

Evolutionary Dynamics as a Component of Stage-Structured Matrix Models: An Example Using *Trillium grandiflorum*

Tiffany M. Knight,^{1,2,*} Michael Barfield,^{1,†} and Robert D. Holt^{1,‡}

1. Department of Zoology, University of Florida, P.O. Box 118525, Gainesville, Florida 32611;

2. Department of Biology, Box 1137, Washington University, St. Louis, Missouri 63130

Submitted September 25, 2007; Accepted March 17, 2008;
Electronically published August 4, 2008

ABSTRACT: Evolution by natural selection improves fitness and may therefore influence population trajectories. Demographic matrix models are often employed in conservation studies to project population dynamics, but such analyses have not incorporated evolutionary dynamics. We project evolutionarily informed population trajectories for a population of the perennial plant *Trillium grandiflorum*, which is declining due to high levels of herbivory by white-tailed deer. Individuals with later flowering times are less often consumed, so there is selection on this trait. We first incorporated selection analyses into a deterministic matrix model in three ways (corresponding to different methods that have been used for analyzing evolution in structured populations). Because it is not clear which of these methods works best for stage-structured models, we compared each with a more realistic, individual-based model. Deterministic models using fitness averaged over the phenotypic distribution gave trajectories that were similar to those of the individual-based model, whereas the deterministic model using fitness at the mean phenotype gave a much faster rate of evolution than that which was observed. This illustrates that subtle differences in the way in which one splices evolution into demographic models can have a large effect on expected outcomes. This study demonstrates that, by combining demographic and selection analyses, one can gauge the potential relevance of evolution to population dynamics and persistence.

Keywords: flowering phenology, natural selection, contemporary evolution, evolutionary rescue, matrix modeling, herbivory.

* Corresponding author; e-mail: tknight@wustl.edu.

† E-mail: mjb01@ufl.edu.

‡ E-mail: rdholt@zoo.ufl.edu.

There is increasing recognition that evolution can occur on ecologically relevant timescales (Ashley et al. 2003; Stockwell et al. 2003; Carroll et al. 2007). Several studies have documented rapid evolution in introduced species (e.g., Holt and Hochberg 1997; van Klinken and Edwards 2002; Garcia-Rossi et al. 2003; Cox 2004; Gilchrist et al. 2004; Maron et al. 2004), in response to changes in predation pressure (e.g., Reznick and Bryga 1996; Reznick et al. 1996; Hargeby et al. 2004), and in response to global change (Bertheaux et al. 2004). All of these examples include organisms that have relatively rapid generation times in which it is possible to directly observe populations over multiple consecutive generations and thus possible to measure responses to selection (Bone and Farres 2001; Stockwell et al. 2003). In longer-lived species, in contrast, direct observation of a multigenerational response to selection is impossible, and a modeling approach must be employed to determine whether evolution is expected to significantly influence population dynamics.

The concept of incorporating evolution (including extinction) into population dynamics has received considerable theoretical attention in recent years. Persistence in a substandard environment is a race between demographic processes that cause a population to decline and evolutionary processes that can sometimes permit the population's adaptation (Holt 1990; Lynch and Lande 1993; Gomulkiewicz and Holt 1995; Boulding and Hay 2001; Holt et al. 2003). Most theoretical studies have focused on organisms that have simple demographies (e.g., discrete generations). However, longer-lived organisms generally have more complex life histories, which is reflected in variation in demographic rates as a function of age or stage. Many threatened species have long generations. To examine the role of evolution in modifying the population trajectories of longer-lived species, models must incorporate more realistic assumptions about demography.

Van Tienderen (2000) outlined a straightforward approach for linking selection analyses with demographic matrix models for structured populations. He connected traits to population growth rate (λ) using mean standardized selection gradients (which connect traits to fitness

components) and to elasticity analyses (which connect demographic vital rates to λ). Here we apply this approach to an empirical system for which we have information about stage-specific vital rates, trait distribution, and a selection differential, and we ask whether natural selection can rescue a declining population from extinction.

We suggest that van Tienderen's (2000) approach may provide overly optimistic assessments of the potential for evolutionary rescue for two reasons. (1) Fitness components are evaluated for the mean phenotype, and phenotypic distribution is ignored. This may lead to higher estimates of the population growth rate and selection gradient than do methods in which fitness components are averaged over the phenotypic distribution. (2) The model assumes that the amount of genetic variance available for selection is constant and that there is no demographic stochasticity. When populations reach small sizes, however, genetic drift and demographic stochasticity may hamper evolution and lead to extinction. We compare van Tienderen's (2000) approach with two other approaches, the first of which explicitly considers the phenotypic distribution when evaluating mean fitness and the second of which is an individual-based modeling approach that incorporates demographic stochasticity, genetic drift, and the potential for heritable trait variation to be exhausted by selection.

To make these issues concrete, we construct a model tailored to assessing the effect of natural selection on the population trajectory of an herbaceous perennial understory plant *Trillium grandiflorum*. Prior study has revealed that populations of this species are declining due to deer herbivory (Knight 2004). Here we show that deer selectively consume early-flowering individuals. Flowering time is known to have a genetic basis in many plant species (Murfet 1977; Geber and Griffen 2003), and so there is the potential for an evolutionary response by this long-lived herb to herbivory via shifts in flowering time. Extensions of standard results in quantitative genetics permit us to determine how selective changes in this phenotypic trait should affect vital rates and, thus, how they should affect λ . In our example population we find that incorporating demographic shifts due to natural selection is indeed predicted to rescue a declining *T. grandiflorum* population from extinction provided that heritability is sufficiently high. However, as noted in "Discussion," we do not expect this to be a typical result. In many circumstances, natural selection may not greatly facilitate population persistence in changed environments because evolution is sluggish compared with the pace of population decline. By focusing on a particular system, we illustrate the range of concrete decisions that must be made to develop demographic models for assessing extinction risks

in organisms with complex life histories that are experiencing evolution by natural selection.

Trillium grandiflorum (Melanthiaceae; hereafter, *Trillium*) is a native perennial herb that occurs in deciduous forests throughout eastern North America. Individuals persist in a dormant state during autumn and winter, and leaves appear aboveground early in the spring before leaf-out of the forest canopy (Case and Case 1997). Reproductive-stage plants produce a single hermaphroditic flower that is self-incompatible and primarily pollinated by bumblebees (Irwin 2000; Sage et al. 2001; Knight 2003b; Steven et al. 2003). Seeds develop within a single fruit and are primarily dispersed by ants (Kalisz et al. 1999). Seeds germinate belowground in their first year, and a single cotyledon leaf appears aboveground in their second year. If plants survive to their third year, a single true leaf appears. These plants are nonclonal and are easily classified into six stage classes: germinant (belowground germinated seed), seedling (a single cotyledon appears above the ground), one-leaf, small three-leaf (leaf length, <5 cm), large three-leaf (leaf length, >5 cm), and reproductive (Knight 2004). Plants can remain in the one-leaf, three-leaf, and reproductive stage classes for multiple years. *Trillium* has been shown to take at least 17 years to reach maturity in forests (Hanzawa and Kalisz 1993) and can live >70 years (Jules 1998).

Trillium is a preferred food item of the white-tailed deer *Odocoileus virginianus* (Augustine and Frelich 1998). White-tailed deer populations have increased dramatically throughout eastern North America over the past 50 years as a result of a variety of anthropogenic factors, including the additional food provided by modern agricultural practices, the eradication of large carnivores, and habitat fragmentation (McShea et al. 1997; Côté et al. 2004). Thus, the levels of herbivory currently experienced by *Trillium*, as well as by many other forest understory plants, are much greater than the likely levels in the evolutionary past of this species.

Deer consume plants only in the reproductive and large three-leaf stages and primarily consume plants in the former stage (Knight 2003a), typically removing all leaf and flower tissue. After consumption, plants do not resprout in that growing season. Although complete defoliation does not usually kill the plant, reproductive-stage plants experiencing herbivory do lose all reproductive success for the current growing season and are more likely to regress to a nonreproductive stage in the following growing season (Knight 2003a; Rooney and Gross 2003). Observed mortality almost entirely occurs in these regressed, nonreproductive stages.

Trillium is one of the earlier understory species to emerge each spring (in late April in Pennsylvania) and, as such, is one of the first forest food resources available to

white-tailed deer. We hypothesized that the flowering time of reproductive-stage plants would determine their likelihood of being consumed by deer. Specifically, we expected individuals with later flowering times to experience less herbivory. Early-flowering plants bloom during a time when few other food resources are available for deer in the forest, whereas plants flowering later in the season bloom during a time when other edible understory plant species have also emerged. Moreover, late-flowering *Trillium* can be hidden underneath the foliage of later emerging species; in contrast, early-flowering individuals are noticeably conspicuous against the sparse ground cover of early spring (T. M. Knight, personal observation).

The particular population considered in this case study was chosen from among 12 populations in Pennsylvania that were examined in a broader demographic study (Knight 2003b) because it experienced moderate herbivory that was exclusively focused on the reproductive-stage individuals of the population. The population occurred in a small woodlot in Crawford County, Pennsylvania. In 2001, deer consumed 26% of the reproductive-stage plants, which suggested a potential for a substantial impact of herbivory on plant population and evolutionary dynamics. In this population it seemed plausible that selection on flowering time could influence its population dynamics.

Methods

Demography of *Trillium*

In 1999 we established 10 1-m² plots, tagged all of the plants within those plots, and classified individuals by stage. Plots were located at 30-m intervals along a single transect through the population. At least 40 plants per stage were monitored. We tagged additional reproductive-stage plants (outside of the plots) in 2001 to increase the sample size (to achieve a total of 155 reproductive-stage plants) of the flowering time survey.

For each tagged plant, we monitored its stage in the following 2 years (2000 and 2001). Stage was determined in late April, when plants first emerged and before they experienced consumption by deer. For all reproductive-stage plants, we recorded whether they produced a fruit, and we counted the seeds within each developed fruit to determine fecundity. We estimated seed germination rate in 1999 by placing 30 seeds and soil from the site in each of 20 25-cm³ seed baskets (composed of 1-mm² mesh). Seed baskets were covered for the first 3 weeks to prevent removal of the seeds by ants. Because *Trillium* that germinate produce roots but no aboveground structures in their first growing season, 10 of these seed baskets were destructively sampled in 2000 to estimate germination rate (our measure of germination rate combines seed sur-

vorship and probability of germination). The remaining 10 seed baskets were examined in 2001 to estimate the proportion of seeds that survived to the seedling stage. No seed dormancy was observed, so we assume there was no persistent seed bank.

Using this field data, we constructed a six-stage demographic matrix model with 13 nonzero matrix entries (for more details, see Knight 2004). The demographic matrix model relates population sizes in consecutive years by $\mathbf{n}_{t+1} = \mathbf{A}_t \mathbf{n}_t$, where the vector \mathbf{n}_t gives the number of individuals in each stage at time t and the matrix entries a_{ij} in the matrix \mathbf{A}_t give the vital rates: the average number of individuals in stage i at time $t + 1$ produced per individual in stage j at time t (which may depend on t). For all stages except the reproductive stage, these vital rates were calculated separately for each time interval (1999–2000 and 2000–2001) and then averaged. The vital rates of reproductive-stage plants depended on the intensity of herbivory.

Standard matrix manipulations (Caswell 2001) permit one to address many questions with such data, including both the expected trajectory of the population (were the environment to remain constant) and its potential demographic response to evolution by natural selection operating on particular vital rates. If we assume that \mathbf{A} is constant, the population growth rate λ is given by the dominant eigenvalue of \mathbf{A} ; this is the rate of increase (per year) once the population reaches its stable stage distribution (SSD), after which the population size recursion is $N_{t+1} = \lambda N_t$ (where N_t is the total population size). When $\lambda > 1$, the population grows, and when $\lambda < 1$, the population size declines and may face extinction.

Relationship between Flowering Time and Herbivory

Each year, we monitored tagged plants for herbivory. Deer herbivory is easily distinguished from other types of insect and mammalian herbivory because deer leave a flat cut on the stem. Plants were observed daily for evidence of deer herbivory during late April and early May (when >90% of the episodes of herbivory occurred) and once every 2 weeks for the remainder of the growing season. In 2001, we documented plant size (leaf length) and the date at which each reproductive-stage plant first opened its flower (ranging from April 29 [which we designate as day 1] to May 12 [day 14]). We used logistic regression to test for a relationship between flowering time and whether plants were consumed by deer. The relationship between flowering time and the incidence of herbivory was best described using a linear equation (as described in “Results”), which we used in our models; this follows van Tienderen (2000), who regressed matrix elements on phenotype (see eq. [4]). We censused reproductive-stage

plants in 2002 to determine their stage and size (leaf length). To determine whether flowering time in the absence of herbivory influences stage transitions and relative growth rates of reproductive-stage plants, we used logistic regression and linear regression, respectively.

Effects of Flowering Time on Vital Rates

We found no effects of flowering time on demographic rates except those related to browsing (see “Results”). To determine the effects of flowering time on the vital rates of reproductive-stage plants, we calculated vital rates separately for consumed and not-consumed plants. Let $p(z)$ be the probability that a reproductive-stage plant with flowering time z is browsed (as obtained from the linear equation described above, truncated at 0 probability). Using the average vital rates of consumed plants (a_{ij}^c) and not-consumed plants (a_{ij}^n) as determined from the 1999–2000 and 2000–2001 census interval data, the expected vital rates of a reproductive-stage plant with flowering time z are calculated as

$$a_{ij}(z) = p(z)a_{ij}^c + [1 - p(z)]a_{ij}^n. \quad (1)$$

Browsing, and therefore flowering time, affected the fecundity and the probability that a reproductive-stage plant would remain in a reproductive stage or would regress to the large three-leaf stage, so equation (1) was used for matrix entries a_{16} , a_{56} , and a_{66} .

Models of Flowering Time Evolution Assuming a Normal Phenotypic Distribution

Because differential consumption of flowering plants by deer is leading to population decline (see “Results”), evolutionary shifts in flowering phenology could mitigate the demographic effects of deer. To address this, we must make assumptions about the genetic architecture underlying population variation in the trait of flowering time. In general, many traits of ecological significance are polygenic in nature (Lande 1982). Flowering time has been shown to be influenced by many loci, each with a small effect, in a number of different species (e.g., Fu and Ritland 1994; Clarke et al. 1995; Laurie et al. 1995; Camargo and Osborn 1996; Mitchell-Olds 1996; Yamanaka et al. 2000; Geber and Griffen 2003). We will therefore assume that the distribution of flowering time can be described using the standard protocols of quantitative genetics and that the phenotypic distribution is Gaussian, with mean \bar{z} and variance Z . We assume that Z is fixed but that \bar{z} can change with time as evolution occurs.

Selective changes in flowering time will alter the exposure of plants to deer herbivory and thus shift popu-

lation growth rates. To approximately determine the trajectory of population size and the evolution of flowering time, we need to determine the growth rate λ as a function of mean flowering time \bar{z} . For a population of plants with the same flowering time z , equation (1) gives the transition matrix elements at that z , and the dominant eigenvalue of that matrix is the growth rate $\lambda(z)$. However, to calculate the approximate population growth rate for a population of plants that have different flowering times, there are several ways that have been previously used to model structured populations. It is not clear which method is best for stage-structured models, so we will use three of them and compare them with a more realistic model. The simplest method is to evaluate $\lambda(z)$ at the mean phenotype \bar{z} , giving $\lambda(\bar{z})$. This is the approach taken by van Tienderen (2000). Alternatively, we can average $\lambda(z)$ over the distribution of z (Lande [1976] similarly averages fitnesses over the phenotypic distribution). We refer to this average as $\bar{\lambda}(\bar{z})$. With respect to projecting population dynamics, using this average in effect assumes that all plants mate only with other individuals who are flowering at the same time as they are (i.e., assortative mating by flowering time). When $\lambda(z)$ is approximately linear over the distribution of z (as is likely when selection is weak), then $\lambda(\bar{z})$ is a good approximation to $\bar{\lambda}(\bar{z})$; however, this approximation may be less valid when selection is strong. A third method is to replace each matrix element $a_{ij}(z)$ with its average over the realized distribution of z . We refer to the dominant eigenvalue of this matrix as $\tilde{\lambda}(\bar{z})$. The population trajectory can then be generated using any of these growth rates using

$$N_{t+1} = \lambda_t N_t \quad (2)$$

where N_t is the total population size at time t .

The relationship between growth rate and mean flowering time also determines the strength of selection on flowering time and can be used to estimate the potential for evolution of this trait. Van Tienderen (2000) defines the selection gradient (which is used to predict trait evolution) as $\beta = d \ln \lambda / d \bar{z}$, and he notes that the derivative in this expression can be calculated in two different ways that correspond to the first two definitions of growth rate given above. The change in average phenotype (flowering time) during one year of selection is the product of the genetic variance G and the selection gradient (selection differential in our case, because we are considering a single trait). Therefore, the average phenotype recursion is

$$\bar{z}_{t+1} = \bar{z}_t + G \left. \frac{d \ln \lambda}{d \bar{z}} \right|_{\bar{z}_t}, \quad (3)$$

where λ here could be any of the three growth rates defined

above, depending on the life-history details. Using $\bar{\lambda}(\bar{z})$, this equation is correct for a simple iteroparous life history (Lande 1976), whereas using $\lambda(\bar{z})$ is equivalent to using $\bar{\lambda}(\bar{z})$ if growth rate is a linear function of phenotype (which is approximately true with weak selection; de Jong 1994). Lande (1982) showed that evolution in an age-structured population follows equation (3) using $\bar{\lambda}(\bar{z})$. We have found that, for stage-structured populations, the population size recursion is described with equation (2) using $\bar{\lambda}(\bar{z})$ (see appendix). However, for the stage-structured case, evolution of \bar{z} in general does not appear to be reducible to the compact form of equation (3) (M. Barfield, unpublished results). Here, we will contrast these approximations using selection differentials with simulations based on an individual-based model.

We used all three of these definitions of fitness and the corresponding selection differentials to calculate the trajectories for population size and average phenotype using equations (2) and (3). The initial mean and variance of flowering time of the simulated populations were set equal to the measured values of the studied population. We began with a population of 5,000 individuals (natural *Trillium* populations often have >5,000 individuals; T. M. Knight, personal observation). Because the heritability h^2 of flowering time in *Trillium* is unknown, we considered four different heritability values: 0.25, 0.5, 0.75, and 1. The genetic basis for flowering time has been established for a wide range of species, and this trait is likely heritable in most angiosperms (Lawrence 1963; Murfet 1977; Carey 1983; Pors and Werner 1989; Laurie 1997; O'Neil 1997; Van Berloo and Stam 1999; Geber and Griffen 2003). However, the magnitude of narrow-sense heritabilities differs within and across species; values as low as 0.04 have been found for *Chamaecrista fasciculata* (Kelly 1993), whereas values as high as 0.97 have been found for *Raphanus raphanistrum* (Mazer et al. 1987). Several studies have shown a response to selection on flowering time in natural populations (reviewed in Rathcke and Lacey 1985). The genetic variance was the product of the assumed heritability and the measured phenotypic variance ($G = h^2Z$, where G , Z , and h^2 were assumed to be constant). The population size and mean flowering time were then calculated over 2,000 years using equations (2) and (3) for the three definitions of growth rate, recalculating the transition matrix each year to track changes in mean flowering time.

Van Tienderen (2000) calculated the selection gradient as a sum of products of elasticities and regression slopes relating matrix elements to phenotypes and developed a framework for analyzing evolution with multiple correlated traits. The proportional contribution of each matrix entry to λ is described by a matrix of elasticities, where the ij th matrix element e_{ij} is $\partial \ln \lambda / \partial \ln a_{ij}$. Although we have only one trait, we followed this procedure, recalculating

elasticities for every year of the simulation. The selection differential using this method can be written as

$$\beta = \sum \frac{\partial \ln \lambda}{\partial \ln a_{ij}} \frac{\partial \ln a_{ij}}{\partial z} = \sum \frac{e_{ij}}{a_{ij}} \frac{\partial a_{ij}}{\partial z}, \quad (4)$$

where the values of $a_{ij}(z)$ are given by equation (1) evaluated at the mean phenotype, λ is the dominant eigenvalue of the matrix with elements a_{ij} , e_{ij} is the elasticity of element a_{ij} , and the sums are over all nonzero matrix elements. The last derivative can be found using equation (1) and the browsing probability as a function of flowering time. Our results were identical to those obtained with equations (2) and (3) using the first definition of growth rate $\lambda(\bar{z})$. This was expected because equation (4) is equivalent to the selection differential calculated using the growth rate at each phenotype (without averaging the growth rate or the matrix elements over the phenotypic distribution). This approach gives insight into which matrix elements contribute most to the selection differential. Van Tienderen (2000) used a slightly different formulation that uses mean-normalized regressions of fitness components on traits.

If evolution did not occur, then λ would remain constant, and if $\lambda < 1$, then the population would eventually become extinct. However, with selection, \bar{z} and, therefore, λ increased every year (until \bar{z} reached the value at which there was no browsing, for the first model). Because selection was directional and genetic variance was constant, λ eventually exceeded a value of 1 in all cases and the populations rebounded. If this rebound occurred before population size reached a low critical density (as in Goumukiewicz and Holt 1995), evolution would have rescued the population from extinction. Thus, in our evolutionary analysis of a declining population using stage-structured matrix models, initial population size, initial λ , heritability, and the relationship between flowering time and λ collectively determine whether the population is at risk for extinction.

Individual-Based Model of Selection on Flowering Time

The above approach is heuristically valuable, but it does require one to identify a critical population size below which extinction is likely. Moreover, it also rests on some genetic assumptions (e.g., constant heritabilities) that may be questionable in small, declining populations. To complement the stage-structured models, we created an individual-based model (IBM) that incorporated demographic stochasticity, genetic drift, and the potential for heritable variation in flowering time to be exhausted by selection. Because this model tracks the fates of discrete

individuals, actual extinctions do occur. The model was derived from the discrete genetic model described by Holt et al. (2003; based on an earlier model of Burger and Lynch [1995]), which was modified here to incorporate the six life-history stages of *Trillium*, selection on flowering time, and a closed population. We assumed that quantitative genetic variation in flowering time was influenced by multiple loci. We compared the course of adaptive evolution for this IBM with the simpler demographic models described above and assessed extinction risks.

In the IBM, flowering time was determined by a single polygenic character determined by 10 freely recombining diploid loci with additive allelic effects within and among loci (i.e., no dominance or epistasis). The phenotypic value of flowering time for an individual z was the sum of the contribution of each of its alleles and an environmental effect. The initial phenotypic variance was again the measured variance in flowering time. The initial genetic variance was the assumed heritability multiplied by the phenotypic variance ($G = h^2Z$). Values for G , Z , and h^2 were then free to change over time as evolution proceeded. A variety of initial heritabilities was used (as in the stage-structured matrix model) because this parameter was unknown for the *Trillium* population.

The environmental effect e was assumed to have a Gaussian distribution with a mean of 0 and variance E equal to the difference between the initial phenotypic and genotypic variances (E did not change with evolution). For each individual, e was randomly assigned at birth and remained constant throughout the individual's lifetime (i.e., there is no within-individual variation in flowering time). This could arise from fixed microsite effects or initial conditions that influenced flowering time (e.g., how deeply the tuber of the plant is initially buried). We assumed that there were no mutational or migrational inputs of heritable variants; if we relax this assumption, then evolution would likely be faster, so our results should describe a worst-case scenario (at least with respect to this genetic assumption). The phenotype did not affect an individual's fitness until it reached the reproductive stage. The stage-specific probabilities of survival and growth were identical to those of the matrix model. Each reproductive-stage individual had a probability of being eaten by deer that was entirely determined by its flowering time.

We assumed that individuals were hermaphroditic and that mating was monogamous within each year. Each year every reproductive-stage plant that was not consumed by deer produced a number of seeds drawn from a Poisson distribution with a mean of 9.47 (the actual mean observed for natural plants not eaten by deer; see "Results"). The father of all seeds from a given fruit was chosen randomly each year from the pool of unbrowsed reproductive-stage plants (it is possible for browsed plants to be fathers, and

we performed additional simulations in which both browsed and unbrowsed plants can be fathers; the results differed little from those reported here). Although polygamous mating is possible for *Trillium*, an allozyme study by Kalisz et al. (1999) showed that 1.2 effective males sire seeds within a fruit. This suggests that monogamous mating is a reasonable qualitative assumption for *Trillium*.

If eaten, a reproductive-stage plant in the simulations had a probability of 0.31 of remaining in the reproductive stage in the next year, and if not eaten, the probability was 0.78 (as observed for natural plants; see "Results"). Plants that did not remain in the reproductive stage regressed to the large three-leaf stage. We assumed "ceiling" density-dependent regulation of the number of germinants (i.e., new recruits). With more than K germinants, K survivors were sampled randomly. We set K equal to the initial total population size; therefore, density dependence should not affect the population size during the phases of decline and evolutionary rescue that are of interest.

We began with 5,000 individuals in the population and then followed population size and mean flowering time for 2,000–3,000 simulated years. We started at the SSD for the population as determined by the demographic matrix at the empirically observed mean flowering time. The initial flowering time of reproductive-stage individuals in the simulated population had a mean of 7.6 days (with day 1 being the date of earliest flowering) and variance of 10.54 days² (as measured in the natural population described above). The initial mean genotypes and phenotypes of other stages were set to their steady state values relative to that of the reproductive-stage individuals (see appendix). We also examined the probability of extinction for populations that varied in initial size and heritability of flowering time. We performed 400 runs for each set of conditions and calculated the fraction of those runs that went extinct by 3,000 years. The initial population sizes ranged from 100 to 5,000 individuals, with initial heritabilities of 0.25, 0.5, 0.75, or 1.

Results

Demography of Trillium and Effects of Herbivory

The demography of *Trillium* is typical of a perennial plant; individuals in smaller stages (germinant, seedling, one-leaf, small three-leaf) have a high probability of mortality, whereas plants in larger stages (large three-leaf, reproductive) have much lower mortality. Indeed, *Trillium* in the large three-leaf and reproductive stages had no detectible mortality in our field study (see demographic matrix below; survival of stage i is the sum of a_{ii} and the elements above and below it). However, plants in these

stages can regress to smaller stages, where they do experience a risk of mortality.

Herbivory increases the probability that reproductive-stage plants regress to the large three-leaf stage. Only 21% of plants not consumed by deer regress to the large three-leaf stage, compared with 69% of consumed plants. Plants eaten by deer experienced a removal of their entire flower or developing fruit and therefore produced no seeds in that year. Plants not consumed by deer produced an average of 10.52 seeds, 90% of which germinated in the next year. Because both seed production and germination occur within a 1-year time step, these two rates are multiplied together to determine the number of germinants at time $t + 1$ produced by reproductive-stage plants at time t (9.47 for this population).

In 2001, 26% of the reproductive-stage plants in this population were consumed by deer. At this level of herbivory, the growth rate λ of the population is 0.991. Thus, this population is expected to decline in size by 0.9% each year. However, if herbivory ceased for this population, the population size would increase by 0.37% each year ($\lambda = 1.0037$). Therefore, in this population, the absence or presence of deer herbivory determines whether the population grows or declines, respectively. If the ambient density of deer was to stay at its current level and there was no evolutionary shift in flowering time, the matrix model projects that extinction for this population is inevitable.

Plants That Flower Later Experience Less Herbivory

Reproductive-stage plants flowering earlier were much more likely to be consumed by deer (odds ratio = 0.676, $P < .001$; fig. 1). The range of flowering times observed was 14 days (from April 29 to May 12). All of the plants that flowered on the earliest date were eaten. None of the plants that flowered later than May 8 (day 10) was eaten by deer. The regression equation for the probability that a plant with flowering time z was browsed was $p(z) = 0.758 - 0.062z$. We did not detect an advantage to flowering early in the season in the absence of herbivory (see appendix for more discussion). Across all 114 plants that were not eaten by deer, flowering time did not affect their stage in the next year (odds ratio = 1.009, $P = .904$) or their relative growth rate (linear regression: $P = .11$).

Because herbivory decreases with flowering time, the proportion of reproductive-stage plants remaining in the reproductive stage and the average seed production of reproductive-stage plants both increase with flowering time. The vital rates of reproductive-stage plants are functions of flowering time, and these functions can be directly incorporated into the demographic matrix. In this matrix, the vital rates of all other stages are constant values, whereas the vital rates of the reproductive-stage plants are

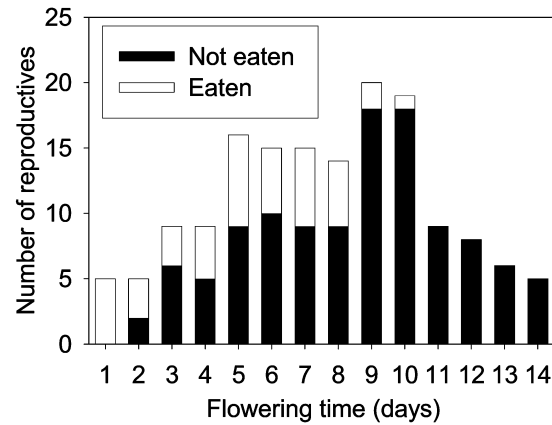


Figure 1: Number of plants eaten and not eaten by deer based on flowering time. Plants flowering later are less likely to be consumed by deer. Results are based on 155 reproductive-stage *Trillium grandiflorum*. Mean flowering time is 7.6 days.

functions of flowering time z that are obtained by substituting the regression equation above and the matrix entries for browsed and unbrowsed plants into equation (1) for the three matrix entries that depend on browsing. The resulting demographic matrix is

$$A = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 2.3 + 0.59z \\ 0.11 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0.33 & 0.65 & 0.04 & 0 & 0 \\ 0 & 0 & 0.15 & 0.84 & 0.20 & 0 \\ 0 & 0 & 0 & 0.10 & 0.64 & 0.58 - 0.029z \\ 0 & 0 & 0 & 0 & 0.16 & 0.42 + 0.029z \end{bmatrix}$$

This applies for $z < 12.23$ (the flowering time that gives 0 browsing using the regression), above which browsing is set to 0 and the three variable matrix entries are fixed at their no-browsing values. The growth rate as a function of flowering time determined from this matrix is shown in figure 2. This curve is the same as the growth rate using the first method described above (for which the average growth rate is the growth rate at the average flowering time). Also shown are the average growth rates as a function of average flowering time using the other two approaches and the parallel selection differentials.

Demographic Model of Selection on Flowering Time

In general, for all approaches, a delay in flowering time increases the growth rate, and a sufficiently large change in flowering time can lead to a population with a growth rate exceeding 1 (fig. 2). The change in $\lambda(z)$ is nonlinear; it accelerates at higher flowering times until it reaches the no-browsing level, when it is capped. Because $\bar{\lambda}(\bar{z})$ is ob-

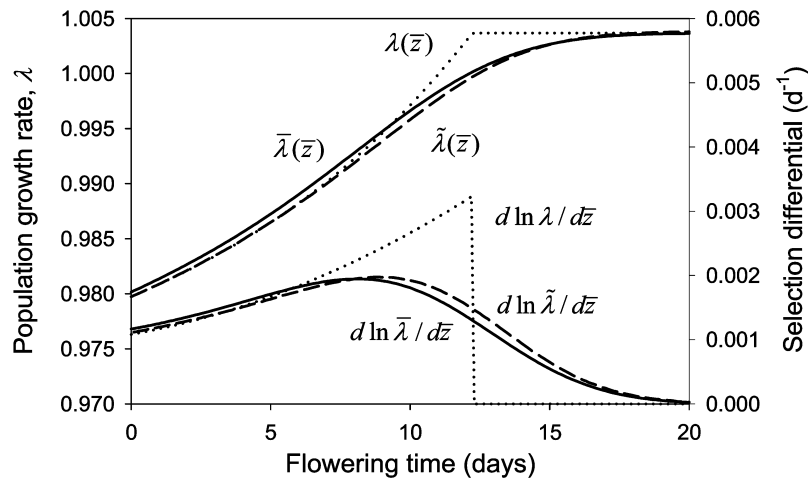


Figure 2: Population growth rate λ and strength of selection on flowering time $d \ln \lambda / d \bar{z}$ as a function of mean flowering time \bar{z} . The three ways to approximate the population growth rate at the mean flowering time are as follows: (1) calculate λ at the mean flowering time $\lambda(\bar{z})$ (dotted line), (2) average λ over the distribution of z ($\bar{\lambda}(\bar{z})$; solid line), or (3) average each element of the demographic matrix over the distribution of z and then calculate λ ($\tilde{\lambda}(\bar{z})$; dashed line).

tained by averaging over $\lambda(z)$, it lies above $\lambda(z)$ at low flowering times (where $\lambda(z)$ is concave upward) and below $\lambda(z)$ at high values of z (where $\lambda(z)$ is concave downward because of the saturation). The third curve $\tilde{\lambda}(\bar{z})$ is very close to $\lambda(z)$ at low flowering times (the two would be identical if the values of a_{ij} were all linear, which they are until saturation is reached, so these two measures of growth rate should be the same far below the zero-browsing flowering time) but below $\lambda(z)$ at higher flowering times. Figure 2 also shows the selection differentials. Overall, the strength of selection appears to be greatest at intermediate flowering times.

Because the measured population has an initial mean flowering time of 7.6 days and will evolve to higher values, the evolution rate using $\lambda(\bar{z})$ will be higher than the alternatives from the beginning of the simulation until it reaches zero browsing (at which point evolution for $\lambda(\bar{z})$ will cease) because the selection differential is highest for $\lambda(\bar{z})$. Evolution using $\tilde{\lambda}(\bar{z})$ should be slightly faster than that using $\bar{\lambda}(\bar{z})$ because of its higher selection differential for values of \bar{z} above the initial value ($\bar{z} = 7.6$).

The increased slope of growth rate at higher values of flowering time can be explained by the increased sensitivity of λ to the vital rates of reproductive-stage plants at higher flowering times. As flowering time increases, the proportion of individuals in the reproductive stage at SSD decreases slightly (because there is more recruitment, which causes germinants to make up a larger fraction of the population; fig. 3A), but the reproductive value of individuals in the reproductive stage increases (fig. 3B). The net effect is a nonlinear increase in the elasticity of stage

transitions of reproductive-stage plants (fig. 3C). Thus, there can be an acceleration of evolutionary impacts on growth rates because, as flowering time increases through selection, the reproductive value of reproductive-stage plants also increases and future changes in flowering time will then cause more dramatic increases in λ .

Population trajectories are shown in figure 4 for a range of heritabilities; the corresponding evolutionary trajectories of flowering time are shown in figure 5. The figures also show the corresponding results from 100 runs of the individual-based model (for population size, a geometric mean is used to prevent runs with higher growth rates from dominating the average). Because this model involves fewer assumptions, we use it here as the standard for judging the accuracy of the other methods. As can be observed in all cases, the projected rate of evolution using $\lambda(\bar{z})$ for growth rate is faster than it is for the other methods (until saturation is reached because all plants have escaped deer herbivory); the population reaches its minimum abundance faster and at a higher density than it does when using the other methods. This result arises because the leveling off of growth rate that occurs when zero browsing is reached does not affect this measure of growth rate until the population mean itself reaches this zero-browsing level. In the other methods, some individuals reach the zero-browsing point before the population mean does, resulting in a reduced growth rate. Using $\lambda(\bar{z})$ for growth rate is a commonly used approximation (as in van Tienderen 2000), but in this case (which is based on real data) it does not actually perform that well.

The other two definitions of growth rate agree much

better with results from the IBM. The method based on averaging elements of the transition matrix ($\tilde{\lambda}(\bar{z})$) tends to produce populations that evolve faster than do the populations for which we use $\lambda(\bar{z})$ (and, generally, the IBM), but the population size also tends to reach lower levels. This agrees with figure 2, for which $\tilde{\lambda}(\bar{z})$ is slightly lower than $\lambda(\bar{z})$ at the initial phenotype (flowering at day 7.6), so that the population initially decreases faster using $\tilde{\lambda}(\bar{z})$ (and, therefore, it reaches lower levels). The selection differential, however, is about the same at the initial phenotype, but for later flowering times the selection differential is higher for $\tilde{\lambda}(\bar{z})$. Therefore, evolution should be faster using $\tilde{\lambda}(\bar{z})$. The growth rates eventually approach equality, and the selection differentials approach zero; therefore, faster evolution does not guarantee that the population projection using $\tilde{\lambda}(\bar{z})$ will eventually catch up with that using $\lambda(\bar{z})$.

For all plots shown here, the size of the population using $\tilde{\lambda}(\bar{z})$ remains below that of the population using $\lambda(\bar{z})$. At a heritability of 1, the IBM population consistently exceeds both these alternatives, whereas for 0.75 the IBM is close to the population that emerges using $\lambda(\bar{z})$. For lower heritabilities, the IBM population size is bracketed by the two methods that are based on averaged growth rate. For the IBM, there were extinctions only for a heritability h^2 of 0.25, for which 40% of the populations went extinct. The trajectories shown are averages using only those populations that did not become extinct. Therefore, the IBM results for $h^2 = 0.25$ might be biased toward higher population and phenotype values (because the populations that reach lower levels are more likely to become extinct and so will not be included in the average) compared with the methods based on deterministic growth rates, for which all populations persist.

Figure 5 shows evolution in the average phenotype (mean flowering time). Using $\lambda(\bar{z})$, the rate of evolution is faster than it is for any of the other methods (until the no-browsing threshold is reached). The other methods of calculating growth rate initially agree well with the IBM, but the IBM eventually starts to evolve significantly more slowly than do the other methods. As expected, the model using $\tilde{\lambda}(\bar{z})$ initially evolves more slowly (and remains lower in flowering time) than the model using $\lambda(\bar{z})$. Overall, the results with $\tilde{\lambda}(\bar{z})$ are at least as good as those with $\lambda(\bar{z})$. This is somewhat surprising, because the population size should evolve according to $\tilde{\lambda}(\bar{z})$ (assuming the population is always at its SSD; see appendix). Of course, the evolutionary course does not necessarily follow equation (3) using $\tilde{\lambda}(\bar{z})$, but even in the first few generations that arise before significant evolution has occurred, the population size using $\tilde{\lambda}(\bar{z})$ does not agree with more precise methods, as it should (see appendix). However, using $\tilde{\lambda}(\bar{z})$ to project total population assumes that the population is at its SSD,

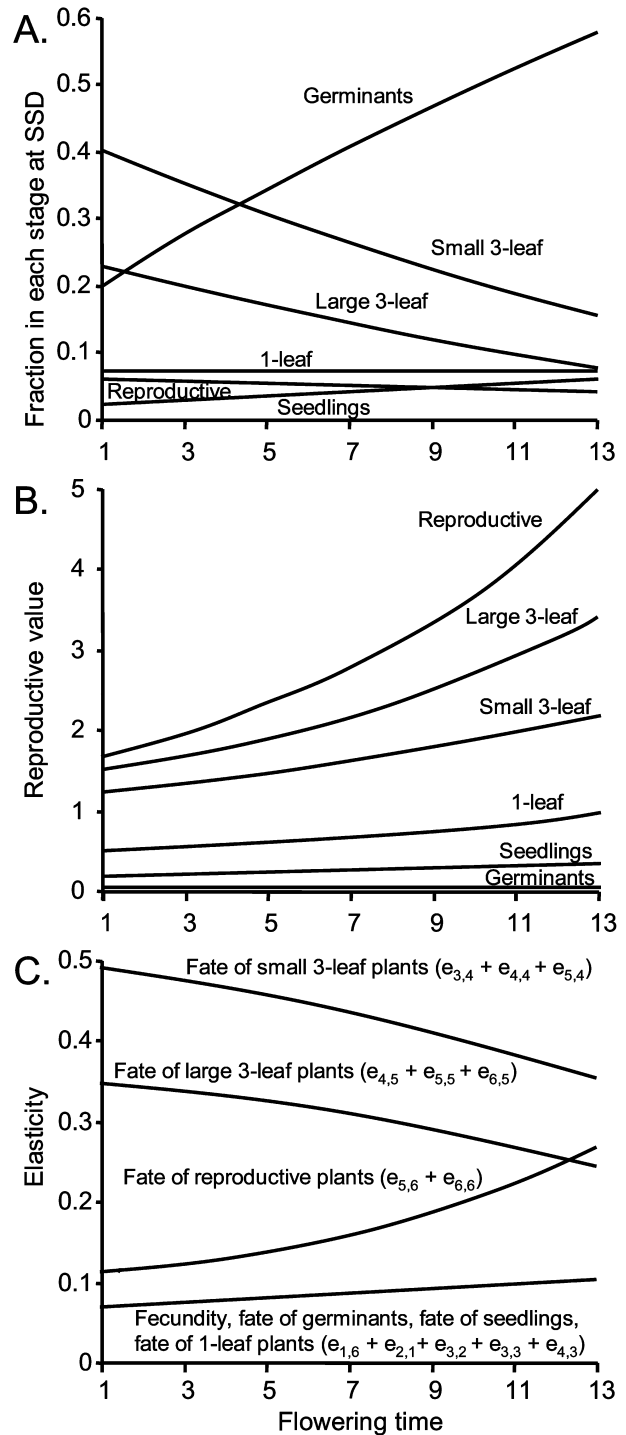


Figure 3: Relationship between eigenvectors and elasticities and mean values of flowering time. A, Proportion of individuals in each stage class at stable stage distribution; B, reproductive values of individuals in each stage class; C, elasticity of λ to groups of matrix entries.

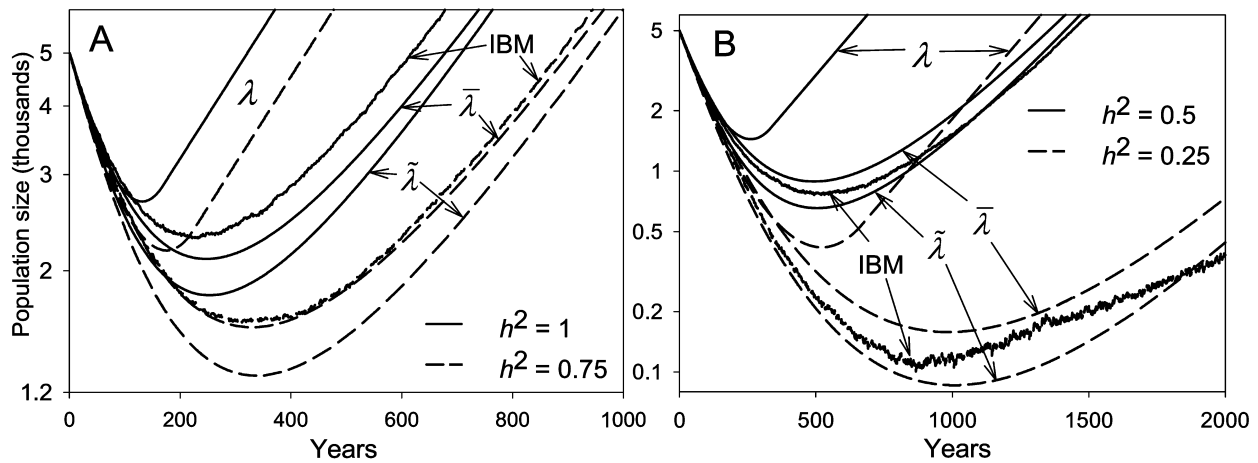


Figure 4: Population size trajectories for (A) the high heritabilities 0.75 (dashed lines) and 1 (solid lines) and (B) the low heritabilities 0.25 (dashed lines) and 0.5 (solid lines), using three different methods for evaluating population growth rate as a function of flowering time ($\lambda(\bar{z})$, $\bar{\lambda}(\bar{z})$, and $\tilde{\lambda}(\bar{z})$, which are abbreviated λ , $\bar{\lambda}$, and $\tilde{\lambda}$ in the figure; see fig. 2 and “Methods” for description) and using an individual-based model (IBM). Results for the IBM are the geometric mean of 100 runs.

and a population will always lag a little behind its SSD if evolution is occurring. We believe that this accounts for some of the differences observed between the population sizes in the early stages of evolution using methods based on growth rates and those using the IBM. In the later stages of the simulations there could be other differences, such as depletion of genetic variance in the IBM, that may further contribute to a slower rate of evolution. For a heritability of 0.25 the population reaches levels around 100—which would correspond to only about six reproductive-stage plants—so there could also be strong effects of demographic stochasticity and genetic drift due to low effective population sizes.

Figure 6 shows the probability of extinction as a function of initial population size, generated using the IBM, for four values of flowering-time heritability. It is no surprise that extinction probability was higher when initial population size was lower. However, when the heritability of flowering time is relatively high, evolution can prevent extinction even at low initial population sizes. With moderate heritabilities, populations of ≥ 500 have a quite reasonable chance of being rescued by evolution. If heritability is very low, however, extinction is the likely outcome.

Discussion

Our demographic models suggest that evolution by natural selection can alter the population trajectory of declining populations of *Trillium* in such a way that selection can rescue these populations from extinction. There are many

factors that collectively contribute to this conclusion for this case study.

1. Flowering time has a large effect on λ . By flowering a few days later in the season, reproductive-stage *Trillium* are much less likely to be consumed by deer, thereby allowing for greater fertility and an increased probability of flowering in future years. A moderate response to selection on this key demographic component thus has a large effect on population growth rates. The value of λ is also sensitive to changes in the probability that reproductive-stage plants will flower in the next year. As *Trillium* evolve a later flowering time, the reproductive value of flowering individuals in the population increases and λ becomes even more sensitive to this vital rate. This positive feedback explains why the relationship between flowering time and λ is nonlinear.

2. The initial size of our *Trillium* population is assumed to be large (5,000 individuals), which substantially increases the probability of evolutionary rescue given genetic variation. In general, we expect that evolutionary rescue is more plausible for large populations facing new demographic threats (i.e., large declining populations; Caughley 1994) than for populations that are initially small (Gomulkiewicz and Holt 1995).

3. The initial rate of decline of this *Trillium* population ($\lambda = 0.991$) is quite slow, in part because of the presence of long-lived stages that escape deer herbivory. Evolutionary rescue would be less likely if the value of λ were initially lower. Some of the 11 other populations of *Trillium* that were considered in a previous demographic study experienced much higher levels of herbivory and lower pop-

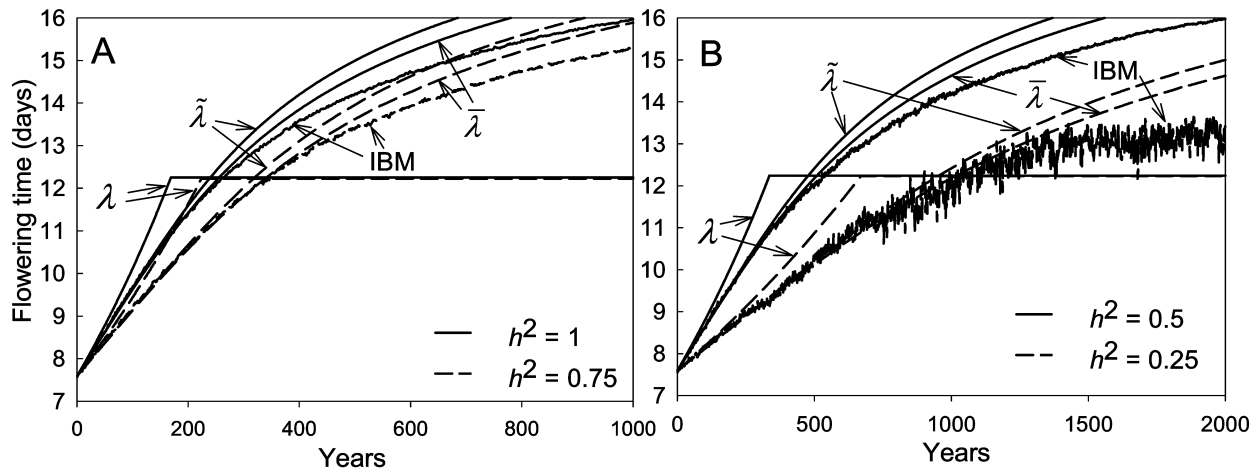


Figure 5: Mean flowering time trajectories for the populations of figure 4.

ulation growth rates (Knight 2003b). It is possible that many of these *Trillium* populations are declining far too quickly for evolutionary rescue to be possible even though appropriate genetic variation to respond to selection is present. In our study population we observed no mortality of plants in the largest two stage classes (which does not imply that the plants are immortal, because they can regress to smaller stages with positive mortality rates). However, even small additional and unmeasured incidences of mortality (which would require longer time series of data to observe and estimate) could cause the population growth rate to decline at a faster rate than we have assumed, which would make evolutionary rescue less likely. Evolutionary rescue is not to be expected even under the most optimistic genetic assumptions (e.g., $h^2 = 1$ and a high initial population size) if the rate of decline in population numbers is too severe; increased mortality in advanced stages could contribute to such a decline.

4. We assumed moderate heritabilities of flowering time; increasing heritability increases the rate of response to selection and so permits the population to more quickly rebound as a result of evolutionary rescue. Species that are less common and less widespread than *Trillium*, as well as those that occur in small or isolated populations, are likely to have lower genetic variation and trait heritabilities than the values we assumed here and thus are less likely to be rescued from looming extinction by selection.

5. Our models ignore both environmental stochasticity and inbreeding depression. These factors could elevate extinction risk and make evolutionary rescue less likely, particularly in small populations. Indeed, one key component of environmental stochasticity that we have not considered is temporal variation in deer herbivory, which would be expected given known temporal variation in deer abun-

dances (Côté et al. 2004). However, in some circumstances, temporal variation in selection pressure might facilitate evolutionary rescue (as occurs in models of evolution in sink environments; Holt et al. 2004b). An important direction for future work is to examine the interplay of stage structure, selection, and temporal environmental variation.

6. We have ignored other traits of selective importance that may be genetically correlated with flowering time. If increases in fitness due to a later flowering time are countered by decreases in fitness due to changes in correlated traits, then evolutionary rescue is hampered and may not occur at all. Our data showed no effect of flowering time on demographic parameters other than through the observed effect on vulnerability to browsing by deer. However, this result is relative to the range of flowering times actually occurring in the extant population. When the distribution of flowering times shifts due to selection, as is predicted by our models, it is likely that unmeasured forces will emerge (see appendix).

7. We projected 2,000 years into the future in our models, which allowed enough time for evolution to alter this long-lived perennial's population trajectory. This is useful for explicating the different predictions that emerge from different modeling approaches, but such long time horizons may not typically be useful in a conservation context. It is highly likely that many factors, including the effects of global climate change in particular (Intergovernmental Panel on Climate Change 2007), may cause demographic vital rates to deviate strongly from those assumed to be constant in our model during this long time interval.

The goal of our modeling effort was to characterize the potential role of evolution on modifying the population trajectories of a stage-structured population and to deter-

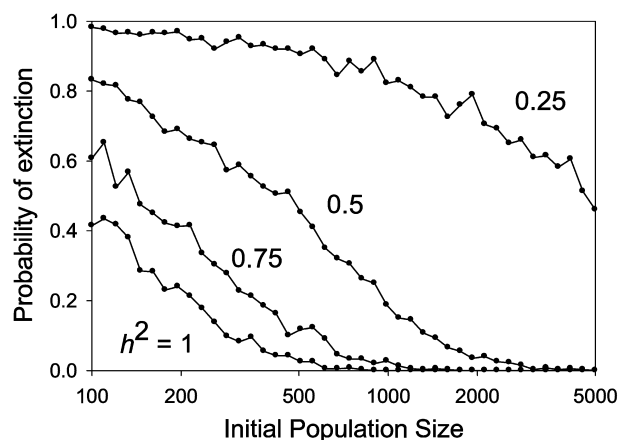


Figure 6: Probability of extinction as a function of initial population size. Extinction is based on 400 runs of the individual-based model. Four values of heritability of flowering time were considered.

mine whether evolutionary rescue of a declining population is possible even under a “best-case scenario” for a real-world example of a declining population of *Trillium*. If one finds that extinction is likely, even given abundant genetic variation and unconstrained selection on particular traits that are key components of fitness, it is fair to conclude that evolution will be unable to rescue the focal population from extinction.

The relationship between a phenotypic trait and λ can be calculated in several different ways. We have shown that the way in which one constructs this calculation has substantial consequences for the expected rate of evolution and the population size trajectory. We applied several modeling approaches with different assumptions to the same *Trillium* population. At higher phenotypic values of flowering time (between 7.6 and 12.23 days), the growth rate and selection differential at each flowering time were higher when fitness was simply evaluated at the mean phenotype (the $\lambda(\bar{z})$ method), compared with their values when fitness or transition probabilities were averaged over the phenotypic distribution (the $\bar{\lambda}(\bar{z})$ and $\tilde{\lambda}(\bar{z})$ methods). Therefore, population size was higher and the rate of evolution was faster using the $\lambda(\bar{z})$ method. The other methods were more in agreement with the individual-based model, which makes fewer assumptions but is also the least accessible to analytic understanding. Thus, we suggest that the $\lambda(\bar{z})$ method (which is the simplest of all of those that we considered) sometimes provides a poor prediction of the rate of evolution, whereas the other two methods (despite their greater complexity) may provide a more realistic description of how selection will modify the trajectory of populations declining toward extinction.

Population viability analysis (PVA) is a collection of

quantitative methods designed to assess the extinction risk of a population (Morris and Doak 2002). A largely unexplored dimension of PVA involves examining potential evolutionary responses by species to changed environments (Ashley et al. 2003). Our individual-based model can be interpreted as the first example of an evolutionarily informed PVA. Conservation biologists have long been concerned with genetic factors, such as the amount of genetic variation within and among populations (reviewed in Hedrick and Miller 1992), as well as demographic factors, which have long been thought to more directly relate to the extinction risk of a population (Lande 1988). Our model provides an example of how one can incorporate both genetic factors and demographic factors into a PVA so as to evaluate the potential for the long-term persistence of a population in an altered environment.

There are two conservation scenarios in which consideration of evolutionary dynamics in a PVA might be particularly beneficial. First, in captive breeding programs, breeders may be able to facilitate evolution by mating individuals with a rare but more fit phenotype. Second, in populations that are projected to experience extinction even if habitat conditions were improved, albeit at a slower rate, the potential for evolutionary rescue may provide justifiable grounds for habitat management. Our model suggests that, by creating an environment in which the population declines at a slower speed, one thereby gives the population a better chance of adapting before extinction occurs. Environmental mitigation that slows the rate of population decline may permit the power of natural selection to be harnessed in the service of species preservation. As a concrete example, there are species of Hawaiian birds for which avian malaria and predation by introduced rodents threaten population persistence. Even with substantial rodent management, bird populations are still expected to decline. However, Kilpatrick (2006) argues that malaria resistance may evolve in these birds and that this evolution is more likely if the rodent population is controlled. Our results support this suggestion. A general expectation of the interplay of demography and evolution is that adaptation is more likely to be observed whenever absolute fitness is raised in unfavorable environments (Holt and Gomulkiewicz 2004). This provides a rationale for striving to maintain populations of threatened species by mitigating unfavorable aspects of their environments, even for those species that seem to be inexorably slipping toward extinction.

In many cases, however, data limitations will preclude incorporating evolutionary dynamics into PVA. For rare and longer-lived species that are often the focus of PVA, adequate genetic information about traits and their heritabilities—and even an understanding of which traits are responsible for declining abundance—is generally not

available. When the goal of the model is to inform urgently needed management decisions for a species, the model ideally should not include parameters for which there are few empirical data (Morris and Doak 2002). If it does, however, a range of values must be considered to span the range of plausible scenarios. If one has at hand a PVA for a threatened species, an understanding of the ecological factors responsible for population decline may suggest key demographic transitions and phenotypic traits that could be the focus of selection. Using the approaches we have explored above, one could incorporate selection acting via these key demographic components for a range of assumptions about heritable variation. In many cases we expect that this will reveal that there is no reason to expect evolution by natural selection to be a strong promoter of population persistence, but in some cases the exercise may provide hope that natural selection may help facilitate persistence. Moreover, there may be key demographic transitions that are not subject to selection that can be improved through management that would increase the potential for selection operating on other demographic transitions to rescue the population from extinction.

Recent reviews of the incidence and importance of rapid evolution have largely ignored longer-lived organisms (e.g., Stockwell et al. 2003); we suspect this is because there are few data on their rates of contemporary evolution and on the influence of evolution on their population trajectories. When the rate of phenotypic change is scaled by generation time, the rate of evolutionary change projected for *Trillium* by our models resembles those observed in shorter-lived organisms. The estimated generation time of the *Trillium* population considered in this study is 56.7 years (calculated using eq. [27] in Cochran and Ellner 1992). Thus, we expect a 0.10 SD change in flowering time per generation, assuming that the heritability of flowering time is 0.25. This rate of phenotypic change is similar to the rate observed for shorter-lived organisms that have undergone “contemporary” evolution, such as fruit flies, guppies, and Pacific salmon (Stockwell et al. 2003).

We assumed a closed population of *Trillium* in our models, but in reality both pollen and seeds may move between populations. Bumblebee pollinators are capable of flying large distances to move pollen, and deer have been shown to act as long-distance seed dispersers (Vellend et al. 2003, 2006). Immigration could either facilitate or hamper the

evolutionary process and thus affect the potential for evolutionary rescue. Immigrants may be maladapted to the focal population if they come from populations that experience lower levels of herbivory or from populations that have not themselves evolved later flowering times. However, theoretical work has shown that immigrants may at times facilitate the evolutionary process because of positive demographic factors (i.e., they add more individuals to the population and buffer it from extinction) and genetic impacts (i.e., immigrants may provide genetic variation on which selection can act; Holt et al. 2005). These benefits of immigrants are particularly pronounced in populations with Allee effects (Holt et al. 2004a). *Trillium* has been shown to experience Allee effects due to lower pollination success at lower population densities (Knight 2003c), and so immigrant pollen and seeds may help facilitate its evolutionary response to deer herbivory.

In longer-lived organisms, it is difficult to determine by direct observation whether evolution by natural selection will occur within ecologically pertinent timescales. We have used an evolutionarily informed stage-structured modeling approach to predict that the evolution of flowering time may substantially alter the population trajectory of a slowly declining population of *Trillium*, and in particular it may suffice to permit this population to persist because of an evolved shift in flowering phenology. We suggest that the modeling techniques we use in this study are necessary tools to use when addressing questions about how evolution will impact the population trajectories of longer-lived organisms (for which multiple generations are not easily observed) and will provide a useful complement to existing models for population viability.

Acknowledgments

We thank S. Andelman, T.-L. Ashman, J. Chase, T. Coulson, N. Friedenber, S. Kalisz, T. Kimbrell, and P. Lorch for discussion and comments and W. Morris and two anonymous reviewers for their insightful comments on this article. This research was supported by McKinley and Darbaker research funds, Botany in Action (Phipps Conservatory and Botanical Garden), the University of Florida Foundation, and the National Science Foundation (DEB-0105000).

APPENDIX

Technical Details

Initializing the Individual-Based Model

For the individual-based model (IBM), we needed to initialize the genotypes and phenotypes of all stages. We had a measured phenotype for only the reproductive stage; at first we used this value as the mean genotype and phenotype

for all stages (and we used the stable stage distribution [SSD] to determine the number of individuals in each stage). This resulted in transients in the population size, genotypes, and phenotypes in the first few generations of the simulation. This occurred because, although we started at the stable distribution of the number of individuals in each stage, we did not necessarily start at a stable distribution of genotypes and phenotypes of each stage. In general, the mean phenotype and genotype differ for different stages (e.g., germinants are produced only by unbrowsed flowers and as such tend to have later flowering times). Because selection is directional, there is no initial genetic equilibrium to use for initialization. However, for our initial conditions it was possible to calculate relative values of the mean genotypes and phenotypes, as described below. These were combined with the measured reproductive-stage plant mean flowering time to give initial values of the mean genotypes and phenotypes of all stages. These values were used to initialize the IBM.

To derive these initial values we developed a more complete model of evolution in stage-structured populations using stage-specific joint probability density functions (PDFs) of genotype and phenotype (which we plan to publish in detail elsewhere). In addition to allowing us a method for sensibly initializing the IBM, this model gave some insight into the expected population size trajectory. Here we provide a summary of this model, which provides a justification for the claim made in the main text that the population size should follow a trajectory based on $\tilde{\lambda}(\bar{z})$.

The model contains recursions for the joint PDF of genotype and phenotype, as well as the population size, for each stage. In our system, selection acts only on the adult stage, but we derived recursions for the population size and joint PDF for each stage for a more general system in which any of the matrix elements a_{ij} could be functions of the phenotype z . We assumed that there was only one reproductive stage (stage r) and one offspring stage (stage 1) and that individuals could not regress into the offspring stage.

Let $f_j(g, z)$ be the joint PDF of genotype g and phenotype z for stage j in the current year. Using the assumptions above, the only element in the first row of the transition matrix is $a_{1r}(z)$, which is the fecundity of the reproductive stage. All other nonzero elements represent transition probabilities; $a_{ij}(z)$ is the probability that an individual with phenotype z in stage j in one year is in stage i the next year. Averaging this function over $f_j(g, z)$ gives the average probability of this transition. Because $a_{ij}(z)$ is independent of g , we can integrate $f_j(g, z)$ over g to give the marginal phenotype PDF ($f_j(z)$) and then average $a_{ij}(z)$ over this. If we multiply the average transition probability by the current number of individuals in stage j , N_j , we get the number of individuals going from stage j to i , which is

$$N'_{ij} = N_j \int \int a_{ij}(z) f_j(g, z) dz dg = N_j \int a_{ij}(z) \left(\int f_j(g, z) dg \right) dz = N_j \int a_{ij}(z) f_j(z) dz,$$

where the integrals are over all values. If we sum these over all stages, j , we get the number of individuals in the next year in stage i :

$$N'_i = \sum_j N'_{ij} = \sum_j N_j \int a_{ij}(z) f_j(z) dz = \sum_j N_j \bar{a}_{ij},$$

where \bar{a}_{ij} is the value of a_{ij} averaged over the phenotypic distribution for stage j . This equation can be written as $\mathbf{n}' = \mathbf{\bar{A}}\mathbf{n}$, where \mathbf{n}' and \mathbf{n} are population size vectors and $\mathbf{\bar{A}}$ is a transition matrix for which each element a_{ij} is equal to \bar{a}_{ij} . The dominant eigenvalue of this matrix is $\tilde{\lambda}(\bar{z})$ in the main text. This is the growth rate over the year assuming that the population is initially at its SSD.

This general model was also solved for recursions of genotypes and phenotypes (which differed from the phenotype recursions based on $\tilde{\lambda}(\bar{z})$). To derive the initial conditions for the IBM, we made the simplifying assumptions that the joint genotype-phenotype distribution of each stage j was Gaussian with the evolving means \bar{g}_j and \bar{z}_j , the fixed variances G_j and Z_j , and the fixed correlation coefficient $h = (G_j/Z_j)^{1/2}$ (the square root of the heritability). We then derived expressions for the mean genotypes and phenotypes of stage i in any year in terms of the values in the previous year

and the elements of the transition matrix, which were

$$\bar{g}'_i = \frac{[\sum_j N_j \bar{g}_j \bar{a}_{ij} + h^2 N_6 P_6 d\bar{a}_{i6}/d\bar{z}_6]}{\sum_j N_j \bar{a}_{ij}},$$

$$\bar{z}'_1 = \bar{g}'_1$$

and for stages $i > 1$

$$\bar{z}'_i = \frac{[\sum_j N_j \bar{z}_j \bar{a}_{ij} + N_6 P_6 d\bar{a}_{i6}/d\bar{z}_6]}{\sum_j N_j \bar{a}_{ij}}.$$

The first terms in the numerators represent the effect of averaging the means of individuals of different classes that move into class i , whereas the last terms represent the effect of selection and are nonzero only for $i = 1$, $i = 5$, and $i = 6$. We used these equations, along with $N'_i = \sum N_j \bar{a}_{ij}$ (from above), to trace the evolution of the population size and mean phenotype using measured values and assumed initial conditions (population size and average genotype and phenotype for each stage, as determined below). We used the measured phenotypic variance of reproductive-stage plants as the initial Z_j for all stages and set $G_j = h^2 Z_j$ using an assumed initial heritability. Using the measured average phenotype of reproductive-stage individuals (\bar{z}_6), we calculated all elements of the transition matrix (using \bar{a}_{ij} for elements that were functions of z) for the initial conditions. The eigenvector corresponding to the dominant eigenvalue of this matrix is the SSD, which was used to initialize population sizes.

To initialize the means of all genotypes and nonreproductive phenotypes, we took the above equations for \bar{g}'_i and \bar{z}'_i , evaluated all terms at the initial reproductive-stage phenotypic mean and variance, and assumed all terms were constant. This led to a set of linear equations

$$\begin{bmatrix} \bar{g}'_1 \\ \bar{g}'_2 \\ \bar{g}'_3 \\ \bar{g}'_4 \\ \bar{g}'_5 \\ \bar{g}'_6 \\ \bar{z}'_2 \\ \bar{z}'_3 \\ \bar{z}'_4 \\ \bar{z}'_5 \\ \bar{z}'_6 \end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & c_{32} & c_{33} & c_{34} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & c_{43} & c_{44} & c_{45} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & c_{54} & c_{55} & c_{56} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & c_{65} & c_{66} & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & c_{32} & c_{33} & c_{34} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & c_{43} & c_{44} & c_{45} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & c_{54} & c_{55} & c_{56} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & c_{65} & c_{66} \end{bmatrix} \begin{bmatrix} \bar{g}_1 \\ \bar{g}_2 \\ \bar{g}_3 \\ \bar{g}_4 \\ \bar{g}_5 \\ \bar{g}_6 \\ \bar{z}_2 \\ \bar{z}_3 \\ \bar{z}_4 \\ \bar{z}_5 \\ \bar{z}_6 \end{bmatrix} + \begin{bmatrix} h^2 b_1 \\ 0 \\ 0 \\ 0 \\ h^2 b_5 \\ h^2 b_6 \\ 0 \\ 0 \\ 0 \\ b_5 \\ b_6 \end{bmatrix},$$

where $c_{ij} = \bar{a}_{ij} N_j / \sum_j N_j \bar{a}_{ij}$, $b_i = (N_6 Z_6 d\bar{a}_{i6}/d\bar{z}_6) / \sum_j N_j \bar{a}_{ij}$, and N_j were at the SSD and the expected values were evaluated at the initial reproductive mean and variance. The rows of the matrix sum to 1, so the dominant eigenvalue is 1 and the eigenvector associated with it is an all-1s vector. After a transient phase, the solution to this system is for the genotypes and phenotypes of all stages to increase in parallel. The solution to the above equations does not approach a stable equilibrium, but the differences in values do. To find the relative values of the average genotypes and phenotypes of the different stages, we made one variable the reference and defined new variables to represent the differences between each of the other variables and the reference. We chose the stage-2 genotype to be the reference because it had a simple equation, and therefore defined $v_i = \bar{g}_i - \bar{g}_2$ for values of i from 1 to 6 and $v_{i+5} = \bar{z}_i - \bar{z}_2$ for values of i from 2 to 6. An equation for the new variables analogous to above matrix equation is $\mathbf{v}' = \mathbf{C}_2 \mathbf{v} + \mathbf{b}$, where \mathbf{C}_2 is the same as \mathbf{C} with all elements of the first column decremented by 1. This equation was then solved for the equilibrium values by setting $\mathbf{v}' = \mathbf{v}$, which gave $\mathbf{v} = (\mathbf{I} - \mathbf{C}_2)^{-1} \mathbf{b}$. The elements of \mathbf{v} are the equilibrium values of all genotypes and phenotypes relative to the stage-2 genotype. The measured average flowering time (7.6 days) is the mean phenotype of stage 6 (\bar{z}_6). Because $v_{11} = \bar{z}_6 - \bar{z}_2$, we used the calculated value of v_{11} to get \bar{z}_2 , and from this we calculated the other initial genotypes and phenotypes using the other elements of \mathbf{v} . The allelic values of each individual of the initial

population were chosen to give the genetic mean calculated for its stage, and the environmental components were chosen to shift the phenotypic mean to its calculated value.

For the results in this article, we show only population size and mean flowering time with the individual-based model. However, the output of the IBM program contained the mean genotype and phenotype of each stage at each generation. With the genotypes and phenotypes initialized in the manner discussed in this appendix, the mean genotype and phenotype of each stage changed smoothly with time—at first as nearly parallel lines—indicating that the above procedure was successful. The population size also initially declined smoothly.

Relationship between Flowering Time and Fitness

Our analysis of a *Trillium* population somewhat surprisingly showed that there was no relationship between time of flowering and fitness in the absence of deer herbivory. At first glance, flowering time would appear to be a neutral trait. If that were not the case, the ability of selection to rescue the population from extinction would be weakened because of countervailing evolutionary processes. It is conceivable that there is stabilizing selection on flowering time such that the observed average flowering time is in fact the evolutionary optimum but that the fitness function relating expected fitness to flowering time is not sharply peaked (but instead is very gently rounded and indeed quite flat), so that in our sample it cannot be distinguished from a completely flat relationship.

An alternative scenario is to imagine that there is frequency-dependent selection at work and that what one sees in the population is a mixed strategy. We will not propose a detailed model of such frequency-dependent selection, but we note that it could arise from shifts in the behavior, population size, species composition, and phenologies of pollinators. For our purposes, what matters about frequency-dependent selection is that, at an ESS, one expects the relative frequency of alternative phenotypes to be adjusted so that they have equal realized fitnesses. In this case, a regression of realized fitness against time of flowering would indeed initially show no relationship because the numbers of flowers at each flowering period are adjusted such that the realized fitness gain per flower is equal. However, as the relative frequencies of different classes change (via shifts in the distribution of flowering times due to selection), this fitness equilibration would be expected to break down.

Finally, as briefly noted in the main text, flowering time could be correlated with other traits (in ways not directly measured in our study) such that stabilizing selection indirectly governs it. All of these possibilities would require much more detailed future study. As also noted in the main text, these alternative evolutionary scenarios would likely make it harder for selection to rescue *Trillium* populations from extinction.

Literature Cited

- Ashley, M. V., M. F. Willson, O. R. W. Pergams, D. J. O'Dowd, S. M. Gende, and J. S. Brown. 2003. Evolutionarily enlightened management. *Biological Conservation* 111:115–123.
- Augustine, D. J., and L. E. Frelich. 1998. Effects of white-tailed deer on populations of an understory forb in fragmented deciduous forests. *Conservation Biology* 12:995–1004.
- Berteaux, D., D. Reale, A. G. McAdam, and S. Boutin. 2004. Keeping pace with fast climate change: can arctic life count on evolution? *Integrative and Comparative Biology* 44:140–151.
- Bone, E., and A. Farres. 2001. Trends and rates of microevolution in plants. *Genetica* 112–113:165–182.
- Boulding, E. G., and T. Hay. 2001. Genetic and demographic parameters determining population persistence after a discrete change in the environment. *Heredity* 86:313–324.
- Burger, R., and M. Lynch. 1995. Evolution and extinction in a changing environment: a quantitative genetic analysis. *Evolution* 49:151–163.
- Camargo, L. E. A., and T. C. Osborn. 1996. Mapping loci controlling flowering time in *Brassica oleracea*. *Theoretical and Applied Genetics* 92:610–616.
- Carey, K. 1983. Breeding systems, genetic variability, and response to selection in *Plectritis* (Valerianaceae). *Evolution* 37:947–956.
- Carroll, S. P., A. P. Hendry, D. N. Reznick, and C. W. Fox. 2007. Evolution on ecological time-scales. *Functional Ecology* 21:387–393.
- Case, F. W., Jr., and R. B. Case. 1997. *Trilliums*. Timber, Portland, OR.
- Caswell, H. 2001. *Matrix population models: construction, analysis, and interpretation*. 2nd ed. Sinauer, Sunderland, MA.
- Caughley, G. 1994. Directions in conservation biology. *Journal of Animal Ecology* 63:215–244.
- Clarke, J. H., R. Mithen, J. K. M. Brown, and C. Dean. 1995. QTL analysis of flowering time in *Arabidopsis thaliana*. *Molecular and General Genetics* 248:278–286.
- Cochran, M. E., and S. Ellner. 1992. Simple methods for calculating age-based life history parameters for stage-structured populations. *Ecological Monographs* 62:345–364.
- Côté, S. D., T. P. Rooney, J. P. Tremblay, C. Dussault, and D. M. Waller. 2004. Ecological impacts of deer overabundance. *Annual Review of Ecology, Evolution, and Systematics* 35:113–147.
- Cox, G. W. 2004. *Alien species and evolution: the evolutionary ecology of exotic plants, animals, microbes, and interacting native species*. Island, Washington, DC.
- de Jong, G. 1994. The fitness of fitness concepts and the description of natural selection. *Quarterly Review of Biology* 69:3–29.
- Fu, Y. B., and K. Ritland. 1994. Marker-based inferences about the

- genetic basis of flowering time in *Mimulus guttatus*. *Hereditas* 121: 267–272.
- Garcia-Rossi, D., N. Rank, and D. R. Strong. 2003. Potential for self-defeating biological control? variation in herbivore vulnerability among invasive *Spartina* genotypes. *Ecological Applications* 13: 1640–1649.
- Geber, M. A., and L. R. Griffen. 2003. Inheritance and natural selection on functional traits. *International Journal of Plant Sciences* 164(suppl.):S21–S42.
- Gilchrist, G. W., R. B. Huey, J. Balanya, M. Pascual, and L. Serra. 2004. A time series of evolution in action: a latitudinal cline in wing size in South American *Drosophila subobscura*. *Evolution* 58: 768–780.
- Gomulkiewicz, R., and R. D. Holt. 1995. When does evolution by natural selection prevent extinction? *Evolution* 49:201–207.
- Hanzawa, F. M., and S. Kalisz. 1993. The relationship between age, size, and reproduction in *Trillium grandiflorum* (Liliaceae). *American Journal of Botany* 80:405–410.
- Hargeby, A., J. Johansson, and J. Ahnesjö. 2004. Habitat-specific pigmentation in a freshwater isopod: adaptive evolution over a small spatiotemporal scale. *Evolution* 58:81–94.
- Hedrick, P. W., and P. S. Miller. 1992. Conservation genetics: techniques and fundamentals. *Ecological Applications* 1:30–46.
- Holt, R. D. 1990. The microevolutionary consequences of climate change. *Trends in Ecology & Evolution* 5:311–315.
- Holt, R. D., and R. Gomulkiewicz. 2004. Conservation implications of niche conservatism and evolution in heterogeneous environments. Pages 244–264 in R. Ferriere, U. Dieckmann, and D. Couvet, eds. *Evolutionary conservation biology*. Cambridge University Press, Cambridge.
- Holt, R. D., and M. E. Hochberg. 1997. When is biological control evolutionarily stable (or is it)? *Ecology* 78:1673–1683.
- Holt, R. D., R. Gomulkiewicz, and M. Barfield. 2003. The phenomenology of niche evolution via quantitative traits in a “black-hole” sink. *Proceedings of the Royal Society B: Biological Sciences* 270: 215–224.
- Holt, R. D., T. M. Knight, and M. Barfield. 2004a. Allee effects, immigration, and the evolution of species’ niches. *American Naturalist* 163:253–262.
- Holt, R. D., M. Barfield, and R. Gomulkiewicz. 2004b. Temporal variation can facilitate niche evolution in harsh sink environments. *American Naturalist* 164:187–200.
- Holt, R. D., M. Barfield, and R. Gomulkiewicz. 2005. Theories of niche conservatism and evolution: could exotic species be potential tests? Pages 259–290 in D. Sax, J. Stachowicz, and S. D. Gaines, eds. *Species invasions: insights into ecology, evolution, and biogeography*. Sinauer, Sunderland, MA.
- Intergovernmental Panel on Climate Change. 2007. *Climate change 2007: the physical science basis*. Cambridge University Press, Cambridge.
- Irwin, R. E. 2000. Morphological variation and female reproductive success in two sympatric *Trillium* species: evidence for phenotypic selection in *Trillium erectum* and *Trillium grandiflorum* (Liliaceae). *American Journal of Botany* 87:205–214.
- Jules, E. S. 1998. Habitat fragmentation and demographic change for a common plant: trillium in old-growth forest. *Ecology* 79:1645–1656.
- Kalisz, S., F. M. Hanzawa, S. J. Tonsor, D. A. Thiede, and S. Voigt. 1999. Ant-mediated dispersal alters pattern of relatedness in a population of *Trillium grandiflorum*. *Ecology* 80:2620–2634.
- Kelly, C. A. 1993. Quantitative genetics of size and phenology of life-history traits in *Chamaecrista fasciculata*. *Evolution* 47:88–97.
- Kilpatrick, A. M. 2006. Facilitating the evolution of resistance to avian malaria (*Plasmodium relictum*) in Hawaiian birds. *Biological Conservation* 128:475–485.
- Knight, T. M. 2003a. Effects of herbivory and its timing across populations of *Trillium grandiflorum* (Liliaceae). *American Journal of Botany* 90:1207–1214.
- . 2003b. Effects of herbivory and pollen limitation on the population dynamics of *Trillium grandiflorum*. PhD diss. University of Pittsburgh.
- . 2003c. Floral density, pollen limitation and reproductive success in *Trillium grandiflorum*. *Oecologia* (Berlin) 137:557–563.
- . 2004. The effects of herbivory and pollen limitation on a declining population of *Trillium grandiflorum*. *Ecological Applications* 14:915–928.
- Lande, R. 1976. Natural selection and random genetic drift on phenotypic evolution. *Evolution* 30:314–334.
- . 1982. A quantitative genetic theory of life history evolution. *Ecology* 63:607–615.
- . 1988. Genetics and demography in biological conservation. *Science* 241:1455–1460.
- Laurie, D. A. 1997. Comparative genetics of flowering time. *Plant Molecular Biology* 35:167–177.
- Laurie, D. A., N. Pratchett, J. H. Bezant, and J. W. Snape. 1995. RFLP mapping of 5 major genes and 8 quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare*) cross. *Genome* 38:575–585.
- Lawrence, C. W. 1963. Genetic studies on wild populations of *Melandrium*. II. Flowering time and plant weight. *Heredity* 18:149–163.
- Lynch, M., and R. Lande. 1993. Evolution and extinction in response to environmental change. Pages 234–250 in P. M. Kareiva, J. G. Kingsolver, and R. B. Huey, eds. *Biotic interactions and global change*. Sinauer, Sunderland, MA.
- Maron, J. L., M. Vila, R. Bommarco, S. Elmendorf, and P. Beardsley. 2004. Rapid evolution of an invasive plant. *Ecological Monographs* 74:261–280.
- Mazer, S. J. 1987. The quantitative genetics of life history and fitness components in *Raphanus raphanistrum* L. (Brassicaceae): ecological and evolutionary consequences of seed-weight variation. *American Naturalist* 130:891–914.
- McShea, W. J., H. B. Underwood, and J. H. Rappole, eds. 1997. *The science of overabundance: deer ecology and population management*. Smithsonian Institution, Washington, DC.
- Mitchell-Olds, T. 1996. Genetic constraints on life-history evolution: quantitative-trait loci influencing growth and flowering in *Arabidopsis thaliana*. *Evolution* 50:140–145.
- Morris, W. F., and D. F. Doak. 2002. *Quantitative conservation biology: theory and practice of population viability analysis*. Sinauer, Sunderland, MA.
- Murfet, I. C. 1977. Environmental interaction and the genetics of flowering. *Annual Review of Plant Physiology* 28:253–278.
- O’Neil, P. 1997. Natural selection on genetically correlated phenological characters in *Lythrum salicaria* L. (Lythraceae). *Evolution* 51:267–274.
- Pors, B., and P. A. Werner. 1989. Individual flowering time in goldenrod (*Solidago canadensis*): field experiment shows genotype more important than environment. *American Journal of Botany* 76: 1681–1688.

- Rathcke, B., and E. P. Lacey. 1985. Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* 16:179–214.
- Reznick, D. N., and H. A. Bryga. 1996. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). V. Genetic basis of parallelism in life histories. *American Naturalist* 147:339–359.
- Reznick, D. N., F. H. Rodd, and M. Cardenas. 1996. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. *American Naturalist* 147:319–338.
- Rooney, T. P., and K. Gross. 2003. A demographic study of deer browsing impacts on *Trillium grandiflorum*. *Plant Ecology* 168: 267–277.
- Sage, T. L., S. R. Griffin, V. Pontieri, P. Drobac, W. W. Cole, and S. C. H. Barrett. 2001. Stigmatic self-incompatibility and mating patterns in *Trillium grandiflorum* and *Trillium erectum*. *Annals of Botany* 88:829–841.
- Steven, J. C., T. P. Rooney, O. D. Boyle, and D. M. Waller. 2003. Density-dependent pollinator visitation and self-incompatibility in upper Great Lakes populations of *Trillium grandiflorum*. *Journal of the Torrey Botanical Society* 130:23–29.
- Stockwell, C. A., A. P. Hendry, and M. T. Kinnison. 2003. Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution* 18:94–101.
- Van Berloo, R., and P. Stam. 1999. Comparison between marker-assisted selection and phenotypical selection in a set of *Arabidopsis thaliana* recombinant inbred lines. *Theoretical and Applied Genetics* 98:113–118.
- van Klinken, R. D., and O. R. Edwards. 2002. Is host-specificity of weed biological control agents likely to evolve rapidly following establishment? *Ecology Letters* 5:590–596.
- van Tienderen, P. H. 2000. Elasticities and the link between demographic and evolutionary dynamics. *Ecology* 81:666–679.
- Vellend, M., J. A. Myers, S. Gardescu, and P. L. Marks. 2003. Dispersal of trillium seeds by deer: implications for long distance migration of forest herbs. *Ecology* 84:1067–1072.
- Vellend, M., T. M. Knight, and J. M. Drake. 2006. Antagonistic effects of seed dispersal and herbivory on plant migration. *Ecology Letters* 9:316–323.
- Yamanaka, N., Y. Nagamura, Y. Tsubokura, K. Yamamoto, R. Takahashi, H. Kouchi, M. Yano, T. Sasaki, and K. Harada. 2000. Quantitative trait locus analysis of flowering time in soybean using a RFLP linkage map. *Breeding Science* 50:109–115.

Associate Editor: John L. Maron
 Editor: Donald L. DeAngelis