

An experimentally introduced population of *Brassica rapa* (Brassicaceae). 2. Rapid evolution of phenotypic traits

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Background and aims – Introduced populations can potentially experience strong selection and rapid evolution. While some retrospective studies have shown rapid evolution in introduced populations in the past, few have directly tested for and characterized evolution as it occurs. Here we use an experimental introduction to directly observe and quantify evolution of multiple traits in a plant population introduced to a novel environment.

Methods – We experimentally introduced seeds of the annual plant *Brassica rapa* L. (Brassicaceae) from a location in southern California into multiple replicated plots in New York. We allowed the populations to naturally evolve for 3 years. Following the resurrection approach, we compared ancestors and descendants planted in common garden conditions in New York in multiple phenotypic traits.

Key results – Within only three generations, there was significant evolution of several morphological, phenological, and fitness traits, as well as substantial variation among traits. Despite selection for larger size during the three years following introduction, there was evolution of smaller size, earlier flowering time, and shorter duration of flowering. Although there were rapid evolutionary changes in traits, descendants did not have greater fitness than ancestors in New York, indicating a lack of evidence for adaptive evolution, at least over the timeframe of the study.

Conclusions – This study found rapid evolution of several morphological and phenological traits, including smaller plant size and shorter time to flowering, following introduction, confirming that evolution can rapidly occur during the early stages of colonization. Many traits evolved in the opposite direction predicted from phenotypic selection analysis, which suggests that the resurrection approach can reveal unanticipated evolutionary changes and can be very useful for studying contemporary evolution.

Key words – Rapid evolution, plants, resurrection approach, morphology, flowering time, *Brassica rapa*, experimental introduction, introduced species.

INTRODUCTION

While once thought to be a slow process, there is now substantial evidence that rapid evolution in natural populations can occur over contemporary timescales (Thompson 2013). Evolution appears to be particularly rapid in cases where there is a mismatch between organisms and their environments (Carroll et al. 2014), as can occur with anthropogenic environmental changes (Palumbi 2001) such as climatic changes (Levitan 2003) or pesticides (Whalon et al. 2008). Thus especially strong selection and rapid evolution is expected for populations introduced to novel environments. Indeed, prior research provides evidence of rapid evolution in introduced populations of invasive species (Maron et al. 2004, Ridley & Ellstrand 2010, Novy et al. 2013, Colautti

& Lau 2015). Despite these examples, evolution could potentially be limited in introduced populations due to factors such as genetic bottlenecks (Barrett 1991, Van Buskirk & Willi 2006, Dlugosch & Parker 2008, Bell & Gonzalez 2009), trade-offs (Blows & Hoffmann 2005, Walsh & Blows 2009), or genetic correlations that oppose selection (Etterson & Shaw 2001). Thus it remains unclear to what extent rapid adaptive evolution occurs in introduced populations.

Detailed information on the rates of evolution of different traits in introduced populations is scarce because much of the prior research in this area has been indirect, coming from populations that have already been introduced and established. Previous studies have used techniques such as population genetic analyses (Dlugosch et al. 2015), quantita-

Table 1 – Summary of environmental variables in the source and introduced environment.

	Source Environment	Introduced Environment
State	California	New York
Latitude	33.661	41.127
Longitude	-117.851	-73.731
Climate type	Mediterranean	Temperate
Soil type	Clay loam	Loam
Vegetation type	Mediterranean Coastal Scrub	Eastern Deciduous Forest
Growing season dates	December–April	April–September
Average high/low temp in January	18°C/9°C	4°C/-5°C
Average high/low temp in July	26°C/19°C	28°C/19°C
Average precipitation in January	7 cm	5 cm
Average precipitation in July	0.6 cm	10 cm

tive genetic analyses (Franks et al. 2008b, 2012), or reciprocal transplants (Maron et al. 2004, Ridley & Ellstrand 2010, Novy et al. 2013, Colautti & Lau 2015) to retrospectively indirectly infer past evolution, rather than directly capturing evolution in action. In contrast, experimental introductions provide the opportunity to directly observe evolution as it occurs (Walsh & Reznick 2011), and allow a focus on the early stages of introduction and colonization not possible in studies where the introduced species is already established.

Experimental introductions of taxa to a new environment have been used to study evolution and colonization success in a variety of animals (Reznick et al. 1997, Herrel et al. 2008, Forsman et al. 2012, Gotanda & Hendry 2014, Stuart et al. 2014, Gordon et al. 2015), but examples of experimental introductions to examine evolution in plants appears to be surprisingly lacking (Campbell et al. 2006, Hovick et al. 2012). Experimental introductions are particularly powerful for studying evolution in introduced species when combined with the resurrection approach (Franks et al. 2008a). In the resurrection approach, ancestors, obtained from stored propagules such as seeds, are compared with descendants in common conditions (Franks et al. 2008a). Differences between ancestors and descendants are strong direct evidence of evolution (Franks et al. 2018). The resurrection approach has several advantages over indirect approaches to investigating evolution (Franks et al. 2018), but we lack prior studies that have combined the resurrection approach with experimental introductions, limiting our ability to characterize evolution in introduced populations as it occurs. Here, we take the novel approach of combining an experimental introduction with the resurrection approach to directly examine contemporary phenotypic evolution following introduction in an introduced plant population.

The species used in this study is *Brassica rapa* L. (syn. *Brassica campestris*, Brassicaceae), a weedy annual plant native to the Middle East that 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. Fast Plants) of *B. rapa*, and populations have become feral or naturalized. This species was chosen due to its demonstrated ability to rapidly evolve in response to artificial (Williams & Hill 1986, Agren & Schemske 1994) and natural (Franks et al. 2007) selection. 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 were able to flower at a smaller size, demonstrating a flexible relationship between size and flowering (Franks & Weis 2008).

This study examines evolution directly following colonization in an experimentally introduced population of *Brassica rapa*. In May 2011, seeds from a population in Southern California were introduced to ten replicated plots in Armonk, New York. These sites are separated by 4500 km from their locality of origin and differ from this in many characteristics, including climate, soil type and species composition (table 1). We thus expect that the introduced population would experience strong selective pressures. The introduced population was allowed to set seed and recruit naturally, and several non-destructive morphological and phenological measurements were taken. During the first three years following introduction, the introduced population experienced strong directional selection for larger size and earlier flowering (Sekor & Franks 2018). However, the strength of direct selection varied among years for plant size and flowering duration (Sekor & Franks 2018). Thus, this prior work demonstrated selection, which could potentially cause rapid evolution, but also found that selection varied among years, which could impede evolution. Building directly on this prior work in this system, the current study characterizes evolutionary change in multiple traits in the introduced population using the resurrection approach.

The aims of this study were to characterize the pattern of evolutionary changes in means and variances of multiple traits and to search for evidence for adaptive evolution. This includes examining which traits exhibit evolution, the rate of

Table 2 – Trait means and standard errors during the first three years following introduction from California to New York. “*F*-statistic” column indicates a general linear model *F*-value and significance on log-transformed data representing variation of trait means among years. Degrees of freedom are 2, 281 for all models. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	2011	2012	2013	<i>F</i> -statistic
Height (cm)	32.9 (1.82)	20.1 (1.09)	15.1 (1.63)	5.34***
Length of longest leaf (cm)	11.6 (0.44)	5.2 (0.23)	2.9 (0.23)	17.56***
Width of longest leaf (cm)	4.2 (0.18)	1.9 (0.091)	1.1 (0.094)	15.12***
Number of leaves	16.0 (0.98)	8.6 (0.57)	4.1 (0.50)	11.26***
Basal stem diameter (mm)	3.0 (0.13)	2.5 (0.099)	1.4 (0.075)	6.48***
Flowering time (days)	43.4 (0.50)	42.1 (0.55)	56.6 (2.96)	3.93***
Duration of flowering (days)	17.2 (0.66)	12.1 (0.61)	16.0 (2.18)	3.95***
Number of seed pods	21.4 (1.67)	23.3 (2.19)	5.0 (0.99)	5.34***

evolution in those traits, correlation among traits, and trait variance. Due to the substantial environmental differences between the home and introduced ranges, as well as the directional selection for increased size and earlier flowering observed during the first three years following introduction, we predicted rapid evolution in morphological and phenological traits. If the evolutionary changes were adaptive, we predicted increased fitness of the descendant compared to the ancestral population in the introduced environment.

METHODS

System and design

The source population used for the introduction in this study is located at the San Joaquin Freshwater Marsh Preserve in Irvine, CA. This population previously showed rapid adaptive evolution in flowering time and produced genome-wide genetic changes following a natural drought (Franks et al. 2007, 2016). In June 2008, seeds were bulk collected from over 1000 individuals and stored at 5°C in paper envelopes until utilized in this study. In May 2011, two thousand *B. rapa* seeds were broadcast over ten replicate 1 m² plots, separated by at least 1 m, at the Louis Calder Biological Field Station in Armonk, NY. Each plot was treated as a block in the experimental design and analyses. The plots were tilled one month prior to the experiment and were regularly weeded to reduce interspecific competition. Throughout the first three growing seasons, the introduced population was allowed to grow and recruit naturally. During these first three seasons, maximum population size consistently decreased (1006 in 2011, 832 in 2012, 251 in 2013). Mean trait values significantly varied among these first three years (table 2) and all size proxies significantly decreased during these three years. During the third season (2013) all seeds were collected by maternal family once they had matured. Despite the preva-

lence of *B. rapa* in the US, field and herbarium searches did not yield any populations within 30 km of the field site, making gene flow into the introduced populations unlikely.

In order to reduce maternal effects in preparation for the resurrection experiment, a “refresher” generation (Franks et al. 2007) was grown in the Calder Center greenhouse. In September 2013, 500 seeds from the original California source population (ancestor generation) and all (474) seeds collected in 2013 from the introduced New York population (descendant generation) were planted in Cone-tainer® pots (Stuewe & Sons, Tangent, Oregon) in a greenhouse. The Cone-tainer® pots were placed in racks at least 3 cm apart and placed on tables in the greenhouse under grow lights on a 16 h:8 h light:dark schedule. The pots were watered regularly and fertilized with Osmocote 14-14-14 slow release fertilizer, followed by Miracle-Gro 15-30-15 fertilizer. Upon the onset of flowering, the plants were bulk pollinated daily within each generation. Bulk pollinating plants from the descendant generation resulted in the interbreeding of plants from the previously replicated plots into a single population. In order to prevent gene flow through pollen transfer between generation groups (ancestors and descendants), each generation was enclosed in screening, dividing the greenhouse. Once the seeds had matured, they were collected by maternal family in coin envelopes.

One month prior to the commencement of the resurrection experiment, ten 1.25 m × 1.25 m plots ~3 m away from the original plots were tilled in the same manner as the original plots. Prior to planting, all seedlings in the plots were removed. The day before planting, four seeds from 125 randomly selected maternal plants of both the ancestor and descendant generation from the refresher generation were attached to toothpicks using water-soluble glue. On 20 May 2014, 50 ancestor and 50 descendant seeds per plot were randomly planted in a 10 × 10 grid with 10 cm between each

seed. The toothpicks were inserted into the ground so that each seed was 0.5 cm beneath the surface and the plots were thoroughly watered to release the seeds from the glue.

The ancestors and descendant generations experienced 49.2% and 48.2% emergence and 42.8% and 41% survival to seed set, respectively. For each plant, germination date and flowering time (days from germination to the onset of flowering) was recorded. Upon the onset of flowering, whether or not each plant had at least one open flower was recorded two to three times a week to determine the duration of flowering. Once a week, measures of morphology, including proxies of overall size (plant height, number of leaves, basal stem diameter) and leaf size (length of longest leaf, width of longest leaf) were taken for each plant, as well as seed pod count per plant.

On 18 June 2014, (~21 days after emergence) pictures of randomly selected leaves were taken of 100 plants per generation to estimate leaf loss due to herbivory. The current leaf area and estimated area prior to herbivory were determined using ImageJ (<https://imagej.net/>). On 14 July (~47 days after emergence), when all plants had flowered, but none had senesced, leaf tissue was collected from the two youngest fully formed leaves of 50 plants per generation. The first leaf was dried and ground, and sent to the Analytical Chemistry Laboratory at the University of Georgia for total carbon, total nitrogen, and $\delta^{13}\text{C}$ analysis, the last being a proxy of integrated water use efficiency (Farquhar et al. 1989). The second true basal leaf was scanned, then dried and weighed. ImageJ was used to determine the area of the leaf. This was then divided by the dry mass to determine specific leaf area (SLA). Once siliques had matured, we counted the total number of siliques per plant. We then randomly selected and collected two to four siliques per plant and counted seeds per silique by hand. We weighed the seeds for each silique in which the seeds were counted to determine mass per seed. We then estimated the total number of seeds for each plant by multiplying the total number of siliques by the average seed count per silique.

Analyses

The morphological trait values 55 days after average emergence were used in all analyses. To determine if evolution, as a change in mean trait values, had occurred since introduction, we compared ancestors and descendants under common conditions, taking differences between ancestor and descendant trait values as evidence for evolution (Franks et al. 2018). We used linear mixed models, with traits as the dependent variables, block and refresher generation/maternal line as random effects, and generation (ancestor/descendant) as a fixed effect. In order to improve the normality of the residuals, some traits were log, square root, or reciprocal square root transformed (electronic appendix 1). Generalized linear mixed models with a Poisson error distribution were utilized for both phenological traits (flowering time and duration of flowering). A significant effect of generation indicates evolutionary change between ancestors and descendants (Franks et al. 2007). An increased fitness in the introduced population would be evidence of adaptive evolution (Franks et al. 2007). Rates of evolution were calculated in haldanes, or trait standard deviations per generation (Gingerich 1993). In order to

examine pairwise relationships among traits, we determined the matrices of phenotypic correlations using Pearson product moment correlations of all sixteen traits measured for both the ancestor and descendant generations, for a total of 240 tests. We used a Bonferroni correction (Benjamini & Hochberg 1995) to adjust the discovery rate due to multiple comparisons, which lowered the *p*-value significance threshold from 0.05 to 0.0002. We used Fisher's *Z* transformation (Snedecor & Cochran 1980) in order to compare individual correlations between ancestor and descendant generations. We also investigated changes in trait variance between ancestors and descendants using Brown-Forsythe tests (Brown & Forsythe 1974). Changes in trait variance were of interest because they can provide information about patterns of selection or possible genetic bottlenecks experienced by the population.

RESULTS

We found evidence for evolutionary changes in some, but not all traits, after only three generations following introduction. There were significant differences between ancestors and descendants, and thus evidence of evolution, in length of longest leaf, width of longest leaf, number of leaves, basal stem diameter, height, flowering time, and duration of flowering, as well as the fitness components number of seeds per pod and number of seeds per plant (fig. 1, electronic appendices 1 & 2). Following introduction, the plants evolved to be smaller and to flower earlier and for a shorter duration. While statistically significant, these changes were small in magnitude. For example, plants evolved to flower on average just over one day earlier (fig. 1). We did not observe evolution in specific leaf area, resistance to leaf herbivory, leaf percent carbon, leaf percent nitrogen, or the fitness component number of seed pods or mass per seed (electronic appendix 1). Thus the degree of evolution differed substantially among traits.

Of the traits that evolved, all morphological and phenological traits had evolutionary rates of 0.05–0.11 haldanes, such as length of longest leaf (0.079), width of longest leaf (0.104), number of leaves (0.057), basal stem diameter (0.072), height (0.061), flowering time (0.110), and duration of flowering (0.106). While the evolutionary rate of number of seeds per plant fitness component also fell within this range (0.056), average number of seeds per pod (0.158) evolved at a faster rate than any other trait.

There was no significant increase in any of the fitness components measured in descendants compared to ancestors, which would have been evidence of local adaptation in the introduced population. While there was no significant difference between ancestors and descendants in the number of seed pods or mass per seed, the average number of seeds/pod and total number of seeds per plant decreased since introduction.

Many of the traits measured were significantly correlated in both the ancestor and descendant generations (electronic appendix 3). As expected, many size traits were positively correlated with one another and many were positively correlated with the fitness components in both ancestors and descendants. In particular, most size traits were positively correlated with number of seed pods and seeds per plant. Also,

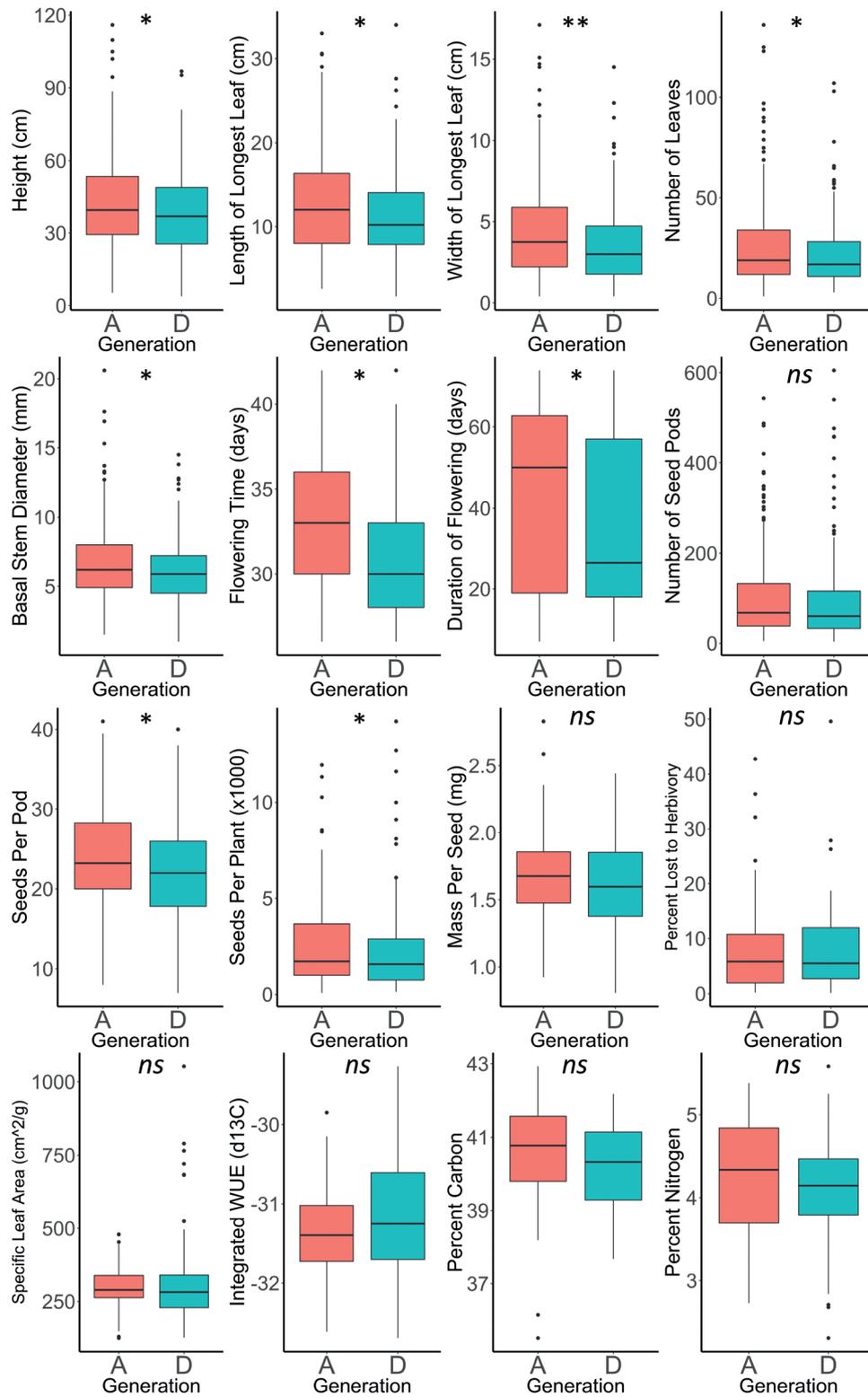


Figure 1– Tukey boxplots depicting trait distributions for the Ancestor (A) and Descendant (D) generations. The lower and upper bounds of the box are the first and third quartile, the band represents the median, and the whiskers represent the highest and lowest datum within 1.5 x interquartile range (IQR) of the upper and lower quartile, respectively. Differences in the trait means between ancestors and descendants were tested using linear mixed models, with traits as the dependent variables, block and refresher generation maternal line as random effects, and generation (ancestor/descendant) as a fixed effect. A significant effect of generation indicates evolutionary change between ancestors and descendants: not significant (*ns*), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 3 – Standard deviation of trait values for ancestor and descendant populations.

The right columns represent the Brown-Forsythe *F*-statistic and *p*-value between traits. WUE = water use efficiency. Values with *p* < 0.05 are indicated with bold.

	Ancestors	Descendants	<i>F</i> -Statistic	<i>p</i> -value
Height (cm)	19.95	16.75	$F_{1,417} = 2.59$	0.11
Length of longest leaf (cm)	6.00	4.72	$F_{1,417} = 12.33$	< 0.001
Width of longest leaf (cm)	2.95	2.45	$F_{1,417} = 4.49$	0.035
Number of leaves	22.38	16.18	$F_{1,417} = 5.35$	0.021
Basal stem diameter (mm)	2.92	2.36	$F_{1,417} = 3.26$	0.072
Flowering time (days)	3.93	3.93	$F_{1,417} = 0.012$	0.91
Duration of flowering (days)	21.15	20.24	$F_{1,417} = 1.26$	0.26
Number of seed pods	99.05	100.96	$F_{1,417} = 0.024$	0.88
Seeds/pod	6.27	6.99	$F_{1,264} = 0.70$	0.40
Seeds/plant	2436.8	2522.5	$F_{1,264} = 0.14$	0.70
Mass/seed (mg)	0.33	0.33	$F_{1,264} = 0.61$	0.44
Leaf herbivory (%)	0.084	0.088	$F_{1,120} = 0.017$	0.90
Integrated WUE	0.58	0.71	$F_{1,93} = 2.31$	0.13
Specific leaf area	77.80	155.62	$F_{1,163} = 5.93$	0.016
Percent carbon	1.55	2.09	$F_{1,93} = 0.36$	0.55
Percent nitrogen	0.725	0.750	$F_{1,93} = 1.62$	0.21

at least one size trait (length of longest leaf in ancestors, and basal stem diameter and number of leaves in the descendants) in both generations was positively correlated with mass per seed. In both the ancestors and descendants, there was a negative correlation between basal stem diameter and flowering time, as well as positive correlations between most size traits and duration of flowering. In the descendants, there was a significant positive correlation between water use efficiency and number of leaves that was approaching significant when corrected for multiple tests (*p* = 0.002 with a significance threshold of *p* = 0.0002) in the ancestors. However, the relationships between the flowering traits and the fitness components were less static and significance varied between ancestors and descendants. For example, in the ancestor generation, there were significant positive correlations between the duration of flowering and the fitness components number of seed pods, seeds per plant, and mass per seed. However, there were no significant correlations between flowering time and any of the fitness components in the ancestor generation. In the descendant generation, there were no significant correlations between duration of flowering and the fitness components, but rather negative correlations approaching significance between flowering time and the fitness components number of seed pods, number of seeds per pod, and seeds per plant. According to the Fisher’s *Z* transformation, the correlation coefficient between number of leaves and length of longest leaf, as well as duration of flowering and seeds per plant, varied between ancestors and descendants.

The Brown-Forsythe tests demonstrated that trait variance was significantly reduced, in the descendants compared to the ancestors, for length of longest leaf, width of longest leaf, and number of leaves, but was significantly larger for specific leaf area (table 3). Height, basal stem diameter, flowering time, duration of flowering, the fitness components, and the remaining physiological traits did not show significant differences in variation.

DISCUSSION

This study demonstrates rapid evolution in an experimentally introduced plant population. There were significant differences between ancestors and descendants, raised under common conditions, for morphological, phenological and reproductive traits, demonstrating evolution since introduction in those traits. While rapid evolution in introduced species has been shown (Reznick & Ghalambor 2001, Prentis et al. 2008, Buswell et al. 2011), prior studies only indirectly inferred evolution. This study, which directly examines evolution using an experimental introduction, demonstrates very rapid evolution in the earliest stages of colonization in an introduced population.

While there is no universally agreed on cut-off for what constitutes “rapid” evolution, prior key examples of what is considered to be rapid contemporary evolution, such as industrial melanism in peppered moths (Kettlewell 1958) and changes in beak size in Darwin’s finches following drought

in the Galapagos (Grant & Grant 1993), have taken place within several to several dozen generations. The evolutionary shifts we observed in this study, which took place within three generations, would thus be considered rapid. Calculated rates of evolution in our study were often around 0.1 haldanes, which is similar to rapid evolutionary change in the literature (Kopp & Matuszewski 2014).

The rates of evolution we detected depended on the types of trait, and were generally fairly comparable to those found in previous studies. We found evolutionary rates of morphological traits to be between 0.05 and 0.11 haldanes, which is somewhat lower than other published accounts of morphological evolution in plants (~0.15 haldane average), but within the 95% credible intervals (Pitchers et al. 2014). For phenological traits, we found rates of about 0.11 haldanes for flowering time and duration of flowering. Although these rates are also somewhat lower than found in prior studies (~0.3 haldane average) (Pitchers et al. 2014), they were still higher than the morphological traits and indicative of rapid evolution. This rapid evolution in phenology in our experimentally introduced population concurs with published accounts of rapid evolution in flowering time (Franks & Weis 2008, Nevo et al. 2012), as well as its importance in colonization success (Griffith et al. 2004, Vandepitte et al. 2014) and maintaining fitness during range expansion (Griffith & Watson 2006, Novy et al. 2013).

In contrast to the morphological and phenological traits, there was no observed evolution of any physiological traits measured, which suggests that these traits may potentially respond more via plasticity than evolution or take a longer time to evolve. While some studies suggest that physiological traits may be more evolvable than morphological traits (Bone & Farres 2001), our results concur with a previous study on *B. rapa*, which observed evolution in flowering time but not water use efficiency in response to drought (Franks 2011).

By examining evolution of multiple traits, we were able to develop a more integrative picture of how this population responded to selection in the new environment. We found evolution of smaller plant size, earlier flowering, and shorter duration of flowering, which suggests that the plants evolved to mature faster and complete reproduction sooner, showing the evolution of an accelerated life cycle since introduction. This result is in line with prior work (Franks et al. 2007, Franks 2011) showing the evolution of earlier flowering within seven generations of a strong multi-year drought. The adaptive evolution of *r*-selected traits (Amundsen et al. 2012) and faster growth strategies (Leishman et al. 2014), thus fast life-history strategies, are common in the new environment of invasive species.

One surprising finding is the fact that the evolutionary changes we observed in this study did not always match predictions from measurements of selection in these populations during colonization. For example, our prior work in this system (Sekor & Franks 2018) showed selection for earlier flowering, and this study showed evolution of earlier flowering, matching this prediction. However, the prior work showed selection for increased size, but the current study found evolution of smaller size. We had expected that

the generally greater water and nutrient availability in the introduced site of New York compared with the source site of southern California would have favoured larger plants. However, size and flowering time were phenotypically correlated in this study, and have been shown to be also genetically correlated in prior studies (Franks & Weis 2008). Given the large number of phenotypic correlations we found in this study, it is possible that genetic correlations opposing selection could have acted as a constraint to evolution (see also Etterson & Shaw 2001). However, additional work would be needed to determine how selection acted on genetic correlations in this system. In any case, it is likely that selection did not act on each trait independently but that selection on some traits could have influenced evolution of correlated traits.

Another surprising finding was that despite rapid evolution, the descendant populations did not have greater fitness in the new environment compared to the ancestors. If the evolutionary changes had been adaptive, we would have expected an increase in fitness. In contrast to this expectation, descendants were significantly less fit than ancestors as estimated by average number of seeds per pod (7% decrease) and number of seeds per plant (5% decrease), and ancestors and descendants did not differ in average seed size. Although fitness is a central concept in evolution, it is notoriously difficult to measure, and it is possible that our fitness estimates did not allow us to detect an increase in actual fitness in these populations. Because it is not possible to measure traits of individuals that did not survive (e.g. died as small seedlings), it is possible that viability selection played an important and unmeasured role in evolution in these populations, or that genetic drift occurred. Also, it is possible that adaptive evolution simply would have taken more time than the three generations of this study.

In addition to evolutionary shifts in plant traits, we found a decrease in the variance of several traits (variances lower in descendants compared to ancestors), including the number of leaves and length and width of the longest leaf, and we found an increase in variance in SLA. A decrease in genetic variance could be caused by a population bottleneck, as observed here during the course of the experiment (see Methods, System and Design). A decrease in variance could also be caused by selection. Genetic variance should decrease with stabilizing selection, although we did not detect stabilizing selection in this system (Sekor & Franks 2018). Directional selection could also decrease genetic variation over many generations, as alleles under selection approached fixation (Falconer & MacKay 1996). However, this would be unlikely after only three generations. In any case, although the cause of the changes could not be determined, it is notable that we found changes in phenotypic variances in multiple traits after only three generations of introduction to a novel environment. Changes in phenotypic variance may indicate changes in additive genetic variance, which could potentially influence the rate of further evolution. Specifically, a loss of additive genetic variance would reduce the ability of populations to evolve in response to future changes in conditions.

While many of the relationships among traits were similar in ancestors and descendants, there were also some differences. In particular, the relationships between flowering traits and fitness proxies seem to vary among generations. In

the ancestor generation, there were significant positive correlations between the duration of flowering and the fitness proxies number of seed pods and number of seeds per plant. However, in the descendant generation, these correlations were only marginally significant ($p = 0.0004$ with a significance threshold of $p = 0.0002$ when corrected for multiple tests). This could suggest that flowering time has become increasingly important to fitness (Ashworth et al. 2016, Franks et al. 2007).

This study found the rapid evolution of smaller size, earlier flowering time, and shorter duration of flowering within three generations directly following the experimental introduction of *Brassica rapa* from California to New York. Future studies that use the model of combining experimental introductions with the resurrection approach have the potential to greatly expand our understanding of evolution directly following introduction. Seed storing efforts, such as Project Baseline (Franks et al. 2008a, Etterson et al. 2016), are promoting the use of the resurrection approach and ensure these studies can continue.

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) mean trait values of ancestor and descendant populations, transformation, error distribution, and Wald F-values and p-values for fixed effect of generation used from generalized linear mixed models; (2) histograms representing trait distributions of the ancestor and descendant generations; and (3) phenotypic correlation matrices for ancestor (A) and descendant (B) generations.

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REFERENCES

Agren J., Schemske D. (1994) Evolution of trichome number in a naturalized population of *Brassica rapa*. *The American Naturalist* 143: 1–13. <https://doi.org/10.1086/285593>

Amundsen P.-A., Salonen E., Niva T., Gjelland K.Ø., Præbel K., Sandlund O.T., Knudsen R., Bøhn T. (2012) Invader population speeds up life history during colonization. *Biological Invasions* 14: 1501–1513. <https://doi.org/10.1007/s10530-012-0175-3>

Ashworth M.B., Walsh M.J., Flower K.C., Vila-Aiub M.M., Powles S.B. (2016) Directional selection for flowering time leads to

adaptive evolution in *Raphanus raphanistrum* (Wild radish). *Evolutionary Applications* 9: 619–629. <https://doi.org/10.1111/eva.12350>

Barrett S.C.H. (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk D.A., Holsinger K.E. (eds) *Genetics and Conservation of Rare Plants*: 3–30. Oxford, Oxford University Press

Bell G., Gonzalez A. (2009) Evolutionary rescue can prevent extinction following environmental change. *Ecological Letters* 12: 942–948. <https://doi.org/10.1111/j.1461-0248.2009.01350.x>

Benjamini Y., Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B* 57: 289–300.

Blows M.W., Hoffmann A.A. (2005) A reassessment of genetic limits to evolutionary change. *Ecology* 86: 1371–1384. <https://doi.org/10.1890/04-1209>

Bone E., Farres A. (2001) Trends and rates of microevolution in plants. *Genetica* 112: 165–182. <https://doi.org/10.1023/A:1013378014069>

Brown M.B., Forsythe A.B. (1974) Robust tests for the equality of variances. *Journal of the American Statistical Association* 69: 364–367. <https://doi.org/10.2307/2285659>

Buswell J.M., Moles A.T., Hartley S. (2011) Is rapid evolution common in introduced plant species? *Journal of Ecology* 99: 214–224. <https://doi.org/10.1111/j.1365-2745.2010.01759.x>

Campbell L.G., Snow A.A., Ridley C.E. (2006) Weed evolution after crop gene introgression: greater survival and fecundity of hybrids in a new environment. *Ecological Letters* 9: 1198–1209. <https://doi.org/10.1111/j.1461-0248.2006.00974.x>

Carroll S.P., Jorgensen P.S., Kinnison M.T., Bergstrom C.T., Denison R.F., Gluckman P., Smith T.B., Strauss S.Y., Tabashnik B.E. (2014) Applying evolutionary biology to address global challenges. *Science* 346: 1245993. <https://doi.org/10.1126/science.1245993>

Colautti R.I., Lau J.A. (2015) Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Molecular Ecology* 24: 1999–2017. <https://doi.org/10.1111/mec.13162>

Dlugosch K.M., Anderson S.R., Braasch J., Cang F.A., Gillette H.D. (2015) The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology* 24: 2095–2111. <https://doi.org/10.1111/mec.13183>

Dlugosch K.M., Parker I.M. (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431–449. <https://doi.org/10.1111/j.1365-294X.2007.03538.x>

Etterson J.R., Franks S.J., Mazer S.J., Shaw R.G., Soper Gorden N.L., Schneider H.E., Weber J.J., Winkler K.J., Weis A.E. (2016) Project baseline: An unprecedented resource to study plant evolution across space and time. *American Journal of Botany* 103: 164–173. <https://doi.org/10.3732/ajb.1500313>

Etterson J.R., Shaw R.G. (2001) Constraint to adaptive evolution in response to global warming. *Science* 294: 151–154. <https://doi.org/10.1126/science.1063656>

Falconer D.S., Mackay T.F.C. (1996) *Introduction to quantitative genetics*. 4th Ed. Harlow, Longmans Green.

Farquhar G.D., Ehleringer J.R., Hubick K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503–537. <https://doi.org/10.1146/annurev.pp.40.060189.002443>

Forsman A., Wennersten L., Karlsson M., Caesar S. (2012) Variation in founder groups promotes establishment success in the

- wild. *Proceedings of the Royal Society B Biological Sciences* 279: 2800–2806. <https://doi.org/10.1098/rspb.2012.0174>
- Franks S.J. (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. *New Phytologist* 190: 249–257. <https://doi.org/10.1111/j.1469-8137.2010.03603.x>
- Franks S.J., Avise J.C., Bradshaw W.E., Conner J.K., Etterson J.R., Mazer S.J., Shaw R.G., Weis A.E. (2008a) The resurrection initiative: storing ancestral genotypes to capture evolution in action. *Bioscience* 58: 870–873. <https://doi.org/10.1641/B580913>
- Franks S.J., Kane N.C., O'Hara N.B., Tittes S., Rest J.S. (2016) Rapid genome-wide evolution in *Brassica rapa* populations following drought revealed by sequencing of ancestral and descendant gene pools. *Molecular Ecology* 25: 3622–3631. <https://doi.org/10.1111/mec.13615>
- Franks, S.J., Hamann E., Weis A.E. (2018) Using the resurrection approach to understand contemporary evolution in changing environments. *Evolutionary Applications* 11: 17–28. <https://doi.org/10.1111/eva.12528>
- Franks S.J., Pratt P.D., Dray F.A., Simms E.L. (2008b) Selection on herbivory resistance and growth rate in an invasive plant. *The American Naturalist* 171: 678–691. <https://doi.org/10.1086/587078>
- Franks S.J., Sim S., Weis A.E. (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America* 104: 1278–1282. <https://doi.org/10.1073/pnas.0608379104>
- Franks S.J., Weis A.E. (2008) A change in climate causes rapid evolution of multiple life-history traits and their interactions in an annual plant. *Journal of Evolutionary Biology* 21: 1321–1334. <https://doi.org/10.1111/j.1420-9101.2008.01566.x>
- Franks S.J., Wheeler G.S., Goodnight C. (2012) Genetic variation and evolution of secondary compounds in native and introduced populations of the invasive plant *Melaleuca quinquenervia*. *Evolution* 66: 1398–1412. <https://doi.org/10.1111/j.1558-5646.2011.01524.x>
- Gingerich P.D. (1993) Quantification and comparison of evolutionary rates. *American Journal of Science* 293A: 453–478. <https://doi.org/10.2475/ajs.293.A.453>
- Gordon S.P., Reznick D., Arendt J.D., Roughton A., Ontiveros Hernandez M.N., Bentzen P., López-Sepulcre A. (2015) Selection analysis on the rapid evolution of a secondary sexual trait. *Proceedings of the Royal Society B Biological Sciences* 282: 20151244. <https://doi.org/10.1098/rspb.2015.1244>
- Gotanda K.M., Hendry A.P. (2014) Using adaptive traits to consider potential consequences of temporal variation in selection: male guppy colour through time and space. *Biological Journal of the Linnean Society* 112: 108–122. <https://doi.org/10.1111/bij.12261>
- Grant B.R., Grant P.R. (1993) Evolution of Darwin's finches caused by a rare climatic event. *Proceedings of the Royal Society B Biological Sciences* 251: 111–117. <https://doi.org/10.1098/rspb.1993.0016>
- Griffith C., Kim E., Donohue K. (2004) Life-history variation and adaptation in the historically mobile plant *Arabidopsis thaliana* (Brassicaceae) in North America. *American Journal of Botany* 91: 837–849. <https://doi.org/10.3732/ajb.91.6.837>
- Griffith T.M., Watson M.A. (2006) Is evolution necessary for range expansion? Manipulating reproductive timing of a weedy annual transplanted beyond its range. *The American Naturalist* 167: 153–164. <https://doi.org/10.1086/498945>
- Herrel A., Huyghe K., Vanhooydonck B., Backeljau T., Breugelmans K., Grbac I., Van Damme R., Irschick D.J. (2008) Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *Proceedings of the National Academy of Sciences of the United States of America* 105: 4792–4795. <https://doi.org/10.1073/pnas.0711998105>
- Hovick S.M., Campbell L.G., Snow A.A., Whitney K.D. (2012) Hybridization alters early life-history traits and increases plant colonization success in a novel region. *The American Naturalist* 179: 192–203. <https://doi.org/10.1086/663684>
- Kettlewell H.B.D. (1958) A survey of the frequencies of *Biston betularia* (L.) (Lep.) and its melanic forms in Great Britain. *Heredity* 12: 51–72. <https://doi.org/10.1038/hdy.1958.4>
- Kopp M., Matuszewski S. (2014) Rapid evolution of quantitative traits: theoretical perspectives. *Evolutionary Applications* 7: 169–191. <https://doi.org/10.1111/eva.12127>
- Leishman M.R., Cooke J., Richardson D.M. (2014) Evidence for shifts to faster growth strategies in the new ranges of invasive alien plants. *Journal of Ecology* 102: 1451–1461. <https://doi.org/10.1111/1365-2745.12318>
- Levitan M. (2003) Climatic factors and increased frequencies of “southern” chromosome forms in natural populations of *Drosophila robusta*. *Evolutionary Ecology Research* 5: 597–604.
- Maron J., Vilà M., Bommarco R., Elmendorf S., Beardsley P. (2004) Rapid evolution of an invasive plant. *Ecology* 74: 261–280. <https://doi.org/10.1890/03-4027>
- Nevo E., Fu Y.-B., Pavlicek T., Khalifa S., Tavasi M., Beiles A. (2012) Evolution of wild cereals during 28 years of global warming in Israel. *Proceedings of the National Academy of Sciences of the United States of America* 109: 3412–3415. <https://doi.org/10.1073/pnas.1121411109>
- Novy A., Flory S.L., Hartman J.M. (2013) Evidence for rapid evolution of phenology in an invasive grass. *Journal of Evolutionary Biology* 26: 443–450. <https://doi.org/10.1111/jeb.12047>
- Palumbi S. (2001) Humans as the world's greatest evolutionary force. *Science* 293: 1786–1790. <https://doi.org/10.1126/science.293.5536.1786>
- Pitchers W., Wolf J.B., Tregenza T., Hunt J., Dworkin I. (2014) Evolutionary rates for multivariate traits: the role of selection and genetic variation. *Philosophical Transactions of the Royal Society B Biological Sciences* 369: 20130252. <https://doi.org/10.1098/rstb.2013.0252>
- Prentis P.J., Wilson J.R.U., Dormontt E.E., Richardson D.M., Lowe A.J. (2008) Adaptive evolution in invasive species. *Trends in Plant Science* 13: 288–294. <https://doi.org/10.1016/j.tplants.2008.03.004>
- Reznick D.N., Ghalambor C.K. (2001) The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* 112: 183–198. <https://doi.org/10.1023/A:1013352109042>
- Reznick D.N., Shaw F.H., Rodd F.H., Shaw R.G. (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* 275: 1934–1937. <https://doi.org/10.1126/science.275.5308.1934>
- Ridley C.E., Ellstrand N.C. (2010) Rapid evolution of morphology and adaptive life history in the invasive California wild radish (*Raphanus sativus*) and the implications for management. *Evolutionary Applications* 3: 64–76. <https://doi.org/10.1111/j.1752-4571.2009.00099.x>
- Sekor M.R., Franks S.J. (2018) An experimentally introduced population of *Brassica rapa* (Brassicaceae). 1. Phenotypic selection over three years following colonization of a novel environment. *Plant Ecology and Evolution* 151: 209–218. <https://doi.org/10.5091/plecevo.2018.1354>

- Snedecor G.W., Cochran W.G. (1980) *Statistical methods*. Ames, Iowa State University Press.
- Stuart Y.E., Campbell T.S., Hohenlohe P.A., Reynolds R.G., Revell L.J., Losos J.B. (2014) Rapid evolution of a native species following invasion by a congener. *Science* 346: 463–466. <https://doi.org/10.1126/science.1257008>
- Thompson J.N. (2013) *Relentless evolution*. Chicago, The University of Chicago Press.
- Van Buskirk J., Willi Y. (2006) The change in quantitative genetic variation with inbreeding. *Evolution* 60: 2428–2434. <https://doi.org/10.1111/j.0014-3820.2006.tb01879.x>
- Vandepitte K., de Meyer T., Helsen K., van Acker K., Roldán-Ruiz I., Mergeay J., Honnay O. (2014) Rapid genetic adaptation precedes the spread of an exotic plant species. *Molecular Ecology* 23: 2157–2164. <https://doi.org/10.1111/mec.12683>
- Walsh B., Blows M.W. (2009) Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. *The Annual Review of Ecology, Evolution, and Systematics* 40: 41–59. <https://doi.org/10.1146/annurev.ecolsys.110308.120232>
- Walsh M.R., Reznick D.N. (2011) Experimentally induced life-history evolution in a killifish in response to the introduction of guppies. *Evolution* 65: 1021–1036. <https://doi.org/10.1111/j.1558-5646.2010.01188.x>
- Whalon M., Mota-Sanchez D., Hollingworth R. (2008) *Global pesticide resistance in arthropods*. Wallingford, CABI. <https://doi.org/10.1079/9781845933531.0000>
- Williams P.H., Hill C.B. (1986) Rapid-cycling populations of *Brassica*. *Science* 232: 1385–1389. <https://doi.org/10.1126/science.232.4756.1385>
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