allowed sunflecks to reach the plants throughout the day. Because light was being manipulated in these plots, leaf litter was not allowed to accumulate (for details see Chapter 2). To understand how light and litter manipulations relate to the conditions the plants naturally experience, 25 additional plots were established. In 15 of these plots leaf litter was left intact. These plots were placed along the natural light gradient with five in the forest interior, five along the southern edge, and five in the full sun. The remaining ten plots, five along the southern edge and five in the forest interior, had all leaf litter removed on a twice weekly basis. Light was more variable in the plots in the forest and along the edge than in the cleared area where light

levels were experimentally manipulated. The mean light availability in the forest interior was about 45% of full sun, while along the edge it was about 70% of full sun. Overall, there were five light levels in the study: low (18%), medium (40%), forest interior (45%), edge (70%), and full sun (100%). The low and medium light levels had all leaf litter removed, while the three highest light levels had both litter and no litter treatments.

In the fall of 1996, I conducted weekly censuses of all naturally emerging *Collinsia verna* seedlings. Plants were tagged with color coded washers. Mortality censuses were done weekly through December, again just prior to flowering in April, and at harvest in June. In April and May of 1997, survivors were tagged for date of first flowering and sub-sampled for specific leaf area. A circular piece of leaf tissue from the youngest fully expanded leaf was collected with a hole punch, air dried, and weighed to determine specific leaf area for approximately 250 randomly chosen individuals in each treatment, 50 from each plot. Plants were harvested prior to seed dispersal in June. After harvest, plants were air dried and scored for length of the mainstem, aboveground vegetative biomass, number of flowers, number of fruits, number of seeds, and total mass of seeds. Multiplicative fitness components (fruits per flower and seeds per fruit) were calculated from these measures for use in path analysis. Reproductive investment was calculated as the proportion of aboveground biomass that was seeds. Because all plants were dying at this point, this trait measures the efficiency with which they were able to convert vegetative biomass to seeds, not simply reproductive allocation.

Data analysis

Phenotypic plasticity-The first goal of the analysis was to determine if the traits were plastic in response to the environmental manipulations. For this analysis, each of

the eight environments was considered a unique treatment. Fixed effects of treatment on emergence date, flowering date, specific leaf area, mainstem length, vegetative biomass, reproductive investment, flowers, fruits/flower, seeds/fruit, and seeds were tested with a one-way MANOVA (SAS GLM procedure, SAS 1989). This approach is conservative, correcting for any correlations among traits. Although specific contrasts are of interest in the selection parameters (below), these phenotypic traits and fitness components were compared across all pairs of environments using univariate post-hoc tests. Traits were log-transformed as necessary to improve normality.

Survival analysis-The effects of leaf litter, light level, and emergence date on survival to flowering were analyzed with proportional hazards regression (SAS PHREG procedure, SAS 1997). Litter was coded as present or absent (0 or 1), and light levels were ordered from low (1) to full sun (5). The lifespan of each individual was determined in days from the date the first seedling emerged. Because seeds that emerged late were not exposed to the same conditions as those which emerged early, individuals that emerged late were treated as missing values in the risk set until they emerged (Allison 1995). Data for plants that survived to flowering was treated as uncensored. There was very little mortality between flowering and harvest, when all plants died. Treating survivors as censored data increased the magnitude of parameter estimates, but signs and significance levels were identical.

Plants growing in the medium and low light treatments experienced high mortality due to grazing by an unknown herbivore in early spring. The shade lattice appeared to provide a refuge for these grazers, who removed the cotyledons and leaves of many plants, killing them (personal observation). Because this mortality is likely an

artifact of the lattice not directly related to the light and leaf litter manipulations of interest, these treatments were excluded from the full analysis.

Phenotypic selection-Natural selection on emergence date, flowering date, specific leaf area, mainstem length, vegetative biomass, and reproductive investment, was analyzed using multivariate episodic selection analysis (Arnold and Wade 1984a). For this analysis, all traits were standardized to a mean of zero and unit variance. Linear and nonlinear selection differentials (S, C) and gradients (β, γ) were calculated with simple (S, C) and multiple regression (β and γ) for two episodes of selection, survival to flowering and fecundity (SAS REG procedure, SAS 1989). Variance inflation factors (VIFs) were less than ten for all terms in all analyses, indicating that there were no problems with multicollinearity (Neter et al. 1985). Although the selection parameters calculated for each episode are not additive (Lynch and Arnold 1988), they do address the fitness consequences of traits independent of selection in other episodes (Koenig et al. 1991). Moreover, because only one trait, emergence date, is common to both selection episodes little would be gained by transforming the selection parameters to make them additive.

Selection gradients are a measure of direct selection on a trait, while the selection differentials measure total selection on a trait including any indirect selection through phenotypically correlated traits. The survival episode was a univariate analysis of emergence date because no other traits were measured in this interval. The fecundity episode included all six traits. Relative fitness was calculated for each episode by dividing a plant's survival (0 = died or 1 = survived) or seed number by the mean survivorship or seed number for all plants within the same environment. Confidence

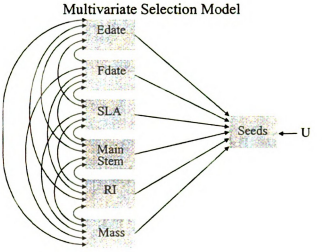
intervals for selection parameters were calculated using bootstrap resampling methods (Noreen 1989, Dixon 1993). The data sets for each of the eight environments were resampled 1000 times using a SAS macro (SAS 1990). The number of observations in each resampled data set was equal to the number of observations in the original data set. Bootstrapping and parametric significance tests produced nearly identical results. Calculation of selection parameters for the survival episode using logistic regression (SAS LOGISTIC procedure, SAS 1997), and back transforming the results (Janzen and Stern 1998) also produced similar results (not reported).

Overall differences across environments in selection parameters were analyzed with heterogeneous slope tests (ANCOVA in SAS GLM procedure, SAS 1989). For traits where the linear selection gradients were significantly different, planned contrasts were constructed using the contrast statement in proc GLM. Six contrasts were evaluated for each trait: the three unmanipulated light environments (forest interior, edge, and full sun) contrasted with each other, and presence versus absence of leaf litter within each light environment.

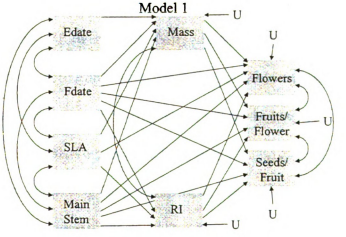
Path analysis-Structural equation modeling was used to investigate the relationships among traits in order to understand the sources of indirect selection in each environment (SAS CALIS procedure, SAS 1997). The multiple regression technique used in selection gradient analysis is analogous to a path model where all traits are directly linked to fitness (Figure 10a). This model assumes no causal relationships among traits and may be misleading when causal relationships among traits are expected. In this study, it is likely that emergence date, flowering date, specific leaf area, and mainstem length are causally related to the performance traits total vegetative biomass

Figure 10. Path models representing possible relationships between traits and fitness. Straight lines with single headed arrows represent hypothesized causal relationships, while curved lines with double headed arrows represent correlations. (a) Multiple regression of all traits on fitness. This path diagram represents the multivariate directional gradient analysis. (b) Path model 1 with vegetative biomass and reproductive investment as intermediate traits, and three multiplicative fitness components. (c) Path model 2 with only vegetative biomass as an intermediate trait. Traits: emergence date (Edate); flowering date (Fdate); specific leaf area (SLA); length of mainstem (Mainstem); reproductive investment (RI); vegetative biomass (Mass); residual unexplained variation (U).

(a)



(b)



(c)

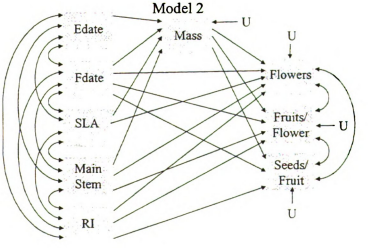


Figure 10.

and reproductive investment. It is also possible that these relationships may change depending on the light environment.

Two *a-priori* path models were analyzed (Figure 10b-c). In model one, vegetative biomass and reproductive investment were treated as intermediate traits linking emergence date, flowering date, specific leaf area and mainstem length to fitness components. In model two, only vegetative biomass was treated as an intermediate trait. Model two may be more appropriate in high resource conditions, where abundant resources make efficient conversion of resources to seeds less important. Model one may be more appropriate in low resource conditions where the ability to efficiently use scarce resources is critical. In both path models, seed number was partitioned into three multiplicative components: flowers, fruits/flower, and seeds/fruit. Correlations among all traits, and correlations of multiplicative fitness components with seed number are provided to aid interpretation, but the analysis was run on the covariance matrices, as this produces more reliable standard errors (Hatcher 1994).

Emergence date, flowering date, specific leaf area, vegetative biomass, and number of flowers were log transformed based on the results of the diagnostics for multivariate normality reported by the CALIS procedure. In structural equation modeling, outliers do not have a strong effect on path coefficients, but can cause highly inaccurate standard errors (Hatcher 1994). Path coefficients were estimated from the full data set using the maximum likelihood method. For significance testing, outliers were identified and excluded using the multivariate kurtosis diagnostics in the CALIS procedure. The significance of individual paths was determined from *t* tests calculated by dividing each path coefficient by its standard error. Because models one and two are not

nested, there is no way to compare them statistically. The fit of each model to the data was compared to that of a null model (no correlations among traits) with Chi-square tests and three goodness-of-fit measures.

RESULTS

Phenotypic plasticity

All traits and fitness components were highly plastic in response to light (MANOVA, $P<0.0001$ for each, Figures 11, 12). The effects of leaf litter were variable. In the forest interior, litter had no effect on early life history traits, but had a dramatic effect on vegetative biomass, reproductive investment, flowers, and seeds per fruit. On the edge, leaf litter affected traits that were expressed throughout the life cycle: emergence date, specific leaf area, mainstem length, and number of seeds. In the full sun, leaf litter only affected emergence date and specific leaf area.

Traits varied in response to light following several different patterns. There were maximums at intermediate light levels for traits closely linked to fitness (vegetative biomass, reproductive investment, number of flowers, and number of seeds), suggesting that the extreme environments were stressful. Mainstem length also had maximum values in intermediate light environments. Emergence date was earliest in the forest interior and edge environments. Flowering date and specific leaf area declined with increasing light. These are characteristic light responses in most plants. Fruits per flower showed a modest increase with light.

Survival analysis

The goal of this analysis was to understand the combined effects of leaf litter, light environment, and emergence date on patterns of mortality. Because mortality in the

Figure 11. Means and standard errors of phenotypic traits. Fixed effects of the eight environments were analyzed in a single one-way MANOVA that included all phenotypic traits and all fitness components (Figure 12) except survival as dependent variables (GLM procedure, SAS 1989). All traits differ across environments at $P<0.0001$. All pairwise means comparisons were made using the tukey option of the means statement of proc. GLM. Columns sharing a letter are not significantly different from each other at $P<0.05$.

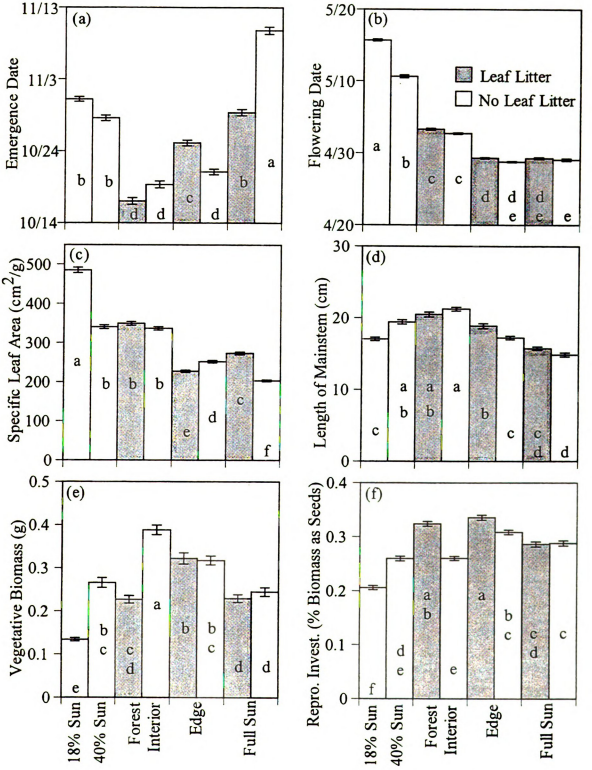

(a)
Emergence Date
11/13
11/3
10/24
10/14
(b)
Flowering Date
5/20
5/10
4/30
4/20
Leaf Litter
No Leaf Litter
(c)
Specific Leaf Area (cm2/g)
500
400
300
200
100
0
(d)
Length of Mainstem (cm)
30
20
10
0
(e)
Vegetative Biomass (g)
0.5
0.4
0.3
0.2
0.1
0
(f)
Repro. Invest. (% Biomass as Seeds)
0.4
0.3
0.2
0.1
0
18% Sun
40% Sun
Forest Interior
Edge
Full Sun

Figure 11.

Figure 12. Means and standard errors of fitness components. All pairwise means comparisons were made as in Figure 11. Survival is not a continuous variable, so it could not be analyzed this way. Environmental effects on survival were analyzed separately using proportional hazards regression (Tables 8 and 9).

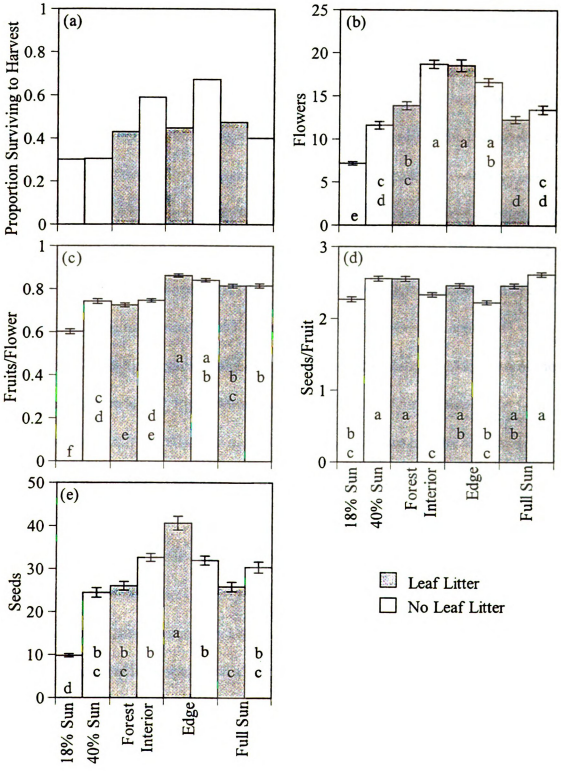

(a)
Proportion Surviving to Harvest
(b)
Flowers
(c)
Fruits/Flower
(d)
Seeds/Fruit
(e)
Seeds
18% Sun
40% Sun
Forest Interior
Edge
Full Sun
Leaf Litter
No Leaf Litter

Figure 12.

medium and low light manipulations was caused by herbivory that was unrelated to light or litter, these two treatments were excluded from the full analysis. Overall, the presence of leaf litter more than doubled the probability that plants would die before flowering (Table 8), but this result was accompanied by a highly significant three-way interaction between leaf litter, light environment, and emergence date. The risk ratio describes the change in hazard of death as a consequence of a one unit change in the variable. Risk ratios greater than one indicate an increasing hazard, less than one indicate a decreasing hazard. The risk ratio of 0.998 for emergence date in Table 8 indicates that the hazard of death before flowering declined 0.2% for every day that emergence was delayed or about 6% per month. The risk ratio of 1.005 for the significant two-way interaction of emergence date and light indicates higher risk of death with greater combinations of these two (late emergence in high light), while the risk ratio of 0.994 for the three-way interaction suggests this relationship did not hold in the presence of litter as seen in Figure 12a. This result is consistent with the hypothesis that leaf litter might reduce the number of diurnal freeze-thaw cycles.

To better understand the three-way litter by light by emergence date interaction, separate analyses of the effect of emergence date on survival were then run within each litter and light level combination, including the low and medium light manipulations (Table 9). When leaf litter was intact, there were no significant effects of emergence date on the hazard function in any light environment. In the absence of leaf litter, the risk of death increased with delayed emergence by 33-36% per month in the forest interior and full sun, but by 63% per month along the edge. In the low light treatment (excluded from the full analysis), the risk ratio of 0.995 indicates a 15% decline in the risk of death for

Table 8. Proportional hazards regression survival analysis for effects of emergence date, leaf litter, and light for forest interior, edge, and full sun environments (N=9836).

Variable	P	Risk Ratio
Emergence Date	0.6564	0.998
Leaf Litter	0.0138	2.099
Light	0.6124	0.969
Emergence Date X Leaf Litter	0.3831	1.007
Emergence Date X Light	0.0003	1.005
Leaf Litter X Light	0.5289	1.051
Emergence X Litter X Light	0.002	0.994

Table 9. Proportional hazards regression survival analysis for effects of emergence date on survival of *Collinsia verna* within environments.

Environment	*N*	*P*	Risk Ratio
Full Analysis			
Forest, With Leaf Litter	1402	0.5037	0.999
Forest, No Leaf Litter	1582	0.0001	1.011
Edge, With Leaf Litter	2100	0.657	0.999
Edge, No Leaf Litter	2165	0.0001	1.021
Full Sun, With Leaf Litter	1466	0.6368	0.999
Full Sun, No Leaf Litter	1121	0.0001	1.012
Herbivore Effects			
18% Sun, No Leaf Litter	2982	0.0001	0.995
40% Sun, No Leaf Litter	2156	0.49	0.999

every month that emergence is delayed. The herbivores preferred earlier emerging seedlings.

Phenotypic selection: Survival episode

The direction of selection on emergence date changed in the unmanipulated light environments depending on the presence or absence of leaf litter (Figure 13a). Moreover, there was significant upward curvature in the fitness surface in all environments (positive nonlinear gradients, Figure 13b). This means that there were dramatic nonlinear increases in survival with late emergence in the presence of leaf litter, but with early emergence in the absence of leaf litter. Overall, this result implies strong disruptive selection on emergence date. In the low and medium light manipulations where herbivory was the primary source of mortality, positive directional selection on emergence date indicates that late emerging plants were less likely to be eaten. These results are generally consistent with the survival analysis, but selection for late emergence by leaf litter appears much stronger here than suggested in the previous analysis.

Phenotypic selection: Fecundity episode

The most striking result of this analysis was that indirect selection differed in sign from direct selection for nearly all traits and environments (Figure 14). In contrast to the survival episode, there were no changes in the direction of direct linear selection except for specific leaf area (Figure 14). Direct selection favored higher specific leaf area (a shade phenotype) in the forest interior and on the edge, but lower specific leaf area in the undisturbed full sun environment (Figure 14c). Total linear selection (open bars, Figure 14) differed significantly in magnitude across environments for all traits except emergence date, and direct selection (shaded bars, Figure 14) differed for specific leaf

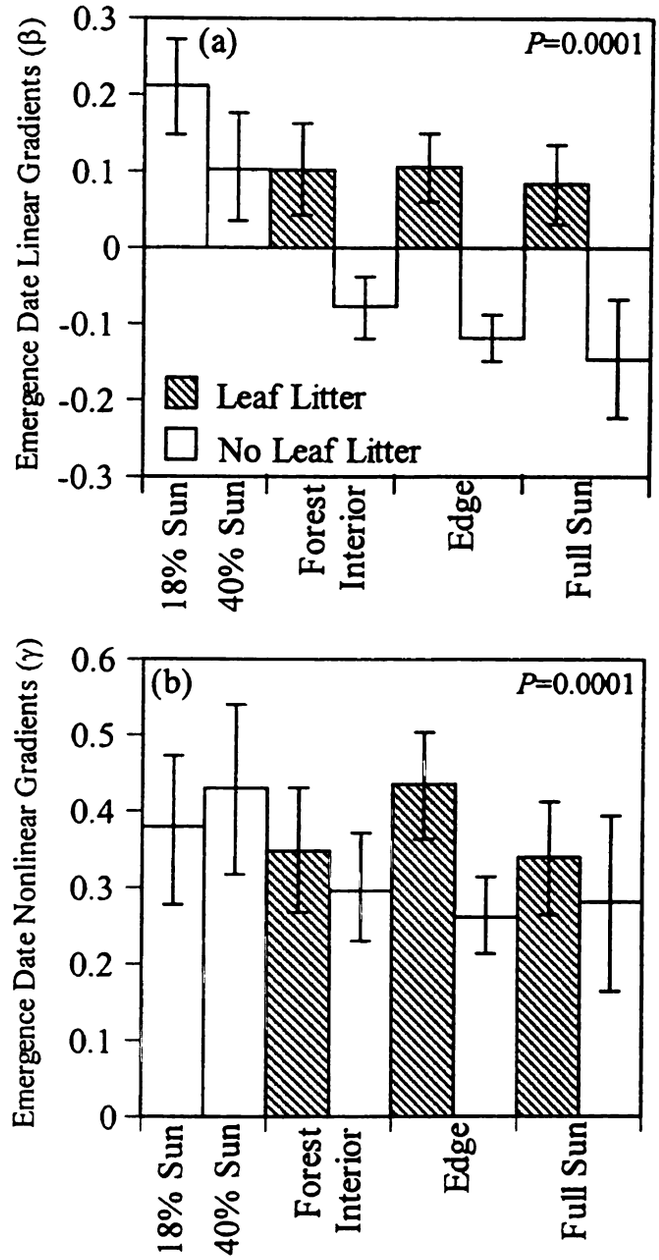


Figure 13. Survival episode standardized linear (β) and nonlinear (γ) selection gradients for emergence date. Because emergence date is the only measured trait that was expressed during this episode, selection gradients and differentials were very similar. Consequently, only the gradients are presented. Bars are 95% confidence intervals based on 1000 bootstrap resampled data sets. *P*-value is the result of an overall ANCOVA testing whether selection gradients differ across environments. The Y-axis indicates the proportion by which relative fitness would change with a change of one standard deviation in the trait. (a) Linear gradients. (b) Nonlinear gradients.

Figure 14. Fecundity episode standardized linear selection differentials (S) and gradients (β). Bars are 95% confidence intervals based on 1000 bootstrap resampled data sets. P-values are the results of ANCOVAs testing wether differentials or gradients differ across environments. The Y-axis indicates the proportion by which relative fitness would increase with an increase of one standard deviation in the trait. Note that the scale of the Y-axis differs between figures. (a) Emergence date. (b) Flowering date. (c) Specific leaf area. (d) Mainstem length. (e) Vegetative biomass. (f) Reproductive investment.

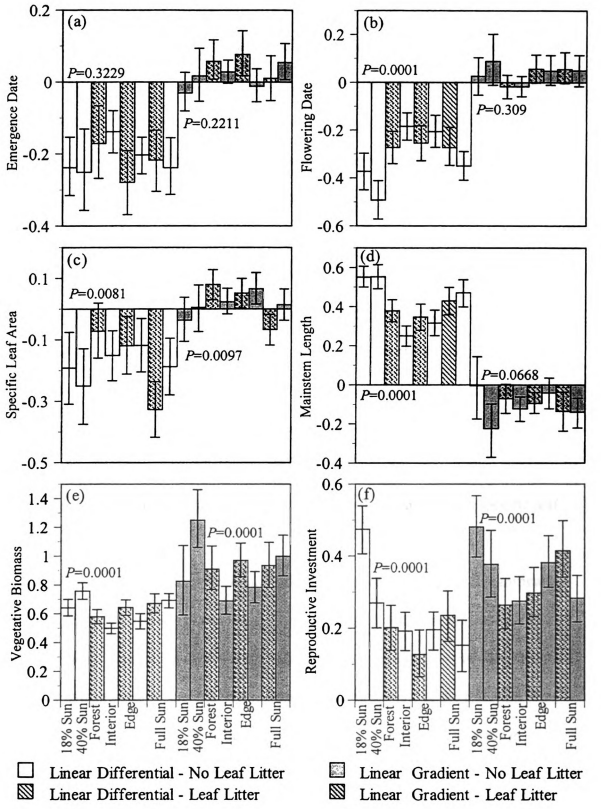

(a)
Emergence Date
P=0.3229
P=0.2211
(b)
Flowering Date
P=0.0001
P=0.309
(c)
Specific Leaf Area
P=0.0081
P=0.0097
(d)
Mainstem Length
P=0.0668
P=0.0001
(e)
Vegetative Biomass
P=0.0001
P=0.0001
(f)
Reproductive Investment
P=0.0001
P=0.0001
18% Sun
40% Sun
Forest
Interior
Edge
Full Sun
Linear Differential - No Leaf Litter
Linear Differential - Leaf Litter
Linear Gradient - No Leaf Litter
Linear Gradient - Leaf Litter

Figure 14.

area, vegetative biomass and reproductive investment.

Planned contrasts for these three traits showed different patterns of change in the linear selection gradients across environments (Table 10). Selection on specific leaf area and reproductive investment differed between the full sun and the other unmanipulated environments (Figures 14c, f). In contrast, selection on vegetative biomass was similar across light environments, but increased in the presence of leaf litter in the forest interior and on the edge (Figure 14e).

There was little direct nonlinear selection (nonlinear gradients, γ_{ii}) on any trait in any environment, except vegetative biomass (Figure 15, shaded bars). There was strong and variable upward curvature in the fitness function for vegetative biomass. This increasing slope suggests that fitness accelerated as plants got bigger across all environments. Total nonlinear selection was generally negative for most traits (Figure 15, open bars). When viewed in conjunction with the negative linear terms, these results suggest that plants with particularly late emergence or flowering, or high specific leaf area had very low fitness.

With one exception, correlational selection gradients (γ_{ij}) were not significantly different from zero (results not shown). There was significant correlational selection to increase the covariance between vegetative biomass and reproductive investment across all environments (range: $\gamma_{ij} = 0.2$ in the forest interior to $\gamma_{ij} = 0.4$ in the full sun). This result simply suggests that plants that were able to allocate resources to seeds in proportion to their size had higher fitness.

Table 10. Significance values (*P*) for planned contrasts of linear selection gradients for light and leaf litter environments.

	Trait		
Contrast	Specific Leaf Area	Vegetative Biomass	Reproductive Investment
With Litter			
Forest Interior vs. Edge	0.5515	0.4043	0.509
Forest Interior vs. Full Sun	0.0008	0.7336	0.0012
Edge vs. Full Sun	0.0048	0.5701	0.0074
Litter vs. No Litter			
Forest Interior	0.2256	0.0017	0.8178
Edge	0.7639	0.004	0.0016
Full Sun	0.0473	0.3382	0.0724

Figure 15. Fecundity episode standardized nonlinear selection differentials (C) and gradients (γ). See Figure 14 for details.

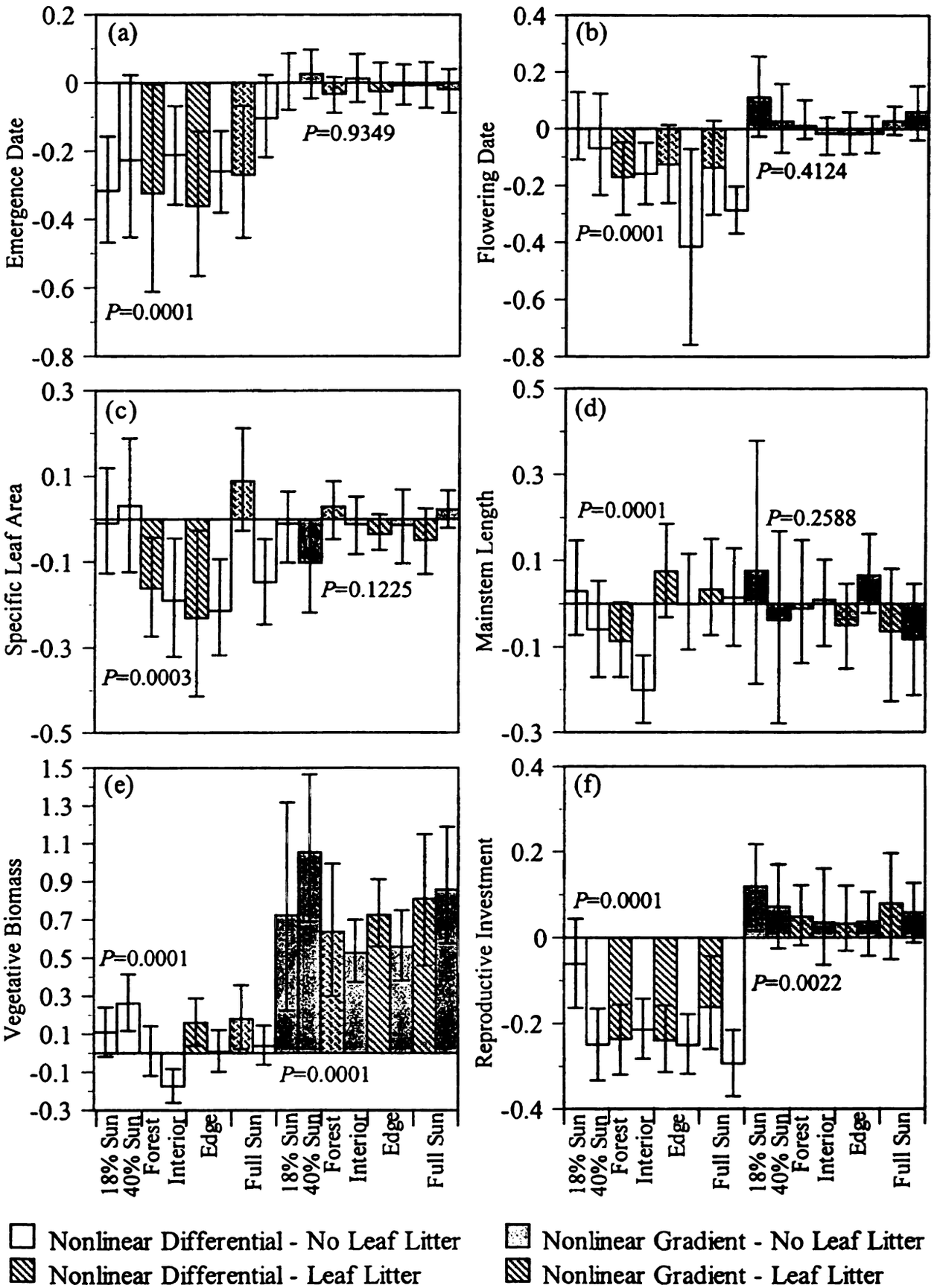

(a)
Emergence Date
P=0.9349
P=0.0001
(b)
Flowering Date
P=0.4124
P=0.0001
(c)
Specific Leaf Area
P=0.1225
P=0.0003
(d)
Mainstem Length
P=0.0001
P=0.2588
(e)
Vegetative Biomass
P=0.0001
P=0.0001
(f)
Reproductive Investment
P=0.0001
P=0.0022
18% Sun
40% Sun
Forest Interior
Edge
Full Sun
Nonlinear Differential - No Leaf Litter
Nonlinear Differential - Leaf Litter
Nonlinear Gradient - No Leaf Litter
Nonlinear Gradient - Leaf Litter

Figure 15.

Path analysis

The path analyses revealed sources of indirect selection on traits in the fecundity episode, and also showed how different traits contribute to the multiplicative fitness components in different ways. As suggested by many reviews of the use of structural equation models (e.g. Petraitis et al. 1996, Shipley 1997, Grace and Pugesek 1998), Table 11 presents the raw data (means, standard deviations, and correlations) used in these analyses. Correlations with seed number are also presented in this table to aid in interpreting paths leading to multiplicative fitness components. Goodness of fit indices for both path models (Figures 10b-c) in all environments are presented in Table 12. The Chi-square statistic reported here is a measure of how well the specified model fits the data, with higher *P* values indicating a better fit. Values of the normed fit index (NFI), non-normed fit index (NNFI), and the comparative fit index (CFI) greater than 0.9 indicate an acceptable fit between model and data (Hatcher 1994). In all cases model one provided a better fit to the data, so only the results of the analysis of this model are presented. Results for model two were essentially identical to those for model one.

Consistent with the results of the fecundity selection episode, the relationships among traits in the path analysis were quite similar across all environments (Tables 13 and 14). The path diagram for the natural forest environment is representative of these results (Figure 16). Earlier emergence was predictive of greater vegetative biomass. Earlier flowering was predictive of greater vegetative biomass and reproductive investment, but later flowering increased number of seeds per fruit. Selection favored higher specific leaf area (shade phenotype) directly through its relationship with flower number and indirectly through reproductive investment. But this selection was balanced

Table 11. Means, standard deviationsb (STD), and correlations within environments between variables in path analysis. Abbreviations are as indicated in the first column. Different environments are above and below the diagonal. (⌘ Variable was log transformed.)

Environment	Trait													
Below Diagonal \ Above	Mean	STD	ED	FD	SLA	MS	RI	VB	FL	FF	SF	SD	Mean	STD
Low Light No Litter (below diagonal) \ Medium Light No Litter (above diagonal)														
Emergence Date (ED)⌘	1.51	0.21	----	0.39	0.08	-0.1	-0.2	-0.2	-0.2	-0.1	-0.2	-0.3	1.48	0.21
Flowering Date (FD)⌘	1.39	0.06	0.38	----	0.32	-0.3	-0.6	-0.2	-0.6	-0.2	-0.2	-0.6	1.29	0.09
Specific Leaf Area (SLA)⌘	2.67	0.1	0.1	0.27	----	-0.2	-0.3	0.14	-0.2	-0.2	0.06	-0.2	2.52	0.1
Mainstem Length (MS)	18	4.55	-0.1	-0.1	-0.1	----	0.7	0.04	0.69	0.18	0.16	0.66	21.4	5.55
Reproductive Investment (RI)	0.21	0.09	-0.1	-0.2	0.08	0.09	----	-0.1	0.07	0.28	0.64	0.35	0.27	0.09
Vegetative Biomass (VB)⌘	2.1	0.27	-0.3	-0.5	-0.3	0.74	0.06	----	0.94	0.25	0.15	0.87	2.39	0.37
Flowers (FL)⌘	0.84	0.26	-0.2	-0.4	-0.1	0.73	0.12	0.82	----	0.13	0.19	0.9	1.06	0.32
Fruits/Flower (FF)	0.66	0.22	0.03	-0.1	-0.3	-0.1	0.4	0.06	-0.2	----	0.14	0.41	0.8	0.16
Seeds/Fruit (SF)	2.36	0.81	-0.1	-0.1	0.09	0.09	0.6	0.09	0	0.05	----	0.5	2.64	0.65
Seeds (SD)⌘	0.96	0.35	-0.2	-0.4	-0.2	0.57	0.64	0.7	0.63	0.38	0.53	----	1.35	0.39

Table 11 (cont'd).

Environment	Trait													
Below Diagonal \ Above	Mean	STD	ED	FD	SLA	MS	RI	VB	FL	FF	SF	SD	Mean	STD
Forest Interior (below diagonal) \ Forest Interior No Litter (above diagonal)														
Emergence Date (ED)⌘	1.27	0.29	----	0.26	0.06	0.1	-0.1	-0.1	-0.1	-0.2	0.07	-0.1	1.25	0.26
Flowering Date (FD)⌘	1.13	0.09	0.29	----	0.22	0	-0.2	-0.3	-0.3	-0.2	-0.1	-0.4	1.11	0.1
Specific Leaf Area (SLA)⌘	2.53	0.09	-0.1	0.19	----	-0.2	0.09	-0.3	-0.2	-0.1	0	-0.2	2.51	0.09
Mainstem Length (MS)	21.6	5.54	-0.1	-0.1	-0.2	----	-0.1	0.55	0.48	0	0	0.37	22.3	5.31
Reproductive Investment (RI)	0.33	0.08	-0.2	-0.2	0.3	-0.1	----	-0.2	0	0.23	0.57	0.37	0.25	0.08
Vegetative Biomass (VB)⌘	2.33	0.27	-0.2	-0.3	-0.3	0.52	-0.2	----	0.9	0.02	0.08	0.76	2.56	0.29
Flowers (FL)⌘	1.14	0.24	-0.2	-0.3	-0.1	0.49	-0.1	0.91	----	-0.1	0.12	0.82	1.24	0.25
Fruits/Flower (FF)	0.73	0.15	-0.1	-0.2	-0.1	0.05	0.32	0.09	-0.1	----	-0.1	0.26	0.77	0.16
Seeds/Fruit (SF)	2.61	0.58	-0.1	0.1	0.17	0.06	0.54	0.03	0.02	0	----	0.51	2.3	0.65
Seeds (SD)⌘	1.39	0.28	-0.2	-0.4	-0.1	0.45	0.28	0.81	0.83	0.28	0.42	----	1.45	0.31

Table 11 (cont'd).

Environment	Trait													
Below Diagonal \ Above	Mean	STD	ED	FD	SLA	MS	RI	VB	FL	FF	SF	SD	Mean	STD
Edge Natural (below diagonal) \ Edge No Litter (above diagonal)														
Emergence Date (ED)⌘	1.38	0.23	----	0.33	0.08	-0.1	-0.1	-0.3	-0.4	-0.1	0.04	0.3	1.29	0.23
Flowering Date (FD)⌘	0.94	0.14	0.34	----	0.2	-0.1	-0.1	-0.3	-0.3	-0.1	0.1	-0.3	0.9	0.15
Specific Leaf Area (SLA)⌘	2.35	0.09	0.06	0.11	----	-0.3	0.31	-0.5	-0.3	-0.1	0.08	-0.2	2.39	0.09
Mainstem Length (MS)	20.3	7.16	-0.1	0	0	----	0	0.44	0.32	0.15	0.15	0.37	17.7	4.79
Reproductive Investment (RI)	0.34	0.09	0	0	0.29	-0.2	----	-0.3	-0.1	0.24	0.52	0.35	0.31	0.09
Vegetative Biomass (VB)⌘	2.46	0.33	-0.4	-0.4	-0.3	0.51	-0.2	----	0.85	0.26	0	0.72	2.43	0.3
Flowers (FL)⌘	1.24	0.31	-0.3	-0.4	-0.2	0.46	0.01	0.92	----	0.17	-0.1	0.8	1.18	0.24
Fruits/Flower (FF)	0.9	0.1	0.1	0	0	0	0.04	0.01	-0.1	----	0.09	0.53	0.85	0.16
Seeds/Fruit (SF)	2.49	0.63	-0.1	0	0.09	0.05	0.54	0.12	0.12	0	----	0.4	2.34	0.63
Seeds (SD)⌘	1.57	0.35	-0.3	-0.4	-0.1	0.43	0.22	0.86	0.92	0.08	0.44	----	1.44	0.31

Table 11 (cont'd).

Environment	Trait													
Below Diagonal \ Above	Mean	STD	ED	FD	SLA	MS	RI	VB	FL	FF	SF	SD	Mean	STD
Full Sun Natural \ Full Sun No Litter														
Emergence Date (ED)⌘	1.47	0.2	----	0.42	0.06	-0.2	0.01	-0.3	-0.3	0	0.05	-0.2	1.58	0.18
Flowering Date (FD)⌘	0.93	0.17	0.35	----	0.23	-0.2	-0.2	-0.4	-0.4	-0.1	-0.1	-0.4	0.9	0.18
Specific Leaf Area (SLA)⌘	2.42	0.11	0.06	0.06	----	-0.1	0.3	-0.3	-0.3	0.01	0.24	-0.1	2.3	0.08
Mainstem Length (MS)	16.7	4.96	-0.1	-0.1	-0.1	----	-0.1	0.7	0.68	0.01	0.08	0.61	16.1	5.7
Reproductive Investment (RI)	0.29	0.09	0	-0.2	0.02	-0.2	----	-0.2	-0.1	0.39	0.59	0.24	0.29	0.09
Vegetative Biomass (VB)⌘	2.29	0.36	-0.3	-0.3	-0.4	0.69	-0.2	----	0.95	0.09	0.04	0.84	2.3	0.36
Flowers (FL)⌘	1.05	0.29	-0.2	-0.4	-0.3	0.59	0.07	0.91	----	0.04	0.07	0.89	1.07	0.31
Fruits/Flower (FF)	0.83	0.16	0	-0.1	-0.1	0.16	0.19	0.23	0.16	----	0.16	0.35	0.84	0.15
Seeds/Fruit (SF)	2.5	0.7	-0.1	-0.1	-0.1	0.05	0.59	0.05	0.07	-0.1	----	0.42	2.66	0.61
Seeds (SD)⌘	1.33	0.37	-0.2	-0.4	-0.3	0.54	0.35	0.81	0.88	0.39	0.43	----	1.39	0.37

Table 12. Goodness of fit indices for path models. *df* = degrees of freedom; NFI = normed fit index; NNFI = non-normed fit index; CFI = comparative fit index.

Model	Chi-square	*df*	*P*	NFI	NNFI	CFI
Low Light No Leaf Litter n=214						
Null Model	941.6317	36	<0.000	0		
Model 1	25.2778	6	0.0003	0.973	0.872	0.979
Model 2	27.2053	7	0.0003	0.971	0.885	0.978
Medium Light No Leaf Litter n=205						
Null Model	1099.373	36	<0.000	0		
Model 1	13.1631	6	0.0405	0.988	0.960	0.993
Model 2	24.4493	7	0.0009	0.978	0.916	0.984
Forest Interior, Natural n=190						
Null Model	761.2273	36	<0.000	0		
Model 1	16.0655	6	0.0134	0.979	0.917	0.986
Model 2	30.5346	7	0.0001	0.960	0.833	0.968
Forest No Leaf Liter n=223						
Null Model	847.4237	36	<0.000	0		
Model 1	9.3355	6	0.1556	0.989	0.975	0.996
Model 2	20.3378	7	0.0049	0.976	0.916	0.984
Edge Natural n=224						
Null Model	893.9886	36	<0.000	0		
Model 1	5.3995	6	0.4937	0.994	1.004	1
Model 2	8.1612	7	0.3186	0.991	0.993	0.997
Edge No Leaf Litter n=231						
Null Model	865.8145	36	<0.000	0		
Model 1	14.0028	6	0.0296	0.984	0.942	0.990
Model 2	33.954	7	0.0001	0.961	0.833	0.968
Full Sun Natural n=316						
Null Model	1407.2935	36	<0.000	0		
Model 1	8.002	6	0.238	0.994	0.991	0.999
Model 2	17.9727	7	0.0121	0.987	0.983	0.992
Full Sun No Leaf Litter n=272						
Null Model	1335.4301	36	<0.000	0		
Model 1	11.304	6	0.0794	0.992	0.976	0.996
Model 2	20.1626	7	0.0052	0.985	0.948	0.990

Table 13. Path model one standardized path coefficients for all causal paths. *P<0.05, **P<0.01, ***P<0.001. Environments: 1. Low light no leaf litter (18% Sun), 2. Medium light no leaf litter (40% Sun), 3. Forest Interior natural, 4. Forest Interior no leaf litter, 5. Edge natural, 6. Edge no leaf litter, 7. Full sun natural, 8. Full sun no leaf litter. Traits: Emergence Date (ED), Vegetative Biomass (VB), Flowering Date (FD), Flowers (FL), Fruits/Flower (FF), Seeds/Fruit (SF), Reproductive Investment (RI), Specific Leaf Area (SLA), Mainstem Length (MS).

	Environment							
Path	1	2	3	4	5	6	7	8
ED>VB	-0.03	0	-0.16**	-0.05	-0.19***	-0.2***	-0.11***	-0.08
FD>VB	-0.29***	-0.37***	-0.22***	-0.21***	-0.26***	-0.1*	-0.19***	-0.15***
FD>FL	-0.08**	-0.03	-0.02	-0.01	0	-0.09***	-0.06***	-0.02
FD>FF	0.1	0.06	-0.11	-0.18***	-0.02	0.01	0.03*	0.09
FD>SF	0.12*	0.1	0.2**	0.08	0.09	0.18*	0.13*	0.11*
FD>RI	-0.2***	-0.23**	-0.23***	-0.18*	-0.07*	-0.14	-0.22***	-0.25***
SLA>VB	-0.2***	-0.11*	-0.19**	-0.18**	-0.26***	-0.35***	-0.28***	-0.23***
SLA>FL	0	0.04*	0.09***	0.04***	0.05**	0.13***	0.04	0.03
SLA>RI	0.14**	0.22**	0.34***	0.11	0.29	0.36***	0	0.34***
MS>VB	0.69***	0.56***	0.47***	0.52***	0.47***	0.32***	0.64***	0.63***
MS>FL	0.29***	0.03	0.03	-0.03*	0	-0.08***	-0.04**	0.02
MS>FF	-0.33**	-0.07	-0.02	0	-0.03	0	0.01	-0.17*
MS>SF	-0.06	-0.03	-0.03	-0.07	0.01	0.13**	0.06	0.02
MS>RI	0.07	0	-0.01	-0.11*	-0.15**	0.05	-0.18*	-0.11*
VB>FL	0.56***	0.93***	0.93***	0.97***	0.97***	1***	0.97***	0.96***
VB>FF	0.33**	0.36*	0.14	0.02	0.03	0.37***	0.27***	0.34***
VB>SF	0.15	0.29**	0.25**	0.27***	0.28***	0.12**	0.15*	0.21**
RI>FL	0.04	0.14***	0.06	0.2***	0.21***	0.24***	0.2***	0.13***
RI>FF	0.43***	0.33***	0.33***	0.21	0.04	0.35	0.24*	0.47***
RI>SF	0.62***	0.68***	0.62***	0.63***	0.6***	0.57***	0.65***	0.66***

Table 14. Path model one unanalyzed correlations. Details as in Table 13.

	Environment							
Path	1	2	3	4	5	6	7	8
ED<->FD	0.39***	0.39***	0.29***	0.26***	0.34***	0.33***	0.35***	0.42***
ED<->SLA	0.1	0.08	-0.05	0.06	0.06	0.08	0.06	0.06
ED<->MS	-0.13*	-0.11	-0.06	0.1*	-0.13	-0.06	-0.07*	-0.15
FD<->SLA	0.27***	0.32***	0.19	0.22*	0.11*	0.2*	0.06	0.23***
FD<->MS	-0.13*	-0.33***	-0.07*	-0.02	-0.02	-0.07	-0.06*	-0.21***
SLA<->MS	-0.05	-0.16*	-0.16***	-0.17**	-0.03	-0.29***	-0.14*	-0.11*
VB<->RI	-0.08	-0.26***	-0.29***	-0.21***	-0.12*	-0.29***	-0.17**	-0.2***
FL<->FF	-0.54***	-0.56***	-0.56***	-0.4***	-0.37***	-0.33***	-0.29***	-0.36***
FL<->SF	-0.27**	-0.21**	-0.17**	-0.26***	-0.44***	-0.49***	-0.35***	-0.24***
FF<->SF	-0.29***	-0.15*	-0.23***	-0.3***	-0.04	-0.1	-0.31***	-0.16*

Figure 16. Representative path diagram showing typical relationships among traits. This diagram is for the natural forest interior plots. The size of the arrow indicates the magnitude of the correlation or path coefficient. Solid lines are positive, dotted lines are negative. "U" represents residual unexplained variance. All values are from structural equation models based on the correlation matrices in Table 11. For other environments see Tables 13 and 14.

Forest Interior With Leaf Litter
Standardized
Path Coefficients
<0.05
0.05-0.15
0.15-0.25
0.25-035
0.35-0.45
0.45-0.55
0.55-0.75
>0.75
Positive
Negative
*P<0.05
**P<0.01
***P<0.001
Emergence Date
Flowering Date
Specific Leaf Area
Main-stem Length
Vegetative Biomass
Repro. Investment
Flowers
Fruits/ Flower
Seeds/ Fruit
U

Figure 16.

by indirect selection for lower specific leaf area (sun phenotype) through vegetative biomass. Lower specific leaf area, and longer mainstems were consistently predictive of greater biomass and consequently more flowers and more seeds per fruit. Earlier flowering and higher specific leaf area were predictive of higher reproductive investment and consequently more fruits per flower and more seeds per fruit.

Vegetative biomass increased fitness primarily through the production of more flowers, while reproductive investment functioned through increases in fruits per flower and seeds per fruit. Seed production was consistently more highly correlated with flower number than with fruits per flower or seeds per fruit (Table 11). There were consistent negative correlations between biomass and reproductive investment and between the three multiplicative fitness components (Table 14). These negative correlations suggest tradeoffs among these various fitness components.

In only two cases were there clear environmentally-dependent changes in the signs of path coefficients. In each case the magnitudes of the path coefficients were small but significant (FD>FF and MS>FL, Table 13). Early flowering plants resulted in more fruits per flower in the forest, but the opposite was true in the sun. In each of these environments plants flowering at these times were more likely to be flowering synchronously with the bulk of the population. Longer mainstems were predictive of more flowers in the low light manipulation, but fewer flowers in three other environments.

DISCUSSION

The results of this study show that the presence of leaf litter caused a change in the direction of direct linear selection on emergence date. Full sun resulted in a change in

the direction of direct linear selection on specific leaf area. These differences in selection provide direct evidence that leaf litter and light were causes of selection in this experiment (Wade and Kalisz 1990). The strength of selection on vegetative biomass and reproductive investment was also environment-dependent in some cases (Table 10). In other cases (flowering date and mainstem length), direct linear selection was surprisingly consistent across all environments, and quite small in magnitude. That flowering date, specific leaf area, and mainstem length are highly plastic, but subject to little direct selection in any environment suggests that the plasticity in these traits is near optimal. Selection always favored greater vegetative biomass and higher reproductive investment. However, there were increasing fitness returns (nonlinear gradients) for greater biomass, but not for greater reproductive investment. Instead, the nonlinear differentials suggest that fitness returns plateau for reproductive investment.

Indirect linear selection was almost always in an opposing direction to direct selection. Consequently, total selection on traits often differed in sign from direct selection. Indirect selection of a greater magnitude and opposite direction from direct selection as seen in this study is a common finding in multivariate selection studies of many species (e.g. *Impatiens pallida* Mitchell-Olds and Bergelson 1990, Bennington and McGraw 1995, Gross et al. 1998, *Lobelia* Johnston 1992, *Diodia teres* Jordan 1991, *Chamaecrista fasiculata* Kelly 1992). In this study, the path analyses revealed consistent relationships among traits across all environments that may lead to the observed patterns of indirect selection.

Traits

Several univariate studies have detected selection for earlier emergence (Kalisz 1986, Miller 1987, van der Toorn and Pons 1988, Biere 1991), and some multivariate studies have found direct influences of emergence time on early survival (Kelly 1992, Stratton 1992). However, most multivariate studies show that selection on emergence date is indirect and occurs through phenotypic correlations with size-related traits expressed later in the life-history (Mitchell-Olds and Bergelson 1990, Kelly 1992, Stratton 1992, Bennington and McGraw 1995, Thiede 1996). In *Collinsia*, the size that seedlings achieve by the onset of winter is an important determinant of overwinter survival (Chapter 2, see also Thiede 1996). Large size at overwintering is also correlated with greater fecundity (Thiede 1996), but at a relatively low level compared with other traits expressed later in the life-history (Chapter 2). Interestingly, there is some evidence that sufficient size at overwintering can be achieved either through early emergence or large seed size (Figure 8 of Chapter 2). Within generations, the phenotypic correlations between these traits are negative (late emerging plants produce small seeds, unpublished data, see also Thiede 1996). However, across generations the phenotypic correlations are positive (large seeds emerge later, Chapter 2, see also Kalisz 1989, Thiede 1996), and the traits have a significant positive genetic correlation (Kalisz 1989, Thiede 1998).

Three episodes of selection on emergence date in an Illinois population of *Collinsia verna* were studied over two generations by Kalisz (1986): survival to spring, survival from spring to fruiting, and fecundity. There was significant direct selection for early emergence in the survival to spring episode of the first year (β=-0.06), and in both fecundity episodes (year 1: β=-0.2, year 2 β=-0.08). In the first year an autumn flood

removed most litter, creating conditions similar to those in the no litter environments in this study, where selection also favored early emergence (Figure 13a). In the fecundity episode, Kalisz found selection for early emergence (range β=-0.05 to β=-0.33). Because Kalisz's study was a univariate analysis, these results are best compared to the selection differentials in this study (Figure 14a). Again, there are striking similarities, both overall, and in her by transect analysis. Kalisz found that total lifetime selection on emergence date at the quadrat scale was highly variable (year 1 range β=-0.55 to 0.2, P<0.01; year 2 range β=-0.96 to 0.68, P<0.89). Considering the small sample sizes in these calculations, it is possible that the presence or absence of leaf litter accounts for these results.

There was no direct selection on flowering date in any environment (Figure 14b). In contrast, many other studies in annual plants have shown that early flowering may have significant fitness benefits (e.g. Mazer 1987, Lechowicz and Blias 1988, Brassard and Schoen 1989, Lotz 1990, Bennington and McGraw 1995, Petit and Thompson 1998, but see Ollerton and Lack 1992). This difference may be due to the size related traits in my analysis. Benefits of early flowering here are accrued indirectly through the greater biomass and higher reproductive investment achieved by early flowering plants. However, some studies still found positive direct benefits of early flowering even when size related traits were included in the analysis (height: Bennington and McGraw 1995, stem height: Petit and Thompson 1998).

Selection on flowering date may depend on variable weather conditions. Spring storms in some years could eliminate the benefits of early flowering. The path analysis suggests that plants flowering later in the spring benefit directly by producing more seeds per fruit (Table 13). It is possible that pollinator service is better later in the season when

conditions are more benign.

Specific leaf area is a trait that integrates over all the physiological and morphological changes that plants make to optimize photosynthetic performance in different light environments. Consequently, it is expected to be highly plastic. The selection differentials and gradients for specific leaf area in this study (Figure 14c) are remarkably similar to the pattern of selection on this trait found in studies of *Iris pumila* (Tucic et al. 1998) and *Diodia teres* (Jordan 1991). Dudley (1996) found similar changes in selection on two other photosynthetic traits, water-use efficiency and leaf area between two moisture environments. The consistency of these results is noteworthy, suggesting physiological and morphological changes in the photosynthetic machinery are critical for success when light and moisture environments are variable. Indirect selection on specific leaf area favored a denser, sun leaf morphology, but the path analysis (Figure 16, Tables 13 and 14) suggested a persistent tradeoff across all environments. Plants with thick leaves produced more biomass, but plants with thin leaves were more efficient at converting vegetative biomass to seeds. Resources invested in thick, dense leaves may be less labile.

Not surprisingly, positive direct selection for size related traits is a common finding in nearly all selection studies (e.g. *Impatiens pallida*, Mitchell-Olds and Bergelson 1990, Bennington and McGraw 1995, Gross et al. 1998; *Erigeron annuus*, Stratton 1992). Interestingly, although overall selection favored taller, heavier plants, direct selection in this study favored shorter plants (Figure 14d). Most multivariate selection studies that include a plant height trait have not found significant direct selection for smaller plants (e.g. Kelly 1992, Bennington and McGraw 1995, Petit and

Thompson 1998, but see Gross et al. 1998). I could find no previous selection studies that have included both measures of plant height and biomass, so it is difficult to assess the generality of this finding. Selection for shorter plants is likely only when an analysis includes other size traits more strongly correlated with fitness.

A-priori, selection would be expected to favor taller plants when inter and intra-specific competition for light is greatest. Indeed, just this sort of adaptive plasticity in stem elongation has been demonstrated in *Impatiens capensis* (selection gradients for height at 25 plants/m^2: $\beta = -0.12$, at 1111 plants/m^2 $\beta = 0.25$, Dudley and Schmitt 1996), and other species (Schmitt et al. 1995, Pigliucci and Schmitt 1999). The plots in the current study spanned a range of densities, yet direct selection consistently favored shorter plants. At harvest, densities ranged from 75 plants/m^2 in the full sun no litter environment to 244 plants/m^2 in the edge no litter environment. It is possible that direct selection would favor longer stems at densities greater than the range encountered in this study.

In contrast to other traits, total selection on vegetative biomass and reproductive investment was less than direct selection (Figures 14e-f). The results of the path analysis provide an explanation for this pattern: there is a tradeoff between these traits, and they contribute to different fitness components (Figure 16, Table 13). The relationship between vegetative biomass and flower number is obvious: larger plants have more flowers. The relationship between reproductive investment and the number of seeds per fruit suggests that reproductive investment may be a measure of the ability to self pollinate (Kalisz et al. 1999) and/or of various display characters that affect the rate of pollinator service (flower size, color, scent, rewards). However, it is difficult to see how

investment in display characters or the ability to self pollinate could result in the negative correlation between vegetative biomass and reproductive investment. Given this negative relationship, it is likely that reproductive investment is in part a measure of the ability to reallocate resources to seeds. The tradeoff reflects a basic energetic constraint: large plants must invest proportionally more resources in structural tissues, which are then unavailable to provision seeds.

Causes of selection

The observation that there is spatial and temporal variation in the magnitude of selection within populations is a common finding, but rarely can the cause of this variation be identified (e.g. Kalisz 1986, Stewart and Schoen 1987, Kelly 1992, Stratton 1992, Gross et al. 1998). Reciprocal transplants can demonstrate environment-dependent selection and can suggest possible causal agents of population differentiation (e.g. Jordan 1991, Bennington and McGraw 1995, Petit and Thompson 1998). However, actual environmental manipulations are necessary to identify specific causal agents of selection (Mitchell-Olds and Shaw 1987, Wade and Kalisz 1990). Surprisingly few studies in plants have used this powerful approach (Dudley 1996, Mauricio and Rausher 1997, Tucic et al. 1998, Winn 1999).

Recently, researchers have manipulated both environment and phenotype to gain a more mechanistic understanding of environmental effects on plant phenotype and fitness (reviews in Schmitt 1999, Schmitt et al. 1999, Schmitt 1997). By inducing the production of the "wrong" phenotype in relevant environments, this approach allows both the identification of causes of selection and the testing of adaptive plasticity hypotheses. By manipulating plant height using mutants (Schmitt et al. 1995, Callahan et al. 1999,

Pigliucci and Schmitt 1999) and light and hormonal cues (Dudley and Schmitt 1996, Cipollini and Schultz 1999), it has been shown that increased plant density selects for shade-avoidance responses, and that plasticity in these traits is adaptive. Other studies of similar design have demonstrated the potential adaptive value of hormonally-induced chemical defenses and the effectiveness of herbivores as selective agents (Baldwin 1998, Agrawal et al. 1999).

The results of this study show that in this population, leaf litter was a selective force on emergence date (Figure 13a). Further, leaf litter and light interact in their effects. In the presence of leaf litter, selection for late emergence was constant across different light levels. Other studies have shown that leaf litter can be a source of mortality, both directly through physical interference and shading, and indirectly through its affect on pathogens and herbivores abundance (Facelli 1994). In this population, seedlings become etiolated and fragile when they emerge beneath litter. The environment under the litter also provides an ideal habitat for foraging slugs which occur in abundance in some autumns (Thiede 1996).

In the absence of leaf litter, the strength of selection for early emergence increased with light availability. Here, the diurnal freeze/thaw cycle in the upper soil layer over the winter may cause mortality, and this cycle may be stronger in high light. Late emerging, shallowly rooted plants are vulnerable to being heaved from the soil (personal observation). That leaf litter selects for later emergence suggests that the litter layer buffers these temperature cycles, and that late emerging seedlings are less likely to become trapped under litter.

Results also showed that light environment was a selective force on specific leaf

area (Table 10, Figure 14c). Low light favored a shade leaf morphology, while full sun favored a sun leaf morphology. Environment-dependence in the pattern of selection on vegetative biomass and reproductive investment was also evident (Table 10), but the causal effects of environment are more difficult to characterize because these results were not consistent across all environments (Figures 14e-f).

When is plasticity adaptive?

Because the plants in this study were not induced to produce the wrong phenotype in each environment, this study cannot answer this question definitively. However, the results for flowering date, specific leaf area, and mainstem length are consistent with the hypothesis that plasticity is adaptive. The lack of strong direct selection on these traits within environments in spite of significant plasticity across environments suggests that plants are producing the appropriate phenotypes in each environment. The apparent conflict between direct and indirect selection for each of these traits may mean that tradeoffs among traits prevent a closer match between phenotype and environment.

In contrast, there is strong evidence that plasticity in timing of emergence is not adaptive. Emergence date shows considerable plasticity in response to both leaf litter and light (Figure 11a), but plasticity is nearly always opposite to the direction favored by selection. Except on the edge, plants emerged earlier in the presence of leaf litter, but selection in these environments favored later emergence. In the absence of litter, plants in high light emerged late, but selection favored early emergence. Positive directional selection on vegetative biomass and reproductive investment across all environments (Figure 14) combined with the intermediate maxima seen for these traits (Figure 11) suggests that plasticity in these traits is not adaptive. Because both the low light and full

sun environments increase mortality and reduce fecundity, these environments can be characterized as stressful for this population (Hoffman and Parsons 1991, Bennington and McGraw 1995).

A weakness of all the approaches used in this study of natural selection is that they are based upon correlations among traits (Mitchell-Olds and Shaw 1987, Wade and Kalisz 1990, Brodie et al. 1995). These relationships may be due to causal relationships among traits and/or between traits and fitness. They may also be caused by selection on phenotypically correlated, but unmeasured traits, or by environmentally induced covariance between traits and fitness (Rausher 1992). Selection on specific leaf area is a good example. Although specific leaf area reflects changes in leaves that occur in response to light availability, the actual traits under selection differ in different light environments. The large thin leaves produced in shady environments where photons are scarce and moisture more abundant probably maximize light harvesting ability (Sultan and Bazzaz 1993). In contrast, smaller thicker leaves produced in more illuminated, drier environments probably maximize water use efficiency (Dudley 1996). Fine-scale variation in water availability in shady environments could result in the positive selection on specific leaf area if plants in moist patches produced larger, thinner leaves and bigger, more fecund plants. Similarly, fine-scale patchy distribution of light due to variation in the density of competitors could be responsible for negative selection on specific leaf area in the full sun if plants growing in more illuminated patches produced thicker leaves and had higher fitness.

These limitations are one reason why experimental manipulations of phenotypes to address questions regarding causal agents of selection and adaptive responses are so

exciting. Still, there are limitations to phenotypic manipulations. Non-lethal mutations and hormonal manipulations can have far reaching pleiotropic effects on the phenotype unrelated to the focal traits (Ketterson and Nolan 1999, Preziosi et al. 1999, Purrington and Bergelson 1999, Tatar 1999). Additionally, they can produce phenotypes outside the natural range, raising questions about their relevance to evolutionary processes in natural populations. Consequently, more studies of the multivariate phenotype in the context of environmental manipulations within natural populations will also be very valuable (Schmitt 1999).

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Chapter 5

CONCLUSION: ENVIRONMENTAL HETEROGENEITY, PHENOTYPIC PLASTICITY, AND THE MAINTENANCE OF GENETIC VARIATION IN A NATURAL POPULATION

Understanding the evolutionary consequences of spatially and temporally variable environments remains a central goal of plant evolutionary ecology. Presently, there is an abundance of theory, but comparatively little empirical data to bear on the outstanding questions. To meet this goal, we must determine the scale, pattern, and predictability of environmental heterogeneity within populations. We must investigate whether environmental heterogeneity results in variable patterns of natural selection. And we must determine the biotic and abiotic causes of natural selection.

Variable selection is most interesting when there is genetic variation that can fuel a response to selection. Therefore, information is needed regarding the genetics of responses to variable environments and the genetic interdependence between traits expressed in different environments. Finally, because maternal effects on seed and offspring traits can be environment-dependant and can have a substantial impact on offspring fitness, more study is needed about the importance of these cross-generation genetic and environmental interactions. In this study I have attempted to answer some of these questions in a natural population of *Collinsia verna*.

ENVIRONMENTAL HETEROGENEITY

Light availability ranged from 25% to 75% of full sun within the population. Natural light environments were correlated across years at a scale appropriate to favor the evolution of plastic maternal effects.

PLASTIC MATERNAL EFFECTS

There were important individual fitness consequences of traits influenced by maternal genotype and environment, and there were genotype-environment interactions for seed size and dormancy. The surviving offspring of intermediate light mothers consistently produced as many or more seeds than offspring of low and high light mothers in all environments. The results suggest that maternal effects in plants can improve offspring performance in variable environments, but also may constrain offspring performance when mothers are stressed. Genetic variation for plastic maternal effects can be maintained by a heterogeneous and unpredictable selective environment.

GENETIC AND ENVIRONMENTAL EFFECTS

There was additive genetic variation in at least some environments for germination, emergence date, flowering date, specific leaf area, mainstem length, mean seed mass, and reproductive investment. There was strong evidence for genotype-environment interactions (genetic variation for plasticity) for flowering date, specific leaf area, mainstem length, and reproductive investment. Finally, there were no genotype-environment interactions for survival, vegetative biomass, or seed number suggesting that there were no strong light environment specialists among the genotypes sampled. However, significant maternal effects on vegetative biomass, seed number, and seed mass without additive genetic variation suggested that maternal genotypes may specialize for different reproductive strategies. The results suggest that genotypes may specialize for particular patterns of germination, emergence, and reproductive investment, while they may be adaptively plastic generalists for flowering date and specific leaf area.

PHENOTYPIC SELECTION

All traits were highly plastic, and selection varied across environments for emergence date, specific leaf area, vegetative biomass, and reproductive investment. The presence of leaf litter reversed the direction of selection on emergence date and increased selection on vegetative biomass. Full sun reversed the direction of selection on specific leaf area, and increased selection on reproductive investment. These differences in patterns of selection provide direct evidence that leaf litter and light availability are selective agents on these traits. Indirect selection on emergence date, flowering date, specific leaf area, and mainstem length was larger in magnitude and in an opposing direction to direct selection.

Together, the parts of this study have simultaneously addressed the relationships between patterns of environmental variation, patterns of variation in phenotypic selection, and patterns of genetic variation and phenotypic plasticity within a natural plant population. Genetic variation for emergence date and plastic maternal genetic effects on seed size and dormancy may be maintained by a heterogeneous and unpredictable leaf litter environment. The presence or absence of leaf litter can directly or indirectly alter the direction of selection on these juvenile and maternal traits. In contrast, the plasticity of the light sensitive traits flowering date and specific leaf area appears to be at or near optimal levels. The absence of substantial genotype-environment interactions for either of these traits supports this argument. Reproductive investment was both heritable and under strong directional selection. Significant genotype-environment interactions appear to maintain genetic variation in this trait.

FUTURE DIRECTIONS

This research has produced several exceptionally rich data sets. Beyond the contents of this dissertation, I have several analyses underway. Moreover, observations during this study and the results above suggest several areas for future study.

Ongoing analysis of this data

Temporal variation in natural selection-Chapter 4 presents results of phenotypic selection for a single growing season, 1996-97. Temperature and soil moisture data I collected during this field season, and anecdotal observations suggest that this was a benign year for all plants compared to the 1995-96 season. There was ample fall moisture leading to earlier emergence and larger over winter size than the previous year. Spring was warm and sunny compared to the previous year. In addition, the density of plants in my study plots was lower in this second year. Together, these factors resulted in plants that were much larger and more fecund in 1996-97 than those in 1995-96. Plants from 1995-96 remain to be processed so that I can compare selection in the two years.

Environmental correlations and bias in selection analysis-The Lande-Arnold multiple regression approach to measuring phenotypic selection (1983) used here in Chapters 2 and 4 can give biased results if environmental factors also contribute to the covariances between traits and fitness (review in Mauricio and Mojonnier 1997). Phenotypic selection analysis in plants may be particularly prone to bias for two reasons. First, plant size varies with local resource conditions, and traits that vary with size or resources may be subject to environmental covariances with fitness. Second, spatial heterogeneity in environmental conditions is expected to result in stronger environmental covariances in sessile organisms like plants than in more mobile organisms (Mauricio and

Mojonnier 1997). Rausher (1992) has developed an approach to selection analysis that can eliminate this bias. Rausher's method is identical to the Lande-Arnold approach, except it uses estimates of breeding values instead of phenotypic values. To be successful, the method requires genetic data for many sires, and the traits of interest must be genetically variable. My 1995-96 data set with 50 sires should be ideal for applying this method. The results can then be compared to the phenotypic selection analysis for natural plants in this year.

Genetic correlations between traits-The next major task that needs to be completed is a multivariate analysis of the genetic data, giving unbiased between trait additive genetic correlations. These correlations are of interest because they would suggest additional genetic constraints on the independent evolution of traits. Using the univariate analysis of the genetic data presented in Chapter 3 I calculated additive genetic correlations from the correlations of breeding values. This approach biases the correlation estimates toward zero. However, all traits had significant genetic associations with at least one trait in at least one year, excepting mean seed mass for which only one year of correlations was available due to a lack of genetic variation (Table 15). Flowering date in year one and specific leaf area in year two were independent of other traits. In contrast to the cross environment genetic correlations, strong negative correlations between traits were common, especially in the second year. These negative relationships are expected, generally occurring between timing traits (emergence and flowering) and size related traits.

Correlational selection-If traits are genetically correlated, then correlated responses to selection may be a very important force shaping the phenotype.

Table 15. Between trait phenotypic and additive genetic correlations. Phenotypic correlations include all data, sample sizes are as indicated in Table 5. Genetic correlations were calculated from BLUP breeding values with environment treated as a fixed effect. Year 1: data from 50 sires from 1995-96. Year 2: data from 12 sires from 1996-97. The sire variance component for mean seed mass in Year 2 was estimated as zero, so no genetic correlations could be calculated. Correlations in bold are significant after a sequential Bonferroni adjustment within years and type of correlation (α=0.05). #P<0.1, *P<0.05, **P<0.01, ***P<0.001.

Table 15.

Trait	Year	Emerge. Date	Winter Size	Flower Date	Specific Leaf Area	Mainstem Length	Plant Mass	Log Seeds	Mean Seed Mass	Repro. Invest.
Emerge. Date	1		**-0.60*** **	0.06	0.08	-0.25#	-0.1	0.19	0.01	0.22
	2		-0.76**	0.75**	-0.12	0.51#	-0.17	-0.71*		**-0.82****
Winter Size	1	**-0.53*** **		-0.11	-0.16	0.33*	0.35*	0.1	0.14	-0.25#
	2	**-0.66*** **		-0.79**	-0.02	-0.2	0.59*	**0.81****		0.61*
Flower Date	1	-0.03	**-0.24*** **		0.09	-0.18	-0.22	-0.11	-0.27#	-0.15
	2	**0.12*** **	**-0.35*** **		0.07	0.43	-0.37	**-0.80****		**-0.85*** **
Sp. Leaf Area	1	0.01	**-0.17*** **	**0.37*** **		-0.28*	**-0.44****	0.31*	-0.18	**0.52*** **
	2	0.06	-0.09*	**0.41*** **		-0.03	-0.18	-0.11		-0.09
Mainstem	1	**-0.09*** **	**0.3*** **	**-0.22*** **	**-0.15*** **		**0.70*** **	0.38**	-0.07	-0.18
	2	**-0.2*** **	**0.38*** **	**-0.14*** **	**0.34*** **		0.59*	-0.04		-0.57#
Plant Mass	1	**-0.08****	**0.29*** **	**-0.28*** **	**-0.37*** **	**0.83*** **		0.38**	0.04	-0.27#
	2	**-0.19*** **	**0.48*** **	**-0.4*** **	**0.21*** **	**0.85*** **		0.63*		0.06
Log Seeds	1	0.06#	**0.15*** **	**-0.34*** **	**-0.14*** **	**0.49*** **	**0.58*** **		0.01	**0.54*** **
	2	**-0.17*** **	**0.44*** **	**-0.54*** **	**0.14*** **	**0.73*** **	**0.85*** **			0.77**
Seed Mass	1	-0.08*	0.04	**0.09****	-0.01	**0.25*** **	**0.21*** **	-0.05		0.26#
	2	**-0.21*** **	**0.25*** **	**-0.14*** **	**0.12****	**0.29*** **	**0.31*** **	**0.22*** **		
Repro. Invest.	1	0.08*	-0.04	**-0.25*** **	**0.18*** **	**-0.18*** **	**-0.31*** **	**0.47*** **	**0.11*** **	
	2	**-0.11****	**0.21*** **	**-0.44*** **	0	**0.14*** **	**0.16*** **	**0.55*** **	**0.41*** **	

In the current study, preliminary genetic correlation data (Table 15) suggests that selection for greater biomass will also select for earlier emergence, earlier flowering, lower specific leaf area, and longer mainstems. These results are in accord with the results of the path analysis (Figure 16). Selection for greater reproductive investment may also select for earlier emergence, earlier flowering, and lower specific leaf area, but shorter mainstem length (opposing indirect selection through biomass). Given that many genetic correlations are small, it seems possible that strong indirect selection in one direction may be balanced by weaker direct selection in the opposite direction resulting in relative stasis in the traits. Vegetative biomass and reproductive investment are unique among the traits in that total selection is smaller than direct selection (Chapter 4 Figures 14e-f). This may be due to negative indirect selection through each other (Chapter 4, Figure 16). However, vegetative biomass and reproductive investment appear to have little direct genetic relationship (Table 15), so the tradeoff between these traits seen in the path analysis may not be evolutionarily important.

The genetic correlations between emergence date and other traits are most interesting. Emergence date was subject to variable selection, it was the most consistently heritable trait in the second year, and the plasticity of this trait appeared to be maladaptive. These observations may be causally related: unpredictable selection on emergence date by leaf liter could maintain genetic variation in the trait. Moreover, variable selection on emergence date may indirectly maintain genetic variation in genetically correlated traits (seed mass, winter size, flowering date, and reproductive investment; Table 15). Interestingly, the three traits with the strongest genetic correlations with emergence date (flowering date, mainstem length, and reproductive

investment) have more genetic variation than the two traits with low genetic correlations with emergence date (specific leaf area and vegetative biomass). Consequently, variable selection on emergence date may be maintaining genetic variation in other traits through correlated responses.

Constancy of genetic parameters-Changes in quantitative genetic parameters across environments may affect predictions about the evolution of phenotypic plasticity and the maintenance of genetic variation (e.g. Via and Lande 1985, 1987, Mitchell-Olds 1992). Moreover, a basic assumption of quantitative genetic models for predicting evolutionary change is that the additive genetic variance-covariance matrix (**G**), is constant (Lande 1979). Several recent studies of natural plant populations demonstrate environment-dependence in quantitative genetic parameters (Mazer and Schick 1991, Shaw and Platenkamp 1993, Anderson and Shaw 1994, Shaw et al. 1995, Bennington and McGraw 1996).

Phillips and Arnold (1999) propose the use of common principle component (CPC) analysis (Flury 1988) for comparing the structure of genetic covariance matrices. The technique represents a powerful new approach to this question, allowing the testing of a hierarchy of hypotheses from unrelatedness to shared principle components, to proportionality, to matrix equality (Arnold and Phillips 1999). I used CPC software (Phillips 1998) to compare pairs of additive genetic covariance matrices across environments and across the two years of this study. Additive genetic covariance matrices were calculated for each environment and each year as the covariance of the BLUP breeding values. As with the calculation of genetic correlations by this method, sampling error can cause covariance components to be significantly underestimated.

Because sampling error may change across environments, this could contribute to differences between matrices. However, the traits that differ most between matrices are emergence date and winter size. These traits have the largest sample sizes in the data sets, and consequently are least subject to underestimation due to sampling error. The number of traits that could be included in the covariance matrix varied from three to seven depending on the number of nonzero additive genetic variance component estimates for the pair of environments or years. I omitted specific leaf area from all matrices because the extreme size of its (co)variances created problems for statistical comparisons between common principle component models.

Based on these analyses, the **G** matrices for the two years in this study and for most environment pairs are unrelated, lacking even a single shared principle component (Table 16). The one exception was the comparison between forest and edge environments in year two, where one test suggested unrelated structure, while the other suggested equality. The low sample size in this year (12 sires) provides little power to compare different models of matrix relatedness. Together, these results suggest that the genetic relationships between traits may change dramatically across different resource environments or between years. A labile **G** matrix would greatly complicate efforts to make evolutionary predictions based on environment dependent selection.

An additional question of interest to quantitative geneticists is whether phenotypic correlations are reasonable estimates of genetic correlations (Cheverud 1988, Roff 1995, 1996, 1997, Waitt and Levin 1998). Estimates of phenotypic correlations are significantly easier to obtain and are much more precise than genetic correlations. The patterns in this data set match those of these previously published reviews (Table 15).

Table 16. Comparison of genetic covariance matrices using common principle components analysis. Only matrices with at least three traits in common were compared. Traits: emergence date (ed), winter size (ws), flowering date (fd), mainstem length (ms), vegetative biomass (vb), seeds (sd), mean seed mass (sm), reproductive investment (ri).

G Matrix 1		**G** Matrix 2			
Year	Environment	Year	Environment	Traits in Matrices	Best Model
1	----	2	----	ed, ws, fd, ms, vb, sd, ri	Unrelated
1	Medium Light	1	High Light	fd, ms, vb, sd, ri	Unrelated
2	Low Light	2	Edge	ed, ws, fd	Unrelated
2	Medium Light	2	Edge	ed, ws, ri	Unrelated
2	High Light	2	Edge	ws, fd, sm, ri	Unrelated
2	Forest	2	Edge	ed, fd, ms, ri	Equal/Unrelated
1	Medium Light	2	High Light	ws, fd, ri	Unrelated
1	Medium Light	2	Forest	fd, ms, ri	Unrelated
1	Medium Light	2	Edge	ws, fd, ms, sd, ri	Unrelated
1	High Light	2	High Light	fd, sm, ri	Unrelated
1	High Light	2	Edge	fd, ms, sd, sm, ri	Unrelated

Phenotypic and genetic correlations are generally of the same sign, while genetic correlations are often larger in magnitude (in spite of their tendency to be underestimated by the methods used here). Phenotypic and genetic correlations are significantly correlated in each year (Pearson correlations: r=0.77, P<0.0001 in year 1; r=0.67, P<0.0001 in year 2).

Reviews have found more congruence between phenotypic and genetic correlations for morphological traits than for life-history traits (Roff 1995, Simons and Roff 1996). There was no evidence for this pattern in this data set. To address this question, log plant mass and log seeds were selected as major fitness components. The relationships between phenotypic and genetic correlations involving at least one of these traits were little different from the whole data set (Pearson correlations: r=0.81, P=0.0003 in year 1; r=0.82, P=0.0006 in year 2). Although these similarities between phenotypic and genetic correlations are striking, there are convincing reasons why phenotypic correlations should not be used to predict phenotypic evolution (Willis et al. 1991). Moreover, although the phenotypic correlations were very similar across years (r=0.81, P<0.0001, n=36), the results of the CPC analysis show a significant change in the genetic architecture of these traits across years.

It has also been argued that due to antagonistic pleiotropy the genetic correlations between major fitness components and other traits will be negative more often than genetic correlations between non fitness components (Roff 1996). This pattern is not supported in this study (Table 15). In the two years, 27% and 54% of the genetic correlations including log plant mass or log seeds were negative, while 57% and 67% of the genetic correlations between other traits were.

Costs of plasticity-The ability to respond adaptively to environmental variation may be costly (review in DeWitt et al. 1998). Van Tienderen (1991) proposed a method of measuring these costs that combines genetic data and phenotypic selection analysis in a way that is similar to Rausher's (1992) technique for reducing bias in the measurement of selection. Recent applications of this technique in snails (DeWitt 1998) and *Daphnia* (Scheiner and Berrigan 1998) have found little evidence of costs. However, a study in *Iris* (Tucic et al. 1998) found evidence for a fitness cost of producing plastic change in leaf length. My genetic data sets are ideal for the application of this method.

Inbreeding depression in variable environments-There is considerable interest in plant mating system evolution. Models focusing on the role of inbreeding depression suggest mixed mating should be rare (reviews: Lande and Schemske 1985, Charlesworth and Charlesworth 1987, Uyenoyama et al. 1993), but other models suggest that mixed mating is an evolutionary stable strategy when pollinator service is unpredictable (Lloyd 1979, Schoen and Brown 1991, Sakai 1995). Species like *Collinsia verna* with mixed mating systems are ideal for tests of the theory (Kalisz et al.1999). The frequency of self fertilization and the expression of inbreeding depression could both be environment-dependent. The results of chapter one are consistent with this idea: delayed flowering in low light mothers may have resulted in more inbred offspring. These offspring performed as well as offspring of other mothers in the intermediate light environment, but their seed production was reduced in the extreme environments (Chapter 2, Figure 6c).

In 1996-97 I planted all the self fertilized offspring of each sire. These offspring germinated at the same rate as their outcrossed half-sibs in all environments. Selfed and outcrossed progeny did not differ in timing of emergence, survival, or specific leaf area in

any environment. However, in each environment, selfed offspring were smaller at overwintering, flowered later, were smaller at maturity, and produced fewer, smaller seeds. The coefficient of inbreeding depression ($\delta = 1 - w_{selfed} / w_{outcrossed}$) was modest in the natural forest (0.28) and edge (0.23) environments, but was more substantial in the manipulated environments (low = 0.45, medium = 0.51, high = 0.41). These higher levels of inbreeding depression in extreme environments are unlikely exert selection against self-fertilization if outcrossing is rare in these environments.

Evolutionary demography in variable environments-As part of this research I have collected detailed demographic data for each of the light and leaf litter environments. I hope to apply population dynamic models and explore the impact of variation in these environmental factors on demographic processes. Surprisingly, results from Chapter 4 suggest that even in the low light environment many plants can produce enough seeds to guarantee persistence for a few more generations (Chapter 4 Figure 12a, e).

Outstanding questions

Together, these chapters and ongoing analyses address only some of the factors that will be important in the future evolution of this population. Like much scientific research, field observations during this study and the results suggest at least as many questions as are answered. There are several other areas of potentially fruitful research in this population.

Heterogeneity of leaf litter and other environmental factors-Leaf litter is clearly an important factor in this population, but the frequency and predictability of different leaf litter environments are unknown. Also unexamined is variation in selection or genetic parameters associated with other variable aspects of the environment such as

moisture, nutrient supply, and interspecific competition.

Evolution of plant architecture-Data from this study suggests that there is a simple genetic basis to a major change in plant architecture. Typical plants have paired cotyledons, leaves, and branches, but two mothers had these organs in threes and produced trifoliate offspring. Approximately 0.01% of the plants in the population have the trifoliate phenotype. This phenotype could be advantageous in high light environments where greater leaf area could increase competitive ability and/or additional branches could increase seed production.

Physiology of photosynthetic acclimation-My research has used specific leaf area as a measure of all the physiological and morphological changes that plants make to maximize photosynthesis in different light environments. At a physiological level it is well known that acclimation to high or low light alters the light compensation and saturation points, and water use efficiency. It is less well known if there is genetic variation and/or genotype-environment interaction for light compensation and saturation points, and water use efficiency. Further, the degree to which acclimation is a physiological phenomena and thus highly labile, as opposed to a consequence of developmentally fixed morphological changes is little studied in an ecological genetic context.

Functional ecology of anthocyanin pigmentation-Anthocyanin pigmentation in *Collinsia verna* leaves varies between families, between seasons, and across light levels. Studies in other species have shown that anthocyanin production is cued by low temperatures and high light levels. Besides their importance as a pigment in flowers and fruits, anthocyanins have no proven function in plants. There are several hypotheses in

the literature: 1. A screen against ultra-violet light damage. 2. A mechanism for elevating leaf temperature. 3. A mechanism conferring cold hardiness or freeze tolerance. 4. Defense against herbivory via secondary compounds or camouflage. 5. Aposematic coloration. 6. Part of some physiological mechanism like photosynthesis. 7. An artifact of another physiological process that performs one of these functions, or some other unknown function. These questions should be easy to investigate because the pigments are easily extracted and quantified, and a great deal is known about their biosynthesis.

Multilevel selection-There is no active seed dispersal in *Collinsia*, setting up conditions under which kin selection processes could operate (Thiede 1996). Moreover, in areas of North America uncovered after the last glaciation, a novel mechanism has been introduced that may be intensifying the potential for kin selection. This region has no native earthworms. Alien, midden building earthworms were introduced by European immigrants. Earthworms are long lived (10 years), and their burrows can persist for hundreds of years (Edwards and Bohlen 1996). Earthworms collect leaf litter, twigs, and living plant material for their middens. My research plots each contained dozens of stable earthworm burrows and middens. In the fall of 1995, I observed extraordinarily high densities of seedlings in these middens (3-5/cm^2 or 50,000/m^2). In May 1996 I observed dozens of dying plants being pulled into these middens. At harvest, some middens contained the flower tags, seeds, and decomposing remains of 10-15 plants collected from an area within about 10 cm of the burrows. As a result, worms may be concentrating genetically related seeds in a very small area. Upon germination, the seedlings are likely to compete very intensively with each other. Experiments to investigate if kin selection has reduced the intensity of competition between genetically

related individuals would be simple to design.

Predicting multivariate evolution-Finally, the synthesis of all of the factors affecting the multivariate evolution of traits and their plasticity in a single evolutionary model has never been attempted. Indeed, it may never be a very rewarding or productive venture given that predictions based on quantitative genetic parameters have only short-term, local relevance. However, it should be possible to develop matrix models that incorporate multiple environments, the frequencies of those environments, genetic variances, covariances, and selection gradients within each environment, and genetic covariances across environments. Studies like the present one could provide the data necessary to parameterize such models.

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