

An experimentally introduced population of *Brassica rapa* (Brassicaceae). 1. Phenotypic selection over three years following colonization of a novel environment

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Background and aims – Despite the importance of selection in driving evolution, little is known about the consistency of selection, particularly in the early stages of colonization of novel environments. This study examines the targets and consistency of selection on an experimentally introduced population of the annual plant, *Brassica rapa* L., in its first three years following introduction to a novel environment.

Methods – Phenotypic selection analyses were conducted on a variety of traits collected during the first three years following introduction from California to New York, and the consistency of the strength and direction of selection was examined.

Key results – The introduced population experienced direct selection for increased overall size and earlier flowering in 2011 and 2012, and increased height, earlier flowering, and longer duration of flowering in 2013. While the direction of selection varied for height, inter-annual variation in the strength of selection was observed for a variety of traits, possibly due to changing weather patterns in the introduced environment.

Conclusions – The results suggest that selection is dynamic and can fluctuate over time. Thus multi-year assessments of selection are useful for predicting evolutionary responses, particularly in the early stages of colonization of a novel environment.

Key words – Natural selection, plants, phenotypic selection, morphology, flowering time, *Brassica rapa*, ecological genetics, introduced species, Aster model.

INTRODUCTION

When a founding population is introduced to a novel environment, the mismatch between the traits of the organisms and the environmental conditions can result in strong selective pressures and rapid evolution (Reznick & Ghalambor 2001). In the past, it was thought that introduced populations would not rapidly adapt because of limited genetic diversity when a small number of individuals are introduced (Van Buskirk & Willi 2006). However, recent studies suggest that there is a large potential for adaptive evolution along experimental timescales in introduced populations (Carroll et al. 1998, Lee 2002, Maron et al. 2004, Yeh 2004, Franks et al. 2007, Dlugosch & Parker 2008).

The evolutionary trajectory of an introduced population is dependent on the strength and consistency of phenotypic selection following introduction. Temporal variation in selection can favor generalist genotypes, thereby limiting

the potential for rapid, directional local adaptation (Kassen 2002, Kawecki & Ebert 2004), especially when selection varies in direction (Frank & Slatkin 1990). Recent studies and reviews have produced conflicting results when examining the variation in the strength and direction of selection in natural populations (Siepielski et al. 2009, Morrissey & Hadfield 2012). Siepielski et al. (2009) found substantial interannual variation in both the strength and direction of selection, while Morrissey & Hadfield (2012) found that the majority of this variation was due to sampling error. However, the lack of long-term data sets may also be contributing to the lack of variation observed in natural selection (Kingsolver et al. 2001, Sandring et al. 2007, Gutowsky & Fox 2012). For example, the magnitude of variation in the selection and evolution of body size and beak traits in Darwin's finches (Grant & Grant 2002, 2014) would have never been revealed without a decades-long data set.

Table 1 – Previous studies utilizing phenotypic selection analysis to estimate selection parameters in recently colonized (< 30 years) populations.

Organism type	Organism	Trait	Type of selection	Years since introduction	Citation
Bird	<i>Junco hyemalis</i>	Lay date	Directional	20	Yeh & Price 2004
Bird	<i>Junco hyemalis</i>	Tail white (sexual)	Directional	20	Price et al. 2008
Bird	<i>Phasianus colchicus</i>	Various morphology	Directional	30	Witzzell 1991
Fish	<i>Oncorhynchus tshawytscha</i>	Body size, spawning arrival	Directional	0	Anderson et al. 2013
Fish	<i>Salmo salar</i>	Various morphology	Variable	10	Hendry et al. 2003
Fish	<i>Gasterosteus aculeatus</i>	Armor	Directional	2–18	Bell et al. 2004
Plant	<i>Mimulus guttatus</i>	Floral traits and morphology	Directional	11–35	Murren et al. 2009
Plant	<i>Nicotiana glauca</i>	Floral traits	Directional	28+	Schuell 2007

There are several examples of strong selection following introduction to environments that differ from the native range in such factors as climatic conditions, predation, and sexual pressures (table 1). Much of the research on the selection and evolution of introduced species has been conducted on invasive species (Prentis et al. 2008, Westley 2011). However, invasive species often have higher reproductive output or growth rates than non-invasive introduced species, which could potentially alter their responses to selection (Moran & Alexander 2014). Therefore, research examining evolution in non-invasive introduced species is underrepresented. Also, prior work on selection and evolution of introduced species often takes place well after the introductions have occurred, leaving many environmental factors related to the introduction either uncontrolled or undocumented (Hollingsworth & Bailey 2000, Maron et al. 2004, Colautti & Barrett 2011). In contrast, planned experimental introductions provide the opportunity to directly observe selection and evolution as it occurs (Walsh & Reznick 2011), and allow a focus on the early stages of introduction and colonization.

To examine phenotypic selection in a population shortly after experimental introduction, we used the plant *Brassica rapa* L. (syn. *Brassica campestris* L.; Brassicaceae), a weedy naturalized annual native to the Middle East, which has established populations throughout the United States and the world. There are a variety of important cultivars (canola, turnip, bok choy, rapini, mizuna) and artificially selected lines (e.g. Wisconsin Fast Plants) of *B. rapa*, and populations have become feral or naturalized. A variety of studies of the evolution or plasticity of certain traits of *B. rapa* have been conducted, examining morphological (Dechaine et al. 2007) and phenological traits (Agren & Schemske 1994, Byers 2005). Franks et al. (2007) documented the evolution of earlier flowering time in populations of *B. rapa* following a five-year drought in southern California. The derived phenotypes flowered at a smaller plant size, demonstrating a flexible relationship between size and flowering (Franks & Weis 2008).

In this study, we experimentally introduced *Brassica rapa* plants from a southern California site into New York

and measured selection for three years. The goals of the study were to (1) examine the patterns of selection among morphological and phenological phenotypic traits following introduction to a novel environment, and (2) determine if the selection pressures are consistent among the first three years following introduction. Due to the increased availability of nutrients and rainfall in New York compared to California, we predicted there would be selection for larger size and later flowering time. Also, due to the variable nature of summer weather patterns in temperate New York State, as well as previous studies on the consistency of selection in the field (see Siepielski et al. 2009), we predicted that selection on phenotypic traits would vary in strength and direction.

METHODS

Study system and experimental design

The source population used for the introduction in this study is located at the San Joaquin Freshwater Marsh Preserve in Irvine, CA and was previously studied by Franks et al. (2007). In June 2008, seeds were randomly bulk collected from 1000 individuals and stored at 5°C in paper envelopes until used in this study. In May 2011, 200 *B. rapa* seeds were broadcast over each of ten 1 m² replicate plots at the Louis Calder Biological Field Station in Armonk, NY. The plots were arranged in five rows of two adjacent plots, with 1 m between plots within a row and 3 m between the two rows. The plots were tilled one month prior to the experiment and were regularly weeded to reduce interspecific competition, allowing the populations to establish and providing a focus on the abiotic and other environmental differences between the sites. Throughout the first three growing seasons, several morphological and phenological traits were measured at least once per week. Fifteen plants in each plot (150 plants total) were randomly chosen for trait measurements following the emergence of the first true leaf using randomly selected coordinates and selecting the plant closest to the random point within the plot. Measures of morphology include proxies of overall size (plant height, number of leaves, basal stem di-

ameter) and leaf size (length of longest leaf, width of longest leaf). Measures of phenology included flowering time (days from average emergence to the onset of flowering) and duration of flowering. Number of siliques (seed pods) per plant were used as non-destructive estimates of fitness, given the strong correlations between silique number and seed number (Griffith et al. 2004, Kerwin et al. 2015). The cumulative number of flowers per plant measured during all collection dates was calculated to get an estimation of maximum number of flowers during their lifetime. Destructive trait measurements were avoided to allow the seeds to disperse and recruit naturally during this multi-year study. Weather measurements, including air temperature and precipitation, were collected from a NWS weather station at the Westchester County Airport approximately 6 km from the site.

Analyses

The morphological trait values measured 55 days after average emergence were used in all analyses, when most of the plants reached their maximum size. To determine the strength of selection on phenotypic traits, linear regressions were performed between relative fitness (dependent variable) and standardized traits (independent variable) (Lande 1979, Lande & Arnold 1983) in each year. The slope of the regression line was defined as the selection differential, and represents the strength of total (direct and indirect) selection in units of standard deviations per generation (Lande 1979). To distinguish direct from indirect selection due to correlation among traits, multiple regressions were performed and the resulting partial regression coefficients (selection gradients [b]) represent direct selection on the traits (Lande 1979). Estimates of total and direct quadratic selection (g) were also calculated as half of the coefficients of squared trait terms in separate multivariate regression analyses, to examine any curvature in the fitness function that could provide evidence of stabilizing or disruptive selection within the range of trait values observed (Lande & Arnold 1983). All traits in the regression models tested had VIFs lower than 10, which suggest multicollinearity was low (Neter et al. 1989). To determine the statistical significance of the selection differentials and gradients, as well as the consistency of the differentials and gradients among years, 95% confidence intervals were calculated using a bias-corrected bootstrap method (Dixon 1993) with 4000 intervals (Fox 2002).

We also examined selection using multiple fitness components individually, in fitness components analysis, and together, using Aster models (Geyer et al. 2007). Fitness component selection analysis was performed on all phenotypic traits via maximum number of flowers and the calculated number of siliques/maximum number of flowers. In order to examine the significance of fitness component selection, as well as consistency of fitness component selection among years, general linear models were constructed in the same manner as above. In order to examine selection via comprehensive estimations of fitness, we calculated a metric of lifetime fitness using Aster modelling (Geyer et al. 2007). Aster modelling provides a method of combining multiple life history components of fitness using the appropriate distribution family (Gaussian, Poisson, or binomial) for each component

in order to calculate a comprehensive fitness estimate for each individual. The life history components used in the Aster models are maximum number of flowers, success in producing siliques, and number of siliques. To examine the significance of phenotypic selection via the Aster estimates, we created general linear models with relative Aster-estimated fitness as the dependent variable, and trait as the independent variable. The alpha level used for all analyses was 0.05.

We used Non-Metric Multidimensional Scaling (NMDS) to examine selection simultaneously on multiple traits (Minchin 1987). The scores of the first two coordinate dimensions and the vectors for each phenotypic trait were calculated for each year using the R package *vegan*. The scores for the first two NMDS dimensions were standardized and used in phenotypic selection analyses to determine strength of linear selection on these dimensions. All analyses performed in this study used R version 2.15.2 (R Core Team 2012).

RESULTS

Both temperature (electronic appendix 1A) and precipitation (electronic appendix 1B) varied substantially during the three years of the study both within and among years. The study area experienced moderate temperatures (33 days above 28°C) and elevated rainfall (86.9 cm) during Summer 2011, high temperatures (44 days above 28°C) and reduced rainfall (39.1 cm) during Summer 2012, and moderate temperatures (29 days above 28°C) and rainfall (59.2 cm) during Summer 2013. Of the plants initially chosen for trait measurements, the population experienced 16% mortality in 2011 and 14% mortality in 2012. In late July 2013, there was over 6 cm of rainfall in less than 30 hours, resulting in a 73% plant mortality event, and 81% total mortality in 2013.

There was significant total and direct selection on several of the traits during the three years of the study (table 2, electronic appendices 4 & 5). In 2011 and 2012, there was total and direct selection for increased overall size, especially via the number of leaves and basal stem diameter (fig. 1A). However, there was also direct selection for reduced height and leaf length in 2011. There was direct selection for earlier flowering (fig. 1B) during all three years, as well as total selection for earlier flowering in 2012 and 2013. During the 2013 season, there was total selection for increased size (via height, leaf size, and basal stem diameter), earlier flowering, and longer duration of flowering, and direct selection on increased height, earlier flowering time, and longer duration of flowering. Direct linear selection on leaf count and flowering duration varied in strength among years and direct selection on height varied in direction among years.

There was also total and direct quadratic (non-linear) selection on some traits during the study, but most were significant during only a single year (table 2). Only total quadratic selection on basal stem diameter was significant in more than a single year. All statistically significant direct quadratic selection was only significant in a single year. However, visual inspection of the fitness functions indicated that even when quadratic selection coefficients were significant, maximum/minimum values did not occur within the range of the observed data, so there was no evidence for stabilizing or disruptive selection.

Table 2 – Selection differentials and gradients among the three years of the study.

Columns under years represent the total or partial regression coefficient and standard error. A bold regression coefficient represents a differential or gradient significantly different from zero via bias-corrected 95% confidence intervals (obtained via bootstrapping, n = 4000). A double dagger (‡) next to trait name represents trait differentials or gradients that vary among years. Selection on the first two NMDS dimension was calculated from scores obtained using the R package *vegan*.

	2011	2012	2013		2011	2012	2013
Linear differentials				Quadratic differentials			
Height	0.150 (0.078)	0.438 (0.087)	0.634 (0.165)	Height	-0.056 (0.065)	-0.033 (0.077)	0.157 (0.125)
Length of longest leaf	0.257 (0.076)	0.510 (0.083)	0.530 (0.178)	Length of longest leaf [‡]	-0.084 (0.036)	0.024 (0.053)	-0.003 (0.158)
Width of longest leaf	0.337 (0.073)	0.422 (0.087)	0.595 (0.170)	Width of longest leaf	-0.049 (0.051)	0.054 (0.064)	0.154 (0.161)
Number of leaves	0.537 (0.063)	0.727 (0.070)	0.061 (0.206)	Number of leaves	-0.017 (0.034)	0.020 (0.041)	-0.012 (0.120)
Basal stem diameter	0.441 (0.068)	0.854 (0.057)	0.657 (0.161)	Basal stem diameter [‡]	-0.088 (0.037)	0.085 (0.037)	0.071 (0.176)
Flowering time	-0.093 (0.079)	-0.381 (0.089)	-0.586 (0.172)	Flowering time	0.019 (0.039)	-0.033 (0.065)	0.204 (0.145)
Duration of flowering	0.073 (0.079)	0.456 (0.086)	0.713 (0.152)	Duration of flowering	0.097 (0.056)	0.002 (0.069)	0.126 (0.091)
Linear gradients				Quadratic gradients			
Height [‡]	-0.158 (0.084)	-0.049 (0.064)	0.554 (0.169)	Height	0.000 (0.055)	0.080 (0.045)	0.119 (0.145)
Length of longest leaf	-0.443 (0.137)	-0.020 (0.11)	-0.295 (0.209)	Length of longest leaf	-0.077 (0.050)	-0.018 (0.052)	-0.085 (0.153)
Width of longest leaf	0.003 (0.137)	-0.007 (0.095)	0.346 (0.221)	Width of longest leaf	0.017 (0.070)	0.033 (0.054)	0.091 (0.129)
Number of leaves [‡]	0.496 (0.087)	0.492 (0.082)	-0.192 (0.156)	Number of leaves	0.010 (0.034)	-0.008 (0.034)	-0.044 (0.097)
Basal stem diameter	0.589 (0.137)	0.659 (0.069)	0.010 (0.172)	Basal stem diameter	-0.006 (0.052)	0.067 (0.036)	-0.027 (0.195)
Flowering time	-0.114 (0.066)	-0.178 (0.060)	-0.245 (0.174)	Flowering time	0.000 (0.027)	0.014 (0.036)	0.085 (0.132)
Duration of flowering [‡]	-0.124 (0.069)	0.010 (0.059)	0.366 (0.147)	Duration of flowering	0.057 (0.043)	0.010 (0.039)	0.039 (0.094)
				NMDS dimensions			
				First dimension	0.364 (0.072)	0.267 (0.092)	0.418 (0.190)
				Second dimension	0.372 (0.072)	0.647 (0.076)	0.549 (0.176)

The fitness component selection analyses showed significant direct selection on phenotypic traits, as well as significant variation in component selection among years (table 3). There was significant direct selection for increased leaf count in 2011 and 2012, increased basal stem diameter in 2011, and longer duration of flowering in 2013 via maximum number of flowers. The strength of direct selection on leaf count via maximum number of flowers also varied among years. There was significant direct selection for increased leaf width in 2011, increased leaf count and basal stem diameter in 2012, and longer duration of flowering in 2011 and 2013 when the ratio of silique count: maximum number of flowers was used as the fitness proxy. Direct selection on leaf count and basal stem diameter via the silique count: maximum number of flowers fitness component varied among years. The phenotypic selection analyses calculated using the Aster comprehensive fitness estimates were in most cases very similar to those calculated using the silique count fitness proxy (electronic appendix 2). Only for the morphological traits in 2011 were the selection differentials substantially larger via the aster estimates versus the silique count fitness proxy.

The results of the NMDS for 2011 (stress = 0.184) and 2012 (stress = 0.210) show a separation of the morphological and phenological traits along the first two dimensions (electronic appendix 3). Many of the morphological traits were positively correlated during this study (electronic appendix 6), and were related along the first NMDS dimension, with flowering time and duration of flowering displaying a negative relationship along the second dimension. While the relationships are less clear in 2013 (stress = 0.190), the negative relationship among flowering time and duration of flowering is maintained. Selection on the first two NMDS dimensions was significant in all years (table 2) and the direction of selection was maintained among years. There was no significant variation in the strength of selection on the first two NMDS dimensions among years.

Table 3 – Selection gradients on phenotypic traits among the three years of the study via two fitness components: maximum number of flowers and number of seed pods/maximum number of flowers.

A bold regression coefficient represents a gradient significantly different from zero via bias-corrected 95% confidence intervals (obtained via bootstrapping, n = 4000). A double dagger (‡) next to the trait name represents significant variation among years.

Dependent	Independent	2011	2012	2013
Maximum number of flowers	Height	0.080	0.219	0.252
	Length of longest leaf	0.100	-0.005	-0.274
	Width of longest leaf	0.045	0.096	0.057
	Number of leaves	0.208	0.212	-0.103
	Basal stem diameter‡	0.181	0.090	0.276
	Flowering time	-0.064	-0.033	-0.007
	Duration of flowering	0.098	0.064	0.234
Seed pods per flower	Height	-0.029	0.047	0.052
	Length of longest leaf	0.133	0.041	0.066
	Width of longest leaf	0.209	-0.021	0.060
	Number of leaves‡	-0.106	0.649	0.061
	Basal stem diameter‡	-0.126	0.316	0.187
	Flowering time	-0.123	-0.074	-0.022
	Duration of flowering	0.385	0.060	0.545

DISCUSSION

This study makes the novel contribution of examining inter-annual variation in phenotypic selection in an experimentally introduced plant population. The *Brassica rapa* population introduced from the Mediterranean climate of Southern California into the temperate climate of New York experienced significant selection on a variety of morphological and phenological traits, as well as changes in the targets of selection, among the first three years following colonization. During the mild and wet summer of 2011, there was direct selection for increased size via number of leaves and basal stem diameter, as well as direct selection for earlier flowering time.

During the hot and dry summer of 2012, there was also direct selection for increased size and earlier flowering time. However, during the moderately wet and warm summer of 2013, there was no direct selection on number of leaves or basal stem diameter, but rather direct selection for taller plants, earlier flowering time, and longer duration of flowering.

The changes in the pattern of direct selection in 2013 compared to the previous two years are most likely due to the 73% mortality flood in late July 2013. This mortality event prior to the conclusion of reproduction most likely drove the selection for increased height and duration of flowering during the 2013 growing season. The plants that survived the flood likely suffered reduced fertility during the flood pe-

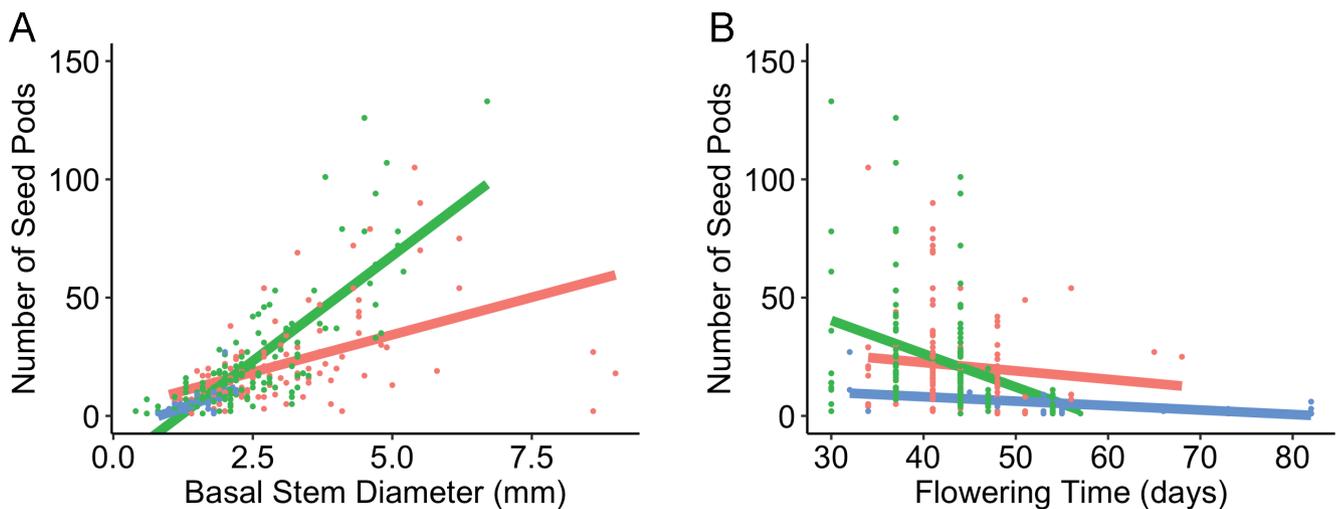


Figure 1 – Relationship between basal stem diameter (A) or flowering time (B) and number of seed pods for 2011 (red), 2012 (green), and 2013 (blue). Regression lines represent total linear selection.

riod, and then continued to flower as long as possible once the water receded. The shift from direct selection for reduced height in 2011 to increased height in 2013 is the only significant change in direction of selection observed in this study. However, larger plants have a tendency to bend at the stem, distorting height measurements, and potentially influencing the direction of selection.

The NMDS plots and corresponding phenotypic trait vectors (electronic appendix 3) for the three years of the study indicate that the first dimension generally represents differences in size while the second dimension represents phenology. However, this trend is not static. In 2011 and 2012, there is a positive relationship among all morphological traits in the first NMDS dimension and a negative relationship between flowering time and duration of flowering in the second NMDS dimension. The selection on these NMDS dimensions mirrors the selection on the individual traits. The first dimension (\approx size) experienced similar positive selection for increased size in 2011 and 2012, but the selection on the second dimension (\approx phenology) was twice as strong in 2012 than 2011 (table 2). However, in 2013, while the negative relationship between flowering time and duration of flowering continued, there was also a positive relationship between duration of flowering and many morphological traits. This suggests that plants that were larger were also able to flower longer, increasing fitness. Selection on both NMDS dimensions was stronger in 2013 than the previous two years, mirroring the increase in direct selection on height and duration of flowering in 2013.

The results of the fitness component selection analyses demonstrate the importance of certain phenotypic traits to the fitness components examined in this study. For the maximum number of flowers fitness component, the number of leaves and basal stem diameter, both proxies for overall plant size, were the significant driving factors. This finding suggests that larger plants will generally have more flowers, which corresponds to the selection analyses performed using silique count as the fitness proxy. In 2013, there was also a significant relationship between duration of flowering and maximum number of flowers, suggesting that maintaining flowering after the flood water receded was important for increased fitness. For the siliques/flower fitness component, earlier flowering time and longer duration of flowering were driving factors. Earlier flowering time and longer duration of flowering could help this transition from flowers to siliques by investing more resources into reproduction or increasing flower exposure to increase attraction to pollinators. There was also significant selection for increased leaf count and basal stem diameter via seed pods/flower during the hot and dry year of 2012. This suggests that larger plants are more likely to be able to produce more siliques per flower in particularly hot and dry conditions.

As expected in introduced environments, the prevalence of directional selection and the absence of stabilizing selection are indicative of the distance between trait distributions of this population and their fitness optimums (Kingsolver et al. 2001). Selection for larger size is consistent with several other studies examining phenotypic selection on plants in introduced environments (Thébaud & Simberloff 2001, Murren et al. 2005) and is often indicative of a benign introduced

environment with plentiful resources (Bossdorf et al. 2005). However, the only trait that demonstrated significant selection during all three years of the study was flowering time. Selection for earlier flowering time is also common in temperate environments (Munguía-Rosas et al. 2011, Anderson et al. 2012), though is often indicative of stress or water limitation (Heschel & Riginos 2005, Sherrard & Maherali 2006). The importance of earlier flowering time in this population is concordant with previous studies showing its selection for earlier flowering in CA populations of *Brassica rapa*, particularly under season-shortening drought conditions (Franks 2011, Franks et al. 2014). However, the flowering time selection differentials estimated in this study ($S = -2.40$ days in 2012 and -9.16 days in 2013) were not considerably higher than in the source environment ($S = -7.67$ days in 2003; Franks et al. 2007). This suggests that introduction to a novel environment may not inherently increase the strength of selection on flowering time.

The variation in the strength of selection observed in this study is consistent with several studies on a variety of different traits, including floral traits (Caruso et al. 2003, Murren et al. 2009), defense traits (Reimchen & Nosil 2002), reproductive traits (Millet et al. 2015), and morphological characteristics (Grant & Grant 2002). However, variation in selection is not always observed (Donovan et al. 2009, Kulpa & Leger 2013). Morrissey & Hadfield (2012) suggest that most of the variation in selection observed is due to sampling error. However, in our study, this is unlikely because we found no relationship between the standard deviation of the selection gradients and their mean standard error, indicating that these results are not strongly affected by sampling error (Millet et al. 2015). Thus this study supports the idea that selection can vary substantially over time.

While this study observed strong phenotypic selection in some traits in an introduced population, there are several factors that could limit the ability of the population to respond to this selection. One factor is the variation in selection among years observed in this study, which could be indicative of shifting adaptive landscapes. However, while it is difficult with this data to assess whether or not selection is moving to a different fitness optimum, the relationship between selection and fitness needs to be considered when evaluating the impact on the introduced population. For example, while the selection regimes in 2013 were significantly different from 2011 and 2012, the introduced population experienced 81% mortality in 2013, which may limit the impact on future generations. This effect may be particularly strong during early colonization of novel environments when the seedbank may not be as robust as in established populations.

Genetic variation can be reduced due to bottlenecks during introduction, which could potentially limit the response to selection (Lande & Shannon 1996), though this effect is not always detected (Harris et al. 2012). Also, strong selection in natural populations is often paired with greater temporal variation in selection (Siepielski et al. 2009), which could potentially limit local adaptation (Kassen 2002, Kawecki & Ebert 2004). However, along with stronger selection, introduced populations could also experience weaker genetic constraints to evolution (Colautti & Lau 2015), which could promote rapid evolution in novel environments. Variation

in selection could also promote phenotypic variation (Bell 2010), increasing evolutionary potential. However, temporal variation in selection is not necessarily indicative of variation in the traits themselves (Gotanda & Hendry 2014). The ability of a population to evolve in response to a changing environment with respect to variation and selection still needs to be thoroughly examined (Shaw & Etterson 2012). Conducting experiments that pair temporally-replicated estimations of selection with assessments of phenotypic evolution can inform our understanding of evolutionary responses to variable selection.

Characterizing selection in introduced populations can potentially aid in the management of invasive species. For example, this information on selection can be used to help determine if invasive populations are under selection to adapt to local conditions or to management activities such as pesticides and biological control (Franks et al. 2008b), or to anthropogenic effects such as climate change (Western 2001). However, our study shows that selection in introduced populations can potentially vary greatly over time, so estimates of selection in one season may not be sufficient to accurately predict evolution over the longer term. Changes in the strength and direction of selection caused by varying climatic conditions could potentially hinder local adaptation. However, there are examples of evolution keeping up with variable climatic conditions, such as ENSO (Grant & Grant 2002).

While this study does shed light on the potential for variation in selection in introduced environments, future research can expand upon these findings. Future studies using multiple sources and several introduced environments would provide information on how characteristics of the source population affect the targets and consistency of selection in novel environments. Research involving experimental crosses would be able to examine genotypic selection, which was not possible in this study. Such results limit our ability to examine bias due to environmental covariance with traits and fitness (Rauscher 1992). However, the qualitative assessments of genotypic and phenotypic selection analyses are often the same, even if the strength of selection differs (Stinchcombe et al. 2002). Also, the priority of this study was to examine selection in a natural population in which only non-destructive measurements were taken. Thus, a fitness proxy (number of seed pods) was used rather than measuring fitness directly. While this is common practice (Griffith et al. 2004, Hereford et al. 2004) and allows comparison to other natural populations, it limits the ability to examine selection on total fitness and fitness components. Lastly, while inter-annual variation in selection could be due to changing weather patterns, the definitive agents of selection cannot be determined. Future studies combining experimental manipulations with observational measurements will be necessary to definitively determine agents of selection (Wade & Kalisz 1990).

This study found that environmental conditions, as well as the targets and strength of selection on phenotypic traits, varies among years. This suggests that not only does the introduced population have to respond to dramatically different conditions, but to variable conditions as well. Dynamic environmental conditions suggest that the adaptive landscapes of phenotypic traits are constantly shifting, with these popula-

tions always trying to hit a moving target. The logical next question is: has there been evolution, and possibly local adaptation, of the introduced population since introduction? In a separate study (Sekor & Franks in press), we address this question in our experimentally introduced population a following study using the resurrection approach of comparing ancestors and descendants in a common garden (Franks et al. 2008a). The resurrection experiment, combined with this study, allows the pairing of selection estimations over multiple seasons with possible evolutionary changes in an effort to examine the process and outcome of natural selection in colonizing populations.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (<https://www.ingentaconnect.com/content/botbel/plecevo/supp-data>) and consist of the following: (1) spring and summer mean maximum daily temperatures (A) and mean monthly precipitation (B) from the NWS weather station at the Westchester County Airport; (2) selection differentials of (A) basal stem diameter and (B) flowering time via silique count (black) and aster fitness estimates (grey) for the three years of the study; (3) biplots representing trait loadings of the first two NMDS dimensions for all measured traits in (A) 2011, (B) 2012, and (C) 2013; (4) mean trait values (and standard deviations) for all phenotypic traits measured over the three years of the study; (5) ninety-five per cent confidence intervals (bias-corrected bootstrap method, $n = 4000$) of linear differentials and gradients, quadratic differentials and gradients, NMDS dimensions, and linear gradients via fitness components; and (6) phenotypic correlations of all morphological and phenological traits in (A) 2011, (B) 2012, (C) 2013.

ACKNOWLEDGEMENTS

We are grateful to the staff of the Louis Calder Center who helped with logistics in performing the field experiment, as well as T. Bleeker, K. Clonan, and M. Clemens for their assistance in conducting the experiment. We would also like to thank E.R. Hekkala, J.D. Lewis, and J.D. Wehr for their helpful suggestions throughout the experimental process. This study was funded by NSF grant DEB-1142784 to S.J. Franks and multiple Calder Center Graduate Research Grants to M.R. Sekor.

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Manuscript received 25 Apr. 2017; accepted in revised version 4 May 2018.

Communicating Editor: Alexandra Ley.