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Global population divergence of a cosmopolian desert plant

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GLOBAL POPULATION DIVERGENCE OF A COSMOPOLITAN DESERT PLANT

by

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B.S. Ecology, Behavior and Evolution

University of California at Los Angeles, Los Angeles, CA, 2007

DISSERTATION

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DEDICATION

I would like to dedicate this dissertation to my maternal grandfather, Leonardo Arañas. Tata Ando, as the older grandchildren call him, was a WWII veteran from the Philippines, was born into and downtrodden by poverty, and did not finish elementary school. Nonetheless, he was the first person to mention the word “science” to me. When I was still living in the Philippines (ca. 1990) he flew to San Jose, California with my grandmother, Mama Concha. For the first time I felt true sadness. Tata Ando was the only person who truly understood my deep interest for nature at that time (a close second was my father, who seeded in me a sense of adventure that I share with my wife and hope to ingrain to my children). Tata Ando always made sure to remind me that he understood my passion. For example, out of many cool things he can send from California in the 1990s, such as Air Jordans and Stussy t-shirts, he sent me a twig from a giant sequoia (*Sequoiadendron giganteum*), which he taped onto a page of National Geographic magazine. If it weren’t for those childhood keepsakes and experiences, I wouldn’t be writing this dissertation in the first place.

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ABSTRACT

Genetic and phenotypic variation can have different patterns within a species if it has populations with contrasting histories. Populations can have discrete differences that are shaped by different evolutionary scenarios, but within each population, range, or region, traits and association with fitness can also be affected by both edaphic and landscape variation. For my dissertation, I surveyed and experimentally analyzed variation and adaptive potential in Sahara mustard (*Brassica tournefortii*), a desert annual that has endemic, invasive, and agricultural populations in Africa, Asia, and the Americas. Although my multi-trait analysis generated complex results, my findings can be applied to other *Brassica* that have both wild and agricultural populations. *B. tournefortii* has both adaptive and maladaptive evolutionary potential that can be harnessed for conservation, invasive species control, and crop development.

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INTRODUCTION

Overview of theoretical background

The study of evolution and experimental populations began some 220 years ago, when Darwin first observed phenotypic variation in the wild, and when Mendel demystified the cause of phenotypic variation in 28,000 *Pisum* plants in an experimental monastery garden. These two fundamental hypotheses were combined to form the Modern Synthesis (Huxley 1943) that became the basis for a unified theory of evolution. The unified theory frames evolution as the result of directional selection of phenotypes with genetic bases of variation. One concept of the unified theory underpins the questions of my dissertation's first chapter. That is, demographic events such as drift or displacement of individuals, can push a population's optimal trait mean off its adaptive peak, allowing directional selection to shape a new peak, thereby forming adaptive landscapes (Wright 1932). The opening chapter examines how trait means and adaptive landscapes can change in different geographic and historical origins, and in the face of aridity (Alfaro and Marshall 2019). In this chapter, I tested extensively how different patterns of selection may have shaped phenotypic variation. In contrast, I examine phenotypic plasticity in the second chapter. Plastic response to extrinsic factors can also produce phenotypic variation (Bradshaw 1965, Sultan 2000), and that the newfound variability can be genetically encoded after the response (Pigliucci 2008). In this chapter I specifically examine how trait means and levels of plasticity can vary among plants with different geographic and historical origins. I also designed a study wherein I examined mechanisms that define the foundation of neutral theory of evolution (Kimura 1979), another mechanism that can contribute to adaptive variation. In the third chapter, I analyzed molecular markers in order to show how neutral evolutionary mechanisms, in

particular, gene flow, may have arranged genetic diversity and population divergence across my study populations. In Chapter 4, I examined whether microsatellite genetic variation affects phenotypic plasticity in the traits I studied. Throughout my work, I approached experimental design using classic plant population biology concepts (Harper 1967, 1977) as my guideposts.

Overview of study system

I used field collections and accessions of *Brassica tournefortii* to create experimental populations, and for molecular analysis. *B. tournefortii* is a mustard that is endemic in the desert habitats of North Africa, Mediterranean regions of Europe, and the Middle East (Abd El-Gawad 2014). In South Asia, this species is cultivated as an oilseed crop (Singh et al. 2015). In the southwestern United States (Trader et al. 2006) and in Australia (Chauan, et al. 2006), this species is classified as a noxious weed. Its diverse historical and geographic backgrounds make it ideal for testing different questions and hypothesis that I addressed in my dissertation.

List of questions and hypotheses

Chapter 1

1. Question: Do the suites of phenology, leaf morphology, branch architecture, size, and reproductive traits vary among ranges, among population nested within ranges, and among maternal families nested within populations within ranges?
2. Question: Which traits have significant fitness functions, and do these fitness functions vary among the native, invasive, and landrace ranges?

3. Question: Do composite trait means (mean factor scores) vary along climate gradients to form clines, and do these potential clines vary among native, invasive, and landrace ranges?
4. Question: Do composite traits vary in strength of selection among populations along climate gradients, and do regression lines of environmental variation versus selection strength differ among native, invasive, and landrace ranges?

Chapter 2

1. Hypothesis: Because of selection for yield stability in domesticated populations, the amount of plasticity due to varying soil moisture in traits related to reproduction, leaf morphology, plant size, and branching architecture, will differ such that native and invasive populations will have linear reaction norms, while landrace populations will have flat or asymptotic reaction norms.
2. Hypothesis: Because there are likely different levels of genetic diversity among the population types leading to different fitness consequences of plasticity, a) the amount of plasticity due to varying soil moisture in traits related to reproduction, leaf morphology, plant size, and branching architecture, would differ such that invasive > native > landrace populations.
3. Hypothesis: Because of likely lower genetic diversity such that response to environmental variation required plasticity, fitness will increase in value with increased plasticity due to varying soil moisture in branching architecture and leaf traits in the invasive populations more than the native and crop populations.

Chapter 3

1. Phylogenetic tree based on population pairwise genetic distances to infer patterns of genetic divergence and evolutionary relationships among study populations of *B. tournefortii*.
2. Hypothesis: The amounts of mean genetic diversity and mean heterozygosity will be in this particular order: native >> landrace > invasive populations.
3. Hypothesis: Focal microsatellites in study populations will be differentiated in this particular order: native < landrace < invasive.
4. Hypothesis: Native accessions will show weak or no gene flow, while the landraces and invasive populations, which are in their same respective regions, will show higher rates of gene flow.

Chapter 4

1. Hypothesis: Microsatellite genetic variation will be associated with overall phenotypic plasticity.
2. Hypothesis: The association of microsatellite genetic variation will have different trends among the native, invasive, and landrace ranges.

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CHAPTER 1

Phenotypic variation of life history traits in

native, invasive, and landrace populations of *Brassica tournefortii*

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Abstract

Varying environments can result in different patterns of adaptive phenotypes. By performing a common greenhouse experiment, we identified phenotypic differentiation on phenology, leaf morphology, branch architecture, size, and reproduction, among native, invasive, and landrace ranges of *Brassica tournefortii*. We first compared trait means and fitness functions among ranges, then we analyzed how trait means and selection strength of populations respond to varying aridity. Most traits varied such that landrace > invasive > native. Excluding reproduction, which was positively selected, most trait PCs experienced non-linear selection in the native range but frequently shifted to directional selection in invasive and/or landrace ranges. Absence of strong clines for trait means in landrace and invasive populations suggests that agricultural practices and novel environments in source locations affected adaptive potential. Selection strength on faster reproductive phenology (negative directional) and leaf margin trait (disruptive) PCs coincided with increasing moisture. In native populations, higher aridity was associated with more days to reproduction, but landrace and invasive populations show stable mean time to reproduction with increasing moisture. A stable adaptive trait can increase range expansion in the invasive range, but stability can be beneficial for future harvest of *B. tournefortii* seed crops in the face of climate change.

Keywords: wild crop relatives, crop evolution, stability, biological invasions, rapid evolution

Introduction

Contrasting evolutionary scenarios among discrete groups of plant populations can produce diverse patterns of phenotypic differentiation. Depending on how (micro)evolutionary and ecological factors interact, local adaptation or phenotypic plasticity can alter correlations between trait values and environmental gradients or trait values and fitness (e.g. Conner and Hartl 2004). When populations of the same species have experienced different histories and environments, we can examine evolution under a variety of selection pressures. For example, evolution of native plant populations can span geological timescales, while adaptations in crops and weeds are shaped by human activity (Meyer and Purugganan 2013). Some varieties have been bred since the rise of civilizations, over a few thousand years (Purugganan and Fuller 2009), while invasive populations can evolve rapidly in the span of a few decades or centuries because of the rapid changes in selective pressures in new environments (Bossdorf et al. 2005; Dlugosch and Parker 2008; Buswell et al. 2011; Colautti and Barrett 2013). Comparing populations of a single species that have evolved under these differing conditions allows us to assess effects of these mechanisms of selection on adaptive trait variation and association of candidate traits with environmental variation.

Because conditions in native, invasive and/or cultivated ranges of a species can vary, we may find different adaptations and associations of traits with environments among these types of populations. Moreover, anthropogenic factors, such as artificial selection and unintentional dispersal, can also affect patterns of phenotypic variation. Although traditional landraces are subjected to artificial selection for success under cultivation, these populations may still have ample evolutionary potential and therefore may show unique responses to environmental variation (Brush 1995; Mercer et al. 2008; Mercer and Perales 2010). A

different evolutionary scenario shapes phenotypic variation in invasive populations. First, human-mediated dispersal of propagules can introduce individuals with limited genetic diversity to a new area. Then, genetic diversity of pioneer populations can increase or show structuring depending on the amount of gene flow from other introduced populations (Bartlett et al. 2002; Valliant et al. 2007; Dlugosch and Parker 2008; Williams and Fishman 2014). There may be introduced genotypes preadapted to original conditions, but if the new habitat is different than the native range (e.g. discrete latitudinal ranges), then environmental filtering can structure traits differently via local adaptation (Maron et al. 2004; Bossdorf et al. 2005; Dlugosch and Hays 2008; Dlugosch and Parker 2008). Plasticity can also result in phenotypic clines across environmental gradients among invasive populations (Matesanz et al. 2012; Colautti and Lau 2015), but this is not always the case (Godoy et al. 2011; Matzek 2012). Whether clines formed by invasive or crop populations will be the same or different than those of native populations will depend on associations of traits with the new environments and how these interactions shape evolution of phenotypes (Colautti et al. 2009).

Pairwise comparisons of invasive, native, and landrace populations have revealed important patterns of phenotypic evolution. For example, similar mean trait values and parallel/continuous clinal responses of invasive and native populations are considered signals that pre-adapted genotypes established in similar habitat conditions in non-native ranges (Bossdorf et al. 2005; van Kleunen et al. 2011). In contrast, differing means among populations or among ranges, and intersecting trait-environment clines indicate genotype-by-environment interaction and/or local adaptation to new environments (Colautti et al. 2009; Colautti and Barrett 2013; Colautti and Lau 2015). On one hand, analysis of clinal responses

can tell us about evolution of invasive species; on the other hand, comparisons of phenotypic and genetic variation in wild and landrace populations allow us to examine the effects of domestication on plant evolution. While pairwise comparisons are informative, a three-way examination of adaptive phenotypic response to environmental factors in native, invasive, and landrace ranges would provide additional insight because it can reveal evolutionary trends of plants potentially subjected to different types of selection. We are aware of no studies that explicitly compare phenotypic means, fitness functions, and clinal patterns of traits and selection strength along environmental gradients among native, invasive, and landrace ranges of a single species.

While testing genetic basis of traits is critical, determining fitness consequences confirms adaptive trait evolution (Conner and Hartl 2004). But, merely describing fitness functions does not detect possible selection agents and how selection can change across landscapes. To determine possible environmental drivers of selection, some have regressed population mean trait values with associated environmental gradients (Maron et al. 2004; Colautti and Barrett 2010). These putative selection agents can then be confirmed by regression of environmental variables versus selection gradients (Stewart and Schoen 1987; Wade and Kalisz 1989; Wade and Kalisz 1990; Conner and Hartl 2004).

To test how variation and selection of phenotypes can be restructured by different histories and climatic gradients, we chose a study system that has both landrace and invasive populations outside of an extant native range. Specifically, we used *Brassica tournefortii* (Sahara mustard) to test whether traits and their fitness, climate variables and traits, or climate variables and selection gradients, have similar or different relationships in native, invasive, and landrace ranges. To assess how adaptive trait variation and strength of selection

can vary among ranges and among climatic gradients, we asked questions about phenotypic evolution in *B. tournefortii*:

1 - Do the suites of phenology, leaf morphology, branch architecture, size, and reproductive traits vary among ranges, among population nested within ranges, and among maternal families nested within populations within ranges?

2 - Which traits have significant fitness functions, and do these fitness functions vary among the native, invasive, and landrace ranges?

3 - Do composite trait means (mean factor scores) vary along climate gradients to form clines, and do these potential clines vary among native, invasive, and landrace ranges?

4 - Do composite traits vary in strength of selection among populations along climate gradients, and do regression lines of environmental variation versus selection strength differ among native, invasive, and landrace ranges?

Material and methods

Study species

Brassica tournefortii (Sahara mustard) is a xerophytic, self-pollinating annual endemic to North Africa, the Middle East, and Mediterranean regions of Europe, is a seed crop in Pakistan and India, and is invasive in Australia and North America (Boutsalis et al. 1999; Dimmitt 2009; Gorecki et al. 2012; Abella et al. 2013; Berry et al. 2014). In the western United States, *B. tournefortii* is an invasive plant that outcompete native desert flora, and impact small animals (Hulton VanTassel et al. 2014). In Australia, it is catalogued as a noxious agricultural weed (Gorecki et al. 2012). It was introduced to the western United

States in the late 1920s and has spread in the last four decades; the invasive populations are therefore quite young. Thus, *B. tournefortii* has a wide global range and populations with diverse histories, making it ideal for examining plant phenotypic evolution. In the invasive ranges it outcompetes endemic plants by having early and rapid phenology (Marushia et al. 2010, 2012), high fecundity (Trader et al. 2006; Bangle et al. 2008), variable germination (Chauhan et al. 2006; Bangle et al. 2008; Gorecki et al. 2012; Abd El-Gawad 2014), and natural and artificial dispersal modes that allow long-distance migration (Berry et al. 2014; Li et al. 2015). Based on our own pilot studies conducted in the greenhouse, different source populations can express variable morphological phenotypes and phenology (Figures 1 a-c). In its invasive range in the deserts of the southwestern United States, a mature plant can grow as an entire diaspore that disperses seeds as a tumbleweed (Figure 1d).

Study area

Our study included populations from native, invasive, and agricultural ranges of *B. tournefortii* (Figure 2, Table 1). For the native range, we used four populations from Morocco, Spain, and Israel. For the agricultural range, we used three populations from India and Pakistan that we call landraces because the seeds were collected from crops grown and bred via traditional practices and not through intensive commercial methods. The seeds we used to grow experimental populations for the native and landrace populations were obtained from accessions provided by the U.S. Department of Agriculture - Agricultural Research Services (USDA-ARS) National Genetic Resources Program. For the invasive range, we used seven populations from the southwestern United States. The seeds from these populations were collected in 2008 by professional biologists who volunteered to sample in

the southwestern U.S. For each site, approximately 10 fruits per plant were collected from 10 to 12 plants per population; these fruits were collected separately for each maternal plant and stored in labeled coin envelopes.

Generation of seed families

To reduce maternal environmental effects and to avoid using plants with unknown parentage, we grew a parental generation in a common environment at the UNM Research Greenhouse for native, invasive, and landrace populations (Figure 3). In March of 2015, the resulting P_1 plants were artificially crossed and their progeny were used for the experiment. For native and landrace populations, we created P_1 generations using seed accessions from the USDA-ARS. Each seed accession originated from field collection of seeds from about 15 to 30 plants per site, which were then maintained by the USDA in plant cages (Laura Marek, USDA, personal communication). We haphazardly drew seeds from each accession envelope, germinated and grew 20 seeds per accession, and then crossed randomly paired individuals assigned as either maternal plants or pollen donors. The resulting F_1 seed families from each artificial cross were used as experimental populations that represent native and landrace ranges. For the invasive range, we used seeds from maternal plants collected in the field. We germinated and grew one seed per maternal plant; for each population, we randomly paired offspring from different maternal plants for crosses and used seeds from the F_1 generation as full-sib families, which we used to represent populations from the invasive range. The steps for artificial crosses are summarized in Figure 2. This generation of seed families was collected from April to May 2015.

Greenhouse experiment

To address our questions, we conducted a greenhouse experiment from August 2015 to February 2016, where seeds from F_1 families were grown in a common environment using a completely randomized design with 14 populations (divided unequally among three ranges) × 5 families/population × 4 replicates/maternal family (n = 280 plants). We planted seeds in the UNM Research Greenhouse in 3.78 liter pots containing a 1:1 mix of sand and Metro Mix ® (SunGro Horticulture ®, Canada). Initially, we used one pot per family (70 pots) and planted approximately 30 seeds in each pot. On the first day of planting, we randomized the location of all 70 pots. As seedlings emerged from each family/pot, we randomly selected and transplanted four seedlings to separate pots. Each seedling that germinated was transferred to a new pot before or at the emergence of the first leaf. After all seedlings were transplanted to individual pots, we randomized pots by using PROC PLAN in SAS 9.3 (SAS Institute, Cary, NC) and R Studio (R Core Team 2018). When we found two or more plants from the same population or maternal family were adjacent to each other, we separated them by assigning new locations. We further controlled for spatial variation in the greenhouse by haphazardly rearranging pot locations for all plants twice at the rosette stage, then twice at the bolting/fruiting stages. We maintained the greenhouse temperature at a minimum temperature of 26.5°C and kept the room at 40% humidity. We supplemented natural lighting with two 1000w sodium halide bulbs, so that the photoperiod is constantly at 14 hr. days and 10 hr. nights.

We hand-watered all pots until the fourth week after planting, and then used an automated drip system twice per day for four-minute periods in the morning and in the late afternoon. As the plants grew larger, we incrementally increased watering time per day. We

administered 25 ml of 1g/L Peters® 20-20-20 General Purpose Fertilizer (The Scotts Company, Marysville, Ohio, USA) once per week until 95% of the plants reached the flowering stage. To ensure that measurements during the adult stage were not recorded when plants were root-bound in their pots, most adult trait measurements, except for aboveground biomass, leaf mass, and leaf margin traits, were collected between the time of first bud appearance and 30 days after first budding. When an individual plant reached 30 days after budding, we collected the entire aboveground structure for biomass measurement.

Trait measurements

Over the lifespan of the plant we measured a total of 33 traits (Table 2). We recognize that some of these traits are correlated with each other and some of these traits may have been affected by pot constraint at some point in the experiment. We corrected for those problems in the following ways. First, we divided variables into five groups, phenological characters, leaf characters, branch architecture, plant size and reproductive characters. We used Principal Component Analysis to generate one or two composite characters for each of these trait groups. Second, while annual *Brassicas* can be root-bound in pots and that pot constraint might confound analysis, we did not simply measure traits at the end of the experiment. We reduced the possibility of systematic error by measuring several traits repeatedly during the growth of the plants and all the measures were combined into the appropriate principal components. We surmised that the PCA identifies via loadings the most variable traits. Traits that had stopped changing due to pot constraint would have been less variable and received low loadings in the composite variables.

Phenology

In the southwestern United States, *B. tournefortii* can outcompete native plants by emerging earlier and reproducing rapidly than the native plants (Marushia et al. 2010, Marushia et al. 2012). We have observed that, in areas that do not experience snow or frost in early spring or late winter, some populations can produce seeds at the onset of the growing season, allowing them to avoid possible mortality from aridity late in the growing season (B. Alfaro pers. observation). But while rapid reproductive phenology is critical for this plant to succeed in its North American range, the crop fields of landrace populations may have led to slower reproductive phenology. In *Brassica* used for canola, increasing day length and warmer temperature is important for development of inflorescences (Burton et al. 2008). Most importantly, the length of the reproductive period is an evolutionary response of many desert annuals to cope with aridity (Kemp 1983). To quantify reproductive phenology, we recorded the date of appearance of the first bud and the first flower. We calculated the days from bud to flower, which marks the days between the appearance of the first bud to the appearance of first petals. To measure senescence for each plant, we counted the number of senesced leaves 30 days after the appearance of the first bud.

Leaf traits

The common limiting factor in all our source populations is aridity. To cope with variability in amount of moisture in hot environments, different species of desert annuals have modified the sizes and shapes of their leaves to increase water use efficiency and reduce leaf damage (reviewed in Wright et al. 2012). Therefore, we included a panel of leaf morphological traits related to leaf size and shape that are critical for plant survival in desert habitats. We

measured length of two tagged leaves for each plant at 6, 12, and 18 days from first bud. For leaf mass and leaf margin traits, we collected the tagged leaves at 30d after budding. We measured leaf margin architecture to assess the potential for adaptation. Specifically, number of lobes per leaf, lobe width, leaf width, number of indentations (teeth) per leaf per plant, distance between indentations, as well as indentation depth per leaf were measured. These characters may be related to leaf function of different temperatures. We used the program LAMINA (Bylesjo et al. 2008) to obtain these leaf measurements. We collected two fresh leaves for each plant, and then obtained digital images by scanning them at 200 dpi using a Hewlett-Packard CP1210 scanner or taking digital photographs of leaves using either an iPhone 6S (Apple Inc., Cupertino, USA) or Samsung Galaxy Note8 (Samsung Group, Seoul, KR) clamped at 0.25 m height on a metal stand with ambient lighting on a white, non-reflective surface. Using a reference image, we analyzed all digitized leaf images using the LAMINA software. C f

In addition to leaf margin structure, we also measured leaf mass per area. To determine leaf mass per area, we collected two leaves per plant, pressed them for 24 hours, scanned them at 200 dpi using a Hewlett-Packard CP1210 scanner, and then used LAMINA to measure area of each leaf. We did not keep track of leaf phenology per plant, so to make sure that we sampled leaves at a consistent phenological age at the time of collection, we chose the largest leaves. Next, we dried the leaves in a desiccator oven at 45˚C for 7 days before weighing them on a Mettler Toledo AG135 analytical balance (Columbus, OH) to the nearest 0.0001 g. To measure lobe width for each dried leaf, we first located the lobes at the midpoint from the base to the tip of each sampled leaf. We then used a digital caliper to

measure the width of the right and left mid-lobes to the nearest 0.01 mm. We used the mean leaf lobe width of two leaves per plant for our analysis.

Branch architecture

Adaptations to disperse seeds for population and range expansion is critical for plant survival and establishment in desert environments (Fllner and Shmida 1981). Invasive *B. tournefortii* in North America is known to disperse fruits and seeds in the southwestern United States by moving as a tumbleweed (Buckley 1981), but traits associated to this dispersal mode have not been shown as adaptive in this species. We chose branch architecture traits based on Baker et al's (2008) finding that variation in branch density and morphology in different populations of tumbleweed species (*Centaurea diffusa*, *Kochia scoparia*, and *Salsola* spp.) was associated with each population's proportion of mobile plants.

We tagged two terminal branches situated at mid-height of the plant for branch measurements. We measured branch length with a meter stick to the nearest 0.1 cm and branch thickness using a digital caliper to the nearest 0.01 mm at 12 and 18 days after the appearance of the first flower bud. If the tagged branch was bent due to the weight of fruits, we straightened it before measurement. To determine total branch number, we counted the total number of terminal (secondary) branches per plant at 6, 12, and 18 days from first bud. In addition to branch length and number of branches, we measured thickness at the base the branch, and branch angle. Thirty days after budding of each plant, when the plants were fully grown, we haphazardly selected two primary branches and measured their angles with respect to the main branch using a protractor to have a rudimentary measurement of branching pattern and plant shape.

Plant size

We included plant size as a trait group because it is known in crop *Brassica* (e.g. Mendham and Scott 1975) that the size of the plant can associate with reproductive output and therefore affect fitness. We measured shoot height for each plant at the appearance of the first bud, and 6, 12, 18, and 30 days from first bud. At 30 d after appearance of the first bud, we counted the total number of basal and cauline leaves per plant. To measure aboveground biomass, each plant was excised from the roots at 30 days after appearance of the first bud. The samples were then placed in a paper bag, cut into smaller sections, stored at room temperature (~25˚C) for 1 month or longer, and then dried for 48 hours in a desiccator oven at 65˚C before weighing. Mass of leaves removed to calculate leaf mass/area was added to these measurements.

Reproduction

For native, invasive, and landrace populations, the number of reproductive parts in *B. tournefortii* produced can determine the survival success, propagule pressure, or yield of a population (e.g. Trader et al. 2006). In preliminary analyses from pilot studies, we have determined that the amount of buds or flowers are associated with the number of fruits produced by a plant, which is associated with seed production in this species. To measure variation in reproductive traits, we counted the total number of flower buds and total number of flowers at 6 and 12 days after the emergence of the first bud for each plant.

Relative fitness

We chose total number of fruits at 18 days after budding as a fitness component and as a proxy for relative fitness. We understand that fruit production does not entirely represent relative fitness. However, fruit production has been shown to contribute to successful establishment in this species (Trader et al. 2006; Bangle et al. 2008; Gorecki et al. 2012; Abella et al. 2013). In landrace populations, increased number of fruits is commonly selected by breeders, especially for seed crops (Tester and Langridge 2010) And, in invasive populations in the southwestern United States, it has been hypothesized that high fruit number results in increased propagule pressure (Trader et al. 2006; Bangle et al. 2008). Although we have repeated measurements of fruit number, measurements earlier than 18 days after budding do not represent total fruiting output because flowers and buds are still present. Measurements at 18 days or later after budding, on the other hand, are taken when all viable flowers have produced fruits. In addition to being fully set with fruit and less pot constrained compared to plants at 30 d post budding, we chose total fruit number per plant at 18 days after budding because of its correlation with other traits that we identified in a pilot study. We determined relative fitness of each plant by identifying the sample plant with the most fruits for our entire study, then calculating the relative fitness of each plant as: fruit number of a plant/fruit number of the plant with the most fruits.

Statistical analysis

We narrowed the number of traits to analyze by using PCA via data matrix. First, we divided traits into five groups: phenology, leaf morphology, branch architecture, size, and reproduction. Using *prcomp* in R Studio (R Core Team 2018), we ran separate PCA procedures for each trait group, and then used the factor scores for the first or first and

second principal component for each group as new variables to have a manageable number of variables for the remaining analyses. The first principal components explained the following proportions of the variance in their trait groups: Phenology PC1, 50.9%, Leaf PC1, 30.7%, Branch PC1, 24.9%, Size PC1, 32.3%, and Reproduction PC1, 46.5%. For Phenology PC1, days to appearance of first bud (-0.70) and days to appearance of first flower (-0.69) were most heavily loaded and both negatively correlated relative with all phenology variables (Table 2). We included second principal components for leaf and branch traits, which explained the following proportions of variance in their trait groups: Leaf PC2, 22.0%, and Branch PC2, 15.0%. For the Leaf PC1, mean leaf length at 30d from first budding (0.46) had the highest loading along with leaf width (0.44, Table 2). For the Leaf PC2, the number of indentations per leaf (0.60) and indentation depth (0.55) had the highest loadings. Because Leaf PC2 differed among imaging devices, we obtained the residuals from a one-way ANOVA (Leaf PC2 = device) and used the residual values for all our analyses. Among all traits in Branch PC1, the number of branches 6d after appearance of first bud (0.55) has the highest loading. For Branch PC2, branch length at 18d after appearance of the first bud (-0.60) and branch angle (-0.45) had the highest loadings and are negatively correlated to all branch architecture variables. For Size PC1, the variable with the highest loading was plant height at 30d after first budding (-0.56), which is negatively correlated with all other size variables. For the Reproduction PC1, the variable with the highest loading (0.59) was total flower number at 12 after first bud.

We performed mixed-model ANOVAs in SAS 9.4 (SAS Institute, Cary, NC) with trait group principal components as dependent variables. The independent variables were range (native, invasive, and landrace) as a fixed effect, experimental population nested within

range as a fixed effect, and maternal family within population as a random effect. We analyzed relationships of phenology, leaf morphology, branch architecture, size, and reproduction principal components with relative fitness using two statistical approaches. We knew from preliminary analysis that some traits have non-linear fitness functions; therefore, our first step was to plot these fitness functions in each range. To avoid forcing regression lines into either linear or quadratic fits and to capture non-linear trends, we used a general additive model (*gam*) approach to smooth regression lines. In particular, we used the *gam* function in the *mgcv* (Wood 2000) and *ggplot2* (Wickham 2016) packages in R. Second, we performed separate type III ANCOVAs via *glm* in R Studio for each trait suite. Based on our *gam* regression lines and preliminary model selection procedures in a pilot study, we used a general model form to test directional and non-linear selection for all trait suites: relative fitness (w) was the response variable, range was the categorical variable, and the linear (β) and quadratic (γ) terms for all composite traits were covariates. We also included the interaction of the covariates with range in our models. While *gam* results test smoothing parameters for predictor variables, they do not include parameter estimates that are relevant for interpreting phenotypic selection. So, we used the ANCOVA results to interpret the regression lines of fitness functions; that is, we used the sign and value of estimates of regression coefficients to indicate the type, direction, and magnitude of phenotypic selection. Specifically, a significant β is interpreted as directional selection, a significant negative curve ($-\gamma$) is interpreted as stabilizing selection, and a significant positive ($+\gamma$) is associated with disruptive selection (Lande 1991; Conner and Hartl 2004).

We used aridity index to determine how desert climate can affect population means and selection strength (Trabucco and Zomer 2019). While climate variables such as BioClim

can be used for our analysis (Hijmans et al. 2005), aridity index is derived from both temperature and moisture, as well as potential evapotranspiration. For desert habitats, this can be more biologically meaningful in terms of fitness of plants. After obtaining the aridity index for all sites, we ran ANCOVA tests using the model form trait group PC = aridity index + range + aridity × range, to test presence and/or changes in clinal trends to detect possible signals of rapid evolution. We used the *ggplot2* package in R Studio to plot models to graphically assess potential clinal trends.

Lastly, we asked whether the strength and direction of selection changed among populations along environmental gradients. We used the same approached proposed by Wade and Kalisz (1990), in that we regressed a climate variable (aridity index) versus linear and quadratic population selection gradients. However, instead of examining variation in selection strength in a habitat, as performed by Steward and Schoen (1987), we extended this approach to the scale of range-wide climatic gradients. To determine differences in selection intensity along climate gradients, we split the dataset by populations, so we could calculate both linear and quadratic slope estimates for each individual population. We were generally interested in how magnitude and direction change across environments, so we did not obtain absolute values of the selection gradients. We then performed ANCOVA (type III SS) to test if slopes of the environment PC versus selection strength (i.e. population selection gradient) for focal trait PCs were different between ranges and used *ggplot2* in R Studio to delineate these patterns.

Results

Variation of trait PCs and relative fitness

The onset of reproduction was earliest (Phenology PC1) in the landrace populations, intermediate in the invasive populations and latest in the native populations. These differences are statistically significant (Table 3, Figure 4a). Leaf PC1, strongly loaded for leaf size, was largest in the landrace populations and smallest in the native populations. These differences are statistically significant (Table 3, Figure 4b). For Leaf PC2, which was highly loaded for number and depth of leaf indentations, the landrace range had the most serrated leaves, but ranges did not differ significantly in indentations (Table 3, Figure 4c). Branch PC1, which was most strongly influenced by number of branches, did not vary significantly among ranges (Table 3, Figure 4d). In contrast, means of Branch PC2 (negatively loaded for lateral branch length and angle) were significantly different among ranges with the native and invasive populations having longer, wider-angled branches than the landraces (Table 3, Figure 4e). While the native range did not differ in mean Size PC1 (plant height) from the invasive populations, the landrace ranges had significantly shorter plants than native and invasive ranges (Table 3, Figure 4f). Landrace and invasive ranges produced more flowers (Reproduction PC1) compared to the native range; this difference approached significance (Figure 4g). Mean relative fitness was highest in the landrace range, intermediate in the invasive range, and lowest in the native range (Figure 4h). These differences were statistically significant (Table 2).

Between-range differences in fitness functions

In the following analyses we were interested in effects of the trait PC values on fitness (a significant trait effect is an overall linear effect and a significant trait^2 effect is an overall quadratic effect) and whether these fitness functions differed among ranges. A significant trait by range effect indicates that the slope of the fitness function differed among ranges and

a significant trait^2 by range effect indicates that the shape of the fitness function differed among ranges. While there were a number of trait, trait^2, and trait-by-range effects, we did not observe significant effects of trait ^{2}-by-range on relative fitness (Table 4). This result would suggest that the fitness functions are similar in shape across ranges; however, when we plotted fitness functions for each range, we observed non-linear trends in most traits (Figure 5). Among the non-linear regression lines, six are from the native range and four of these plots show evidence of stabilizing selection (Figures 5a to 5d). The invasive and landrace ranges, on the other hand, show mostly directional selection. However, in the landrace range relative fitness increases with shorter reproductive periods (Figure 5a), and in the invasive range Leaf PC2 has maximum fitness in extreme leaf margin phenotypes.

Clinal patterns of population means and strength of selection

Of all composite traits that showed genetic bases for variation and relationship with fitness, Phenology PC1 (reproductive phenology) and Leaf PC2 (highly loaded for leaf indentation depth and leaf mass per area) also had statistically significant relationships with aridity index when clinal trends of the two traits were analyzed at the population level. When Phenology PC1 in populations was compared along aridity gradients among the three ranges, the invasive and landrace populations showed no change in timing of reproduction, as indicated by flat trendlines (Figure 6). In contrast, the three native populations had shorter times to reproduction with lower aridity, showing a steep increasing cline (Figure 6). The strength of directional selection, measured as the linear slope of trait value versus relative fitness in each population, varied for phenology. Specifically, selection for shorter reproductive periods increased with increasing humidity for the native, invasive, and landrace populations (Figure

7a), but patterns did not statistically vary among ranges, even though the native and landrace populations had steeper trends than the invasive populations.

While phenotypic means of Leaf PC2 (highly loaded for leaf indentation depth and leaf mass per area) did not have statistically important associations to aridity index, nonlinear selection in populations changed with increasing humidity in some ranges, as indicated by increasing lines (Figure 7b). Both native and invasive populations showed stronger selection for extreme phenotypes with increasing humidity. Landrace populations had an almost flat trendline with an intercept below zero, indicating that nonlinear selection in these populations is weak regardless of the amount of aridity.

Discussion

Associations of phenotypic variation with environmental conditions are commonly observed in plant populations. But, different variability of climate can alter timing of environmental cues that dictate resource availability for plants (i.e. water). Sometimes, this can stimulate evolution of new patterns of phenotypic differentiation and selection (Franks et al. 2007; Nicotra et al. 2010), as we have observed in our comparison of phenotypic means and fitness functions between native, invasive, and landrace populations of *Brassica tournefortii*. While the type of among-range phenotypic differentiation seen in our study system has been attributed to rapid evolution in invasive and landrace plants in other species (Buswell et al. 2011; Colautti and Lau 2015) the reasons why a certain feature will have higher or lower phenotypic means at a certain region are complex. Comparing range means is suggestive, but not conclusive. By including fitness functions in our analyses, we were able to test whether the variability and differentiation in traits among ranges are likely to be associated with fitness. While we expected some fitness functions to differ among ranges, we did not

have specific predictions for each trait for each range**.** The native fitness functions, which showed non-linear patterns, fit Endler's (1986) prediction that phenotypic variation in demes that underwent extended periods of local adaptation will stabilize to intermediate phenotypes, except perhaps reproductive traits. In contrast, invasive and landrace ranges had fitness functions that are mostly directional, which we interpret as indicating rapid evolution.

Our pooled analyses allowed us to describe a snapshot of phenotypic evolutionary potential in entire ranges in terms of composite trait means and fitness functions. We were also able to identify that phenotypic means and selection strength of composites of leaf margin and phenology traits can vary across each range as a response to a critical limiting factor, aridity, in our study area**.** While the three ranges are all hot environments, they vary in vegetation types, topography, and aridity (Laity 2008). Further, the contemporary evolutionary histories are different for the native, invasive, and landrace populations we included in our study. Based on our findings, we assert that the native populations in Israel, Morocco, and Spain have adapted to Mediterranean ecosystems, possibly through millennia, while the younger populations in the southwestern United States have been recently established in mostly roadsides and washes. It is worth noting that the invasive populations we studied are experiencing frequent boom-and-bust cycles due to the highly variable precipitation in this region, which can contribute to genetic differentiation (Li et al. 2015). The clinal patterns we determined indicate that aridity is a likely agent of selection for *B. tournefortii*, which means it may have affected genetic differentiation among populations and among ranges. Thus, we expected to find patterns suggesting adaptive or maladaptive phenotypic differentiation for ecologically important traits, as Winkler et al. 2018 have identified in other populations of *B. tournefortii*. This was true for one composite trait,

Phenology PC1, which had a defined cline for the native populations, but relatively neutral or flat clines for invasive and landrace ranges. While the neutral patterns for the invasive and landrace ranges do not indicate genetic or phenotypic differentiation, mechanisms such as phenotypic plasticity can produce consistent phenotypes, such as in reproductive phenology (Richards et al. 2006).

We found trait means and fitness functions that varied among ranges, but these traits did not show any clinal signal that aridity was critical to their survival. While including other climate features in our study may seem to be a prudent approach, we were not confident that the number and locations of source populations in our study represented the full spectrum of variability required for a three-way analysis. We acknowledge that this study would have stronger implications if the number of populations had been balanced among ranges. We are also aware that the accessions we used were collected in different years, which could have confounded our estimates of selection strength even though seeds used for our study were produced in a common greenhouse. Nonetheless, our common greenhouse experiment shows that even with limited numbers of populations, significant shifts in clinal patterns of trait means and selection gradients between ranges can be detected.

If presence of a cline between a trait mean and an environmental variable is considered a signal of local adaptation, then differences between native, invasive, and landrace clines indicate rapid adaptation to novel environments (Colautti and Barrett 2013; Colautti and Lau 2015). If we examined just regression lines of aridity versus composite trait means, then we would have concluded that invasive and landrace populations both have weak or no signal for local adaptation for reproductive phenology and leaf margin morphology, with respect to aridity index. However, patterns of selection strength across

native, invasive, and landrace aridity gradients tell a different story. We highlight phenology for the rest of our discussion, as it showed signals of adaptive variation among range means, fitness functions, and among clines of population means and population selection gradients.

In some cases, episodes of rapid adaptation occur due to changes in genetic composition driven by a combination of long-distance dispersal events and altered gene flow (Dlugosch and Hays 2008; Colautti and Lau 2015). In *B. tournefortii*, possible bottleneck effects in invasive populations and the intentional selection of maternal phenotypes in the landrace populations may have led to neutral patterns for mean phenology (Figure 6). Neutral patterns of mean time to reproduction in the invasive and landrace ranges suggest a type of plasticity in which different genotypes express the same phenotype in different environments (Richards et al. 2006). In the invasive range, where climate varies dramatically, consistent phenology gives an edge against endemic plants if *B. tournefortii* can reproduce consistently earlier (Marushia et al. 2012).

Traditional agricultural practices in the landrace range of *B. tournefortii* appear to have led to consistent reproductive phenology even with highly variable aridity. That is, the three landrace accessions we studied showed stability. The clines we delineated for landraces suggest that growers may have artificially selected for the most productive plants with the shortest growth periods, which can allow efficient and consistent harvest. As a result, a shorter mean growth period before reproduction may have evolved in landrace *B. tournefortii* allowing plants to rapidly allocate resources to seeds with limited water.

In competition experiments, invasive *B. tournefortii* outcompeted other non-native *Brassicaceae* with its rapid seedling and reproductive phenology (Marushia et al. 2010; Marushia et al. 2012). Based on our findings, invasive mustard is rapidly evolving faster

mean growth periods until reproduction, but the trend is not as strong as native and landrace ranges (Figure 7a). Perhaps plants with fastest phenotypes are the ones that can form monocultures that fill vacant niches in the southwestern deserts of North America (Li et al. 2015). With potentially high intraspecific competition, however, there is the possibility of a fitness cost from a correlated trait that drives negative selection for rapid growth and reproduction (Bossdorf et al. 2004).

Conclusions

The ability to establish in extreme arid habitats makes *B. tournefortii* formidable to control because of diverse niches it can occupy. Although the results are complex, some traits have rapidly diverged among ranges and among populations. Rapid adaptation of phenology to varying degrees of aridity may have resulted in plants that are more suited to their new environments, which is a plausible hypothesis for the spread of *B. tournefortii* in the southwestern United States in less than a century. On the other hand, breeding programs for *Brassica* seed crops should aim to achieve stable phenology to have plants that can withstand the rapid changes in local and global climates.

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Table 1. Source population locations and climatic conditions.

Locality	Range	Latitude	Longitude	Altitude (m)	Total annual precipitation (mm)	Mean annual temperature (°C)	Aridity index
Coachella Valley East (COA)	Invasive	33.65	-116.66	1352	508	13	0.24
Elgin Road, NV (ELG)	Invasive	36.73	-114.43	620	437	14	0.15
Lake Mead, NV (MEA)	Invasive	35.20	-114.57	200	161	19	0.05
North Indian Canyon Rd. (NWI)	Invasive	34.00	-116.57	528	212	19	0.08
Santa Cruz River (SCR)	Invasive	32.40	-111.14	628	316	21	0.12
U.C. Riverside (UCR)	Invasive	33.98	-117.30	491	371	17	0.17
Fateh Jang, Pakistan (FAT)	Landrace	33.57	72.60	507	635	22	0.36
Sammundri, Pakistan (SAM)	Landrace	31.06	72.94	174	367	25	0.18
Uttar Pradesh, India (UTP)	Landrace	26.85	80.91	124	1011	26	0.5
Almeria, Spain (NAJ)	Native	36.96	-2.20	440	139	19	0.08
Madrid, Spain (MAD)	Native	40.40	-3.68	602	98	22	0.06
Palmachim, Israel (PAL)	Native	31.93	34.70	21	209	18	0.11
Tiznit, Morocco (MOR)	Native	29.71	-9.71	211	279	17	0.14

Table 2. Trait groups with their life history characters and principal components loadings. The most strongly loaded trait for each principal component axis used for analyses are indicated in bold.

Composite trait group	Reasons for trait selection	Individual traits	PC1 loading	PC2 loading
Phenology	Early and rapid phenology confers advantage in desert invasive populations (Marushia et al. 2010, Marushia 2012). Phenology determines sowing and harvest time, and yield in *Brassica* crops (Wang et al. 2012, Kirkegaard et al. 2016)	days to appearance of first bud	-0.7	-0.01
		days to appearance of first flower	**-0.69**	-0.06
		senescent leaf: young leaf	0.08	0.78
		days from first bud to first flower	0.17	-0.63
Leaf Traits	Leaf traits are associated with fitness in desert annuals (Angert et al. 2010). Leaf size and leaf margin traits are associated with leaf thermoregulation, especially in hot desert habitats (reviewed in Nicotra et al. 2011 and Wright et al. 2017). Leaf size in seed crops, including *Brassica*, is correlated with yield (e.g. Mendham and Scott 1975).	leaf length mean-6 d from first bud	-0.46	0.03
		leaf length mean-12 d from first bud	-0.41	0.05
		leaf mass per area	-0.14	**0.36**
		number of indentations	-0.14	0.60
		number of lobes	-0.05	0.40
		indentation depth	-0.04	**0.55**
		indentation width	0.25	0.17
		lobe width	0.34	0.05
		leaf width	**0.44**	0.12
		leaf length mean-30 d from first bud	**0.46**	0.03
Branch Architecture	The number of branches, length of branches, and branch angle contributes to shape of *Brassica* (Cai et al. 2006), which can allow a whole *B. tournefortii* plant to disperse seeds by moving as a tumbleweed (Alfaro pers. observation). These traits were identified to affect plant movement (whole plant dispersal) in other tumbleweeds in western United States (Baker 2007; Borger et al. 2007). The number of branches determines yield in *Brassica* seed crop species, branch length associated with inflorescence length in *Brassica* species (Cai et al. 2016).	number of branches-6d from first bud	**0.55**	0.24
		number of branches -18d from first bud	**0.53**	0.10
		number of branches -12d from first bud	**0.46**	-0.07
		branch length mean-12d from first bud	0.40	-0.33
		secondary branch thickness-12 d from first bud	0.19	**-0.43**
		secondary branch thickness-18 d from first bud	0.10	0.25
		mean primary branch angle	-0.03	**-0.45**
		branch length mean-18d from first bud	-0.03	-0.60
Size	Increased size is associated with invasiveness in invasive plant species (Willis et al. 2002). The size of a *Brassica* plant can be used to determine yield in crop species (Cai et al. 2016).	height-30d from first bud	**-0.56**	-0.09
		height-18d at first bud	**-0.52**	-0.21
		above ground dry biomass	**-0.49**	0.03
		total number of leaves	-0.26	0.23
		height-12d at first bud	-0.26	-0.27
		height-6d at first bud	0.11	-0.65
		height at first bud	0.13	-0.64
Reproduction	Related to fitness traits; can be considered as a fitness component; associated with propagule pressure and yield	total flower count-12d from first bud	**0.59**	-0.38
		total bud count-12d from first bud	**0.58**	-0.41
		total bud count-6d from first bud	0.44	0.53
		total flower count-6d from first bud	0.36	0.64

Table 3. Mixed-effects ANOVA results for principal components of phenology, leaf, branch architecture, size, and reproduction traits. Relative fitness was also included (n = 266).

Trait group	Source	*df*	F^{P}	R^2
Phenology PC1 (number of days to first bud)	Range	2	35.79****	
	Population within range	4	17.75****	0.90
	Maternal family	44	3.14****	
Leaf PC1 (mean leaf length)	Range	2	5.43**	
	Population within range	4	12.46****	0.58
	Maternal family	44	2.27****	
Leaf PC2 residuals (number of leaf indentations)	Range	2	0.28	
	Population within range	4	0.51	0.32
	Maternal family	44	1.15	
Branch PC1 (total number of branches per plant)	Range	2	1.71	
	Population within range	4	5.10***	0.48
	Maternal family	44	1.88**	
Branch PC2 (lateral branch length)	Range	2	3.36*	
	Population within range	4	2.43*	0.50
	Maternal family	44	1.22	
Size PC1 (plant height at 30d after first bud)	Range	2	3.47*	
	Population within range	4	34.94****	0.66
	Maternal family	44	1.61*	
Reproduction PC1 (total numbers of flowers per plant)	Range	2	2.58†	
	Population within range	4	4.13**	0.43
	Maternal family	44	1.97***	
Relative fitness, w	Range	2	6.13**	
	Population within range	4	0.73	0.42
	Maternal family	44	1.88**	

$p \leq 0.1^{\dagger}$, $p \leq 0.05^{*}$, $p \leq 0.01^{**}$, $p \leq 0.001^{***}$, $p \leq 0.0001^{****}$

Table 4. ANCOVAs of fitness functions among native, invasive, and landrace ranges (n = 266). The independent variables are range, linear and quadratic terms for composite trait variables (covariates), and the interactions of range with the trait covariates. The dependent variable is relative fitness, calculated as sample number of fruits/maximum number of fruits. Adjusted R^2 values are included.

Composite trait variables	Source	*df*	F^P	R^2
Phenology PC1 (number of days to first bud)	Trait	1	4.69*	0.12
	Trait^2	1	3.17†	
	Range	2	7.42***	
	Trait × Range	2	4.30*	
	Trait^2× Range	2	0.05	
Leaf PC1 (mean leaf length)	Trait	1	0.70	0.24
	Trait^2	1	9.27**	
	Range	2	4.01*	
	Trait × Range	2	7.64***	
	Trait^2× Range	2	0.53	
Leaf PC2 residuals (number of leaf indentations)	Trait	1	0.0089	0.14
	Trait^2	1	2.86†	
	Range	2	13.08***	
	Trait × Range	2	0.03	
	Trait^2× Range	2	0.79	
Branch PC1 (total number of branches per plant)	Trait	1	15.09***	0.34
	Trait^2	1	2.41	
	Range	2	7.02**	
	Trait × Range	2	0.95	
	Trait^2× Range	2	0.91	
Branch PC2 (lateral branch length)	Trait	1	1.90	0.15
	Trait^2	1	2.81†	
	Range	2	8.30****	
	Trait × Range	2	0.99	
	Trait^2× Range	2	2.14	
Size PC1 (plant height at 30d after first bud)	Trait	1	4.49*	0.25
	Trait^2	1	10.58**	
	Range	2	10.71***	
	Trait × Range	2	4.00*	
	Trait^2× Range	2	1.80	
Reproduction PC1 (total numbers of flowers per plant)	Trait	1	6.32*	0.19
	Trait^2	1	0.40	
	Range	2	5.31**	
	Trait × Range	2	0.99	
	Trait^2× Range	2	0.10	

$p \leq 0.1^\dagger$, $p \leq 0.05^*$, $p \leq 0.01^{**}$, $p \leq 0.001^{***}$, $p \leq 0.0001^{****}$

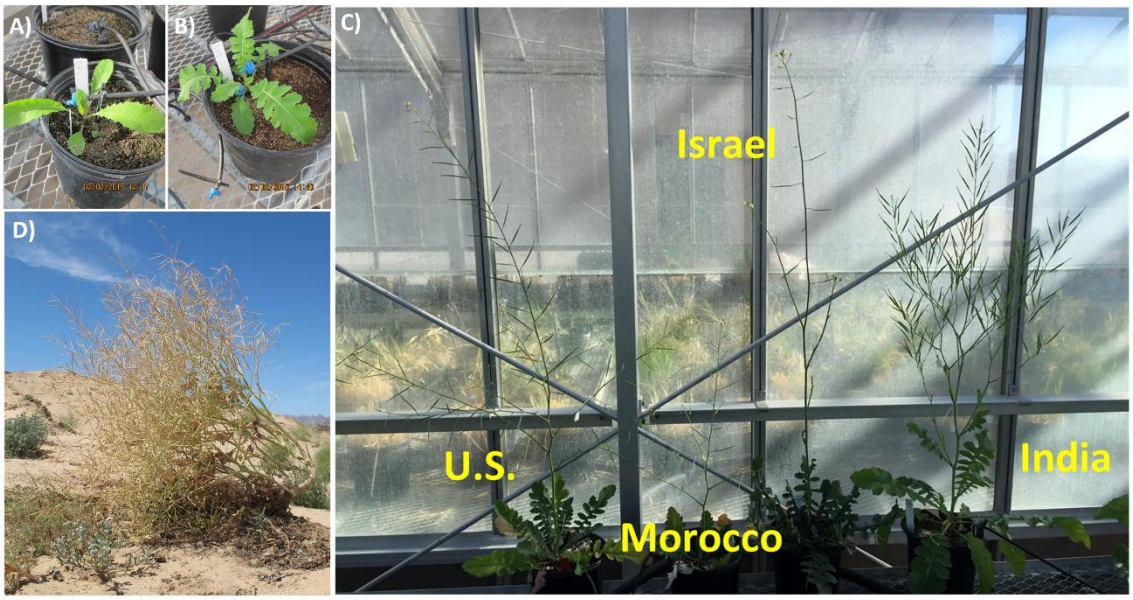

Figure 1. *Brassica tournefortii* seedlings/rosettes used as parental generation (a and b), showing variability in leaf margin morphology, c) bolting/mature seeding plants from common greenhouse study, and d) mature/senesced plant sampled for population genetic study in Mojave Desert, CA.

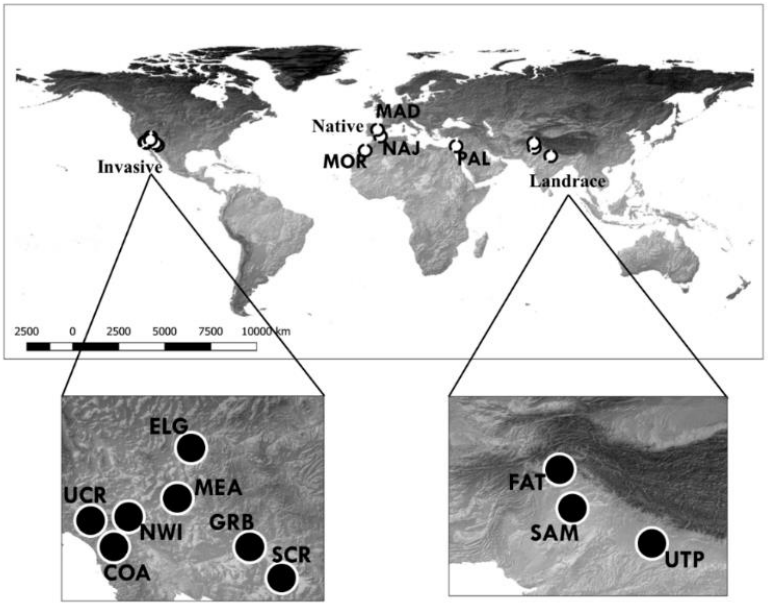

Figure 2. *Brassica tournefortii* sources used for experimental crosses. Invasive range: COA - east Coachella Valley (CA), NWI - North Indian Canyon Rd., (CA) UCR - University of California, Riverside (CA), SCR - Santa Cruz River (AZ), GRB - Gila River Basin (AZ), ELG - Elgin Rd. (NV), MEA - Lake Mead (NV). Native range: MOR - Tiznit, Morocco, MAD - Madrid, Spain, NAJ - Almeria, Spain, PAL - Palmachim, Israel. Landrace range: SAM - Sammundri, Pakistan, FAT - Fateh Jang, Pakistan, UTP - Uttar Pradesh, India.

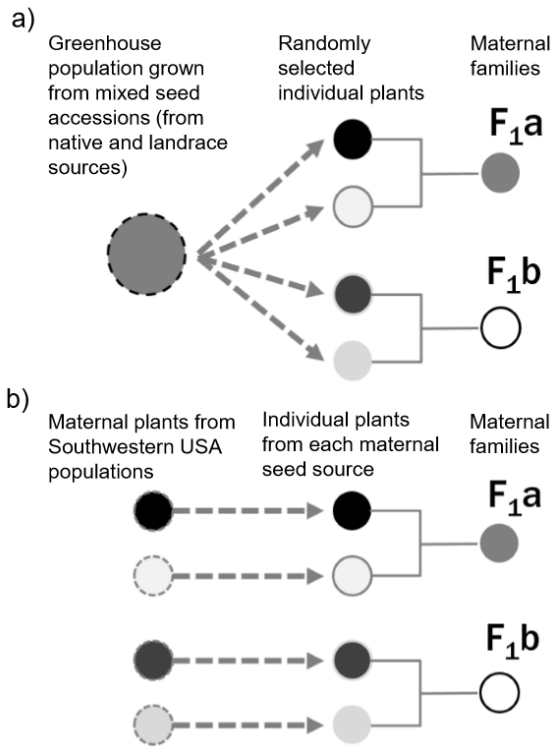

Figure 3. Diagrams of four hypothetical full-sib families to illustrate the types of crosses used to generate seed families for native (a), landrace (a), and invasive (b) populations of *B. tournefortii*.

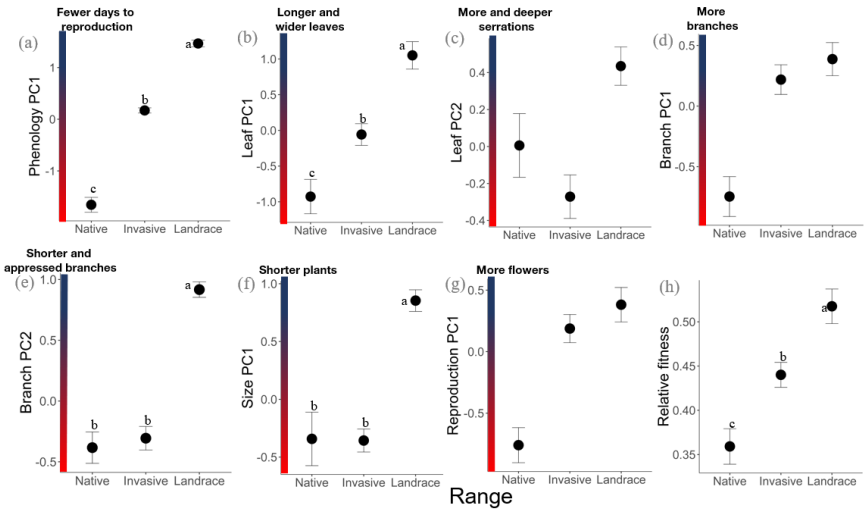

Figure 4. Range means of trait principal components (black circles) in native (*N*=77), landrace (*N*=72), and invasive (*N*=117) ranges: a) Phenology PC1 (days to first bud), b) Leaf PC1 (mean leaf length), c) Leaf PC2 residuals (number of indentations per leaf), d) Branch PC2 (lateral branch length), and e) Size PC1 (plant height at 30d after first bud). The range means of relative fitness are also shown (f). Means within figures that have different superscripts are significantly different in Tukey HSD comparisons.

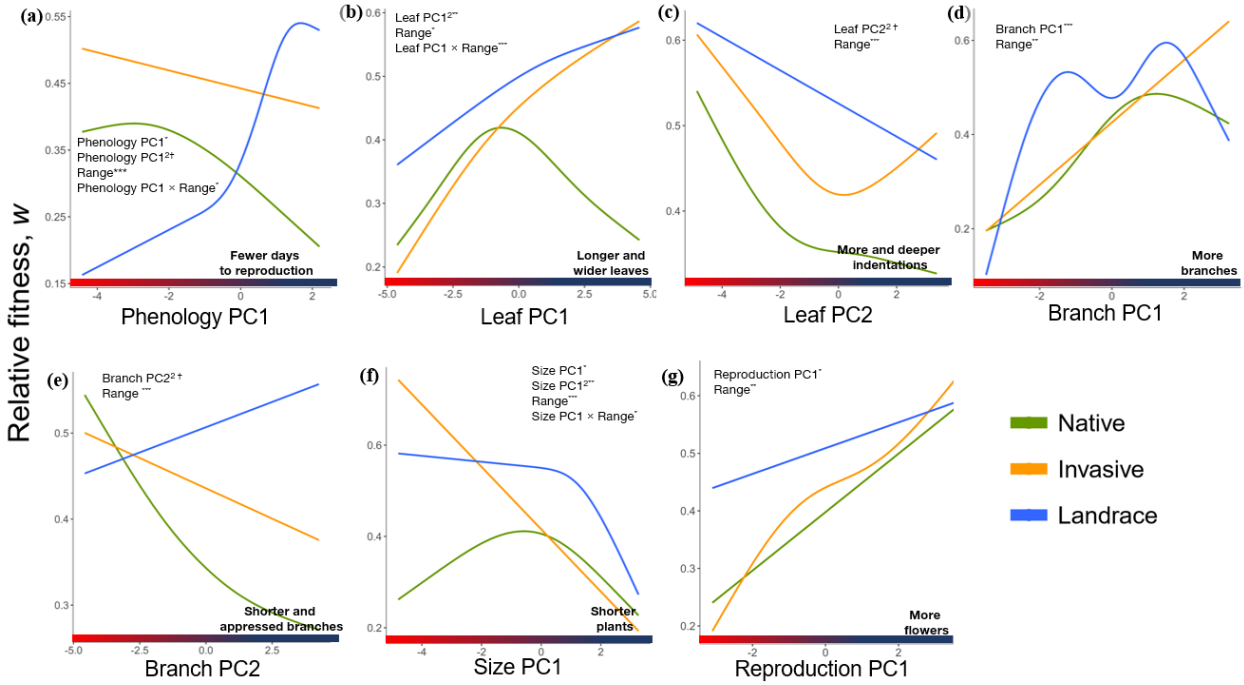

Figure 5 Plots of fitness functions for: a) Phenology PC1 (days to first bud), b) Leaf PC1 (mean leaf length), c) Leaf PC2 residuals (number of indentations per leaf), d) Branch PC1 (number of branches), e) Branch PC2 (lateral branch length), f) Size PC1 (height), and g) Reproduction PC1 (total number of flowers) in native (*N*=77), landrace (*N*=72), and invasive (*N*=117) ranges. The x-axes are values of composite trait groups (PCA scores), and the y-axes are relative fitness (*w*) values derived from maximum total number of fruits per plant. To detect unknown non-linear trends, generalized additive model (gam) function for regression line smoothing (k = 5 dimensions) was used within the *ggplot2* package in R Studio. Full model descriptions are in Table 4. $p \leq 0.1^{\dagger}$, $p \leq 0.05^{*}$, $p \leq 0.01^{**}$, $p \leq 0.001^{***}$, $p \leq 0.0001^{****}$.

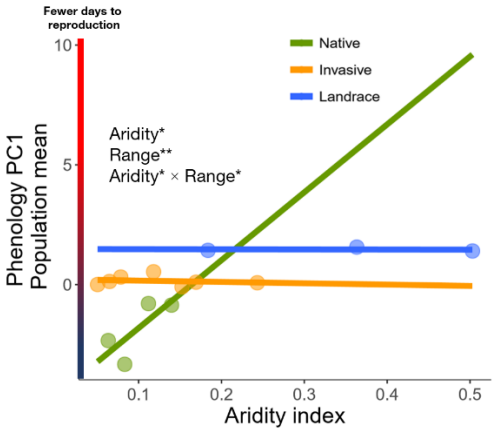

Figure 6. Regression lines of aridity index versus population means of Phenology PC1 (n = 14). Significant main and/or interaction effects from ANCOVA tests are show ($p \leq 0.1^{\dagger}$, $p \leq 0.05^{*}$, $p \leq 0.01^{**}$, $p \leq 0.001^{***}$, $p \leq 0.0001^{****}$).

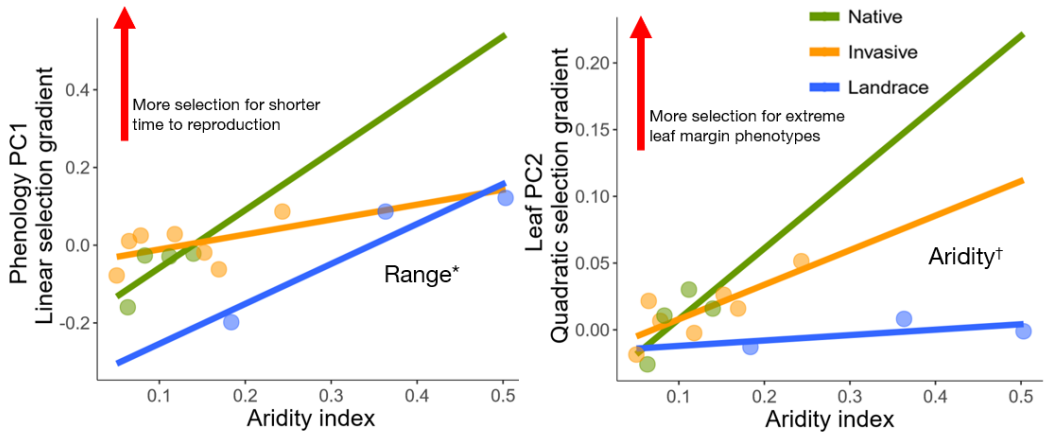

Figure 7. Regression lines of aridity index versus population selection gradients of Phenology PC1 and Leaf PC2 in the native, invasive, and landrace ranges (n = 14). Significant main and/or interaction effects from ANCOVA tests are shown ($p \leq 0.1^{\dagger}$, $p \leq 0.05^{*}$, $p \leq 0.01^{**}$, $p \leq 0.001^{***}$, $p \leq 0.0001^{****}$).

CHAPTER 2

Stable and opportunistic: phenotypic plasticity of composite life history traits in native, invasive, and landrace populations of *Brassica tournefortii*

(Formatted for submission to the American Journal of Botany as

Alfaro, B, and D.L. Marshall, 2020)

Abstract

Premise

The type of reaction norms produced by different environments is critical to understand how phenotypic plasticity can affect ecological breadth. Here, we examined how life history traits in native, invasive, and landrace populations of *Brassica tournefortii* respond to changes in soil moisture.

Methods

We determined in our experimental garden whether soil moisture amount is a source of adaptive phenotypic variation in life history traits of *B. tournefortii*, a desert mustard that is also a crop and invasive weed. We combined individual characters into leaf, branch, and reproductive composite traits to analyze reaction norms. We also analyzed population CVs of individual traits as indices for plasticity.

Key results

Reaction norms and effects of planting time show *B. tournefortii* can thrive in habitats that receive moderate to high moisture. When ample water was administered, native and invasive populations showed stability (asymptotic) in ecological traits (leaf), while landrace populations showed stability in fitness or agronomic traits (branching and reproductive). Alternatively, branching and reproduction were opportunistic (linear) across the moisture gradient in wild populations; landraces were opportunistic for leaf traits.

Conclusions

The presence of both stable and opportunistic reaction norms for native, invasive, and landrace populations illustrate that these populations can exploit favorable environments. Plant conservation and eradication programs should examine plastic responses to key environmental factors to see how much phenotypic variability target populations express. Crop breeding programs should scan for plasticity of target traits to provide growers with versatile crops that can be grown in rapidly changing environments.

INTRODUCTION

Populations with low genetic diversity can sometimes withstand environmental variation by increasing ecological breadth via phenotypic plasticity (Bradshaw 1965, Sultan 2000). Occasionally, the resulting phenotypic variation translates into differential fitness, making trait plasticity itself a potential target for selection in genetically depauperate populations (Schlichting 1986, Scheiner 1993, Sultan 2000). The pattern of variation, however, can depend on both natural and anthropogenic factors that shape adaptive landscapes. When populations of the same species have different evolutionary histories, this produces an array of populations that can be used to study the evolution of plasticity. For example, native, invasive and partially domesticated populations of a species provide an excellent opportunity to study the evolution of phenotypic plasticity.

In native habitats, local adaptation, genetic assimilation, and/or evolutionary reinforcement can remove trait plasticity from populations that remain in a stable environment (Sexton et al. 2002). However, new populations resulting from niche filling or range expansion may introduce previously locally adapted genotypes to new environments where they are less fit (Matzek 2012). Genotypes that can produce varying phenotypes in different environments may have an advantage at the edge of an expanding range, paving the way to gradual adaptive evolution of new trait means relative to the interior of the range. When a small, random sample of native genotypes migrates to a different biome, or cultivated in a homogenous area, an increase in phenotypic variation via plasticity can sometimes lead to rapid evolution.

Domesticated populations of plant species likely evolved under circumstances that produced a different pattern of plasticity than in native populations. Plasticity can be

harnessed in a variety of ways to improve crops (Kusmec et al. 2018). Breeders may breed for consistent yield from a diverse gene pool, selecting for plasticity that results in several genotypes having the same phenotypes (Connor et al. 2011). It is also possible for high yield to be associated with trait plasticity. In northern latitudes, high yield plasticity in spring wheat *(Triticum* spp.*),* oat *(Avena* spp.*),* and six-row barley (*Hordeum*) was related to increases in grain mass and increased amount of grains per square meter (Peltonen-Sainio et al. 2011). In various lines of sunflower (*Helianthus annuus*) and grapevine (*Vitus* spp.), high plasticity in reproductive phenology was associated with increased yield (Sadras et al. 2009). Domesticated plants have different histories compared to native and invasive plants, so associations of plasticity with plant performance can be different between plant types (Grossman and Rice 2012). Commercially bred crops typically experience multiple inbreeding events that can homogenize genetic variation. While landraces are also subject to artificial selection, it is generally weak in that there is still genetic variability that can allow adaptive evolution (Meyer and Purugganan 2013, Mercer and Perales 2010).

There are two common scenarios for the evolution of phenotypic plasticity in invasive plants. First, if the initial colony was comprised of individuals with moderate genetic variability for ecologically important traits, then trait plasticity likely will not become an adaptation. This is because the initial population can become locally adapted (Lande 2015). In this scenario, trait plasticity can still evolve as a neutral or non-adaptive trait that can result in phenotypic variation. Second, if the initial colony was comprised of individuals with limited genotypes, then it may benefit from high phenotypic plasticity. In this scenario, plasticity may evolve as an adaptation that is advantageous to the subsequent generations (Davidson et al. 2011).

Understanding how plasticity evolves in modified gene pools relative to original populations can allow prediction of crop success (Dong et al. 2008) and biological invasions in the face of global climate change (Nicotra et al. 2010, Valladares et al. 2012, Colautti and Lau 2015). To achieve this, the first steps are to experimentally identify 1. the types of traits that respond to selection on plasticity, 2. the pattern of plasticity itself, and 3. the effect of plasticity on fitness or yield in populations of different origins.

We used *Brassica tournefortii*, a desert plant that has discrete ranges of native, introduced, and landrace populations that have divergent life histories, to examine plasticity of life history traits. We conducted a common garden experiment, to compare patterns of phenotypic plasticity of the three types of populations of *B. tournefortii, a* xerophytic mustard native to North Africa and the Middle East. *B. tournefortii* is cultivated and bred as a seed crop in India and Pakistan (Rao et al. 1996), and invasive in western North America and Australia (Boutsalis et al. 1999, Schiermeier 2005, Chauan et al. 2006, Trader 2006, Bangle et al. 2008, Barrows et al. 2009). We know from a greenhouse experiment that *B. tournefortii* from these three range types vary in phenotypic means and adaptive landscapes for several life history traits (Alfaro and Marshall 2019). Analyzing plasticity will provide additional critical information on how different range types of this species respond to varying levels of a limiting factor. We determined in another experiment that precipitation can be a factor in phenotypic selection on some traits in *B. tournefortii*, so we chose soil moisture as a treatment to investigate plastic responses.

For *B. tournefortii*, leaf size, plant size, branch number, and branch length may be very important traits for the spread of invasive populations in this species in the southwestern United States (Li et al 2015, Winkler et al 2018). In our previous paper, we identified

evolutionary signatures demonstrating that leaf traits, branching architecture, size, and reproductive traits are important to the success of this species (Alfaro and Marshall, 2019).

Our goals for this experiment were two-fold. First, we asked whether native, invasive, and landrace populations of *B. tournefortii* showed different patterns and amounts of plastic responses to variation in soil moisture. We already know that different histories and environmental conditions of native, invasive, and landrace ranges of *B. tournefortii* result in variable phenotypes (Winkler et al. 2018, Alfaro and Marshall 2019). For native *B. tournefortii*, phenotypic variability is more likely due to local adaptation than plasticity because it has existed in its habitats for millennia. For young invasive populations that lack genotypic diversity, such as in *B. tournefortii* in southwestern U.S., phenotypic variability is likely due to phenotypic plasticity. Landraces, on the other hand, may have been selected for increased stability for more consistent harvest and larger yields. Second, we evaluated potential relationships between the amount of plasticity and relative fitness for our entire panel of traits, and tested for differences in these associations among native, invasive, and landrace ranges.

We tested three hypotheses:

1. Because of selection for yield stability in domesticated populations, the amount of plasticity due to varying soil moisture in traits related to reproduction, leaf morphology, plant size, and branching architecture, will differ such that native and invasive populations will have linear reaction norms, while landrace populations will have flat or asymptotic reaction norms.
2. Because there are likely different levels of genetic diversity among the population types leading to different fitness consequences of plasticity, a) the amount of

plasticity due to varying soil moisture in traits related to reproduction, leaf morphology, plant size, and branching architecture, would differ such that invasive > native > landrace populations.

3. Because of likely lower genetic diversity such that response to environmental variation required plasticity, we expect fitness to increase in value with increased plasticity due to varying soil moisture in branching architecture and leaf traits in the invasive populations more than the native and crop populations.

METHODS

***Seed sources*—**The source populations of the seed families we used in our garden experiment originated from native, invasive, and agricultural ranges of *B. tournefortii* (Fig.1). First, we planted original field seed collections and USDA seed accessions to create artificial greenhouse populations. The seed accessions from the native populations were collected from Tiznit, (Morocco), Madrid (Spain), Almeria (Spain), and Palmachim (Israel). The seed accessions from the agricultural populations were from Sammundri (Pakistan), Fateh Jang (Pakistan), and Uttar Pradesh (India). Seeds from the invasive populations were field collected in the southwestern United States from Coachella Valley (CA), North Indian Canyon Rd., (CA), near University of California, Riverside (CA), Santa Cruz River (AZ), Gila River Basin (AZ), near Elgin Road (NV), and Lake Mead (NV). We sampled seeds from these collections and grew a parental generation in a common environment. In March of 2015, F_1 seeds from artificial crosses (Fig. 2) from the parental generation were grown in a common greenhouse (Alfaro and Marshall 2019). Based on bud and flower dissections, we know that pollen shedding occurs before anthesis in *B. tournefortii*, so the likelihood of

cross-fertilization was low in the greenhouse where there were no insect pollinators. We allowed the F_1 plants in Alfaro and Marshall's (2019) common greenhouse study to self-fertilize to produce F_2 seeds, which we used as seed families for this experiment.

***Experimental design*—**To germinate the seeds, we used plastic 100 x 15 mm Petri dishes lined with Whatman™ filter paper, which we soaked with 2.5 ml of 5% gibberellic acid solution (to ensure germination) before adding seeds. After 24 hours, we planted seedlings that had developed both radicles and cotyledons in 3.78 liter pots containing a 1:1 mix of sand and Metro Mix ® (SunGro Horticulture ®, Canada). Some seed families were not viable or had longer germination times even with hormone treatment. When an F_2 seed family did not germinate enough replicate seedlings, we planted F_2 seeds from a different plant from the F_1 family. Due to late-germinating or inviable seeds, planting times varied from 1 to 40 days from the start of the experiment. To correct for planting time variation, we included time of planting as a variable.

To include all 14 populations from the native, invasive, and landrace ranges that we used in our previous study, we designed our experiment with 14 populations × 4 families/population × 3 watering levels × 3 blocks, $n = 504$ (1 plant/family/treatment/block). Using PROC PLAN in SAS 9.3 (SAS Institute Inc., Cary, NC), we used a stratified random arrangement of the pots and their treatments so each of the three blocks had one replicate of each population × family × watering level treatment. We set up the garden experiment at a fenced vacant lot in Albuquerque, New Mexico where a water source was available. We let the seedlings grow in standardized conditions until the first two leaves matured. We hand-watered the seedlings with 200 ml of tap water per day (100 ml at 7:00 am and 100 ml at 5:00 pm) and fertilized them using Peters® 20-20-20 General Purpose fertilizer (The Scotts

Company, Marysville, Ohio, USA) once a week until the end of the experiment. In addition to the standard irrigation, after the first two weeks we administered three watering level treatments at 7:00 am daily we added more water by hand-watering pots using plastic beakers:

low –250 ml

medium –450 ml

high –750 ml

In mid-July, the ambient daily temperature and insolation had resulted in some mortality, so to prevent further mortality we placed a 4-meter-high shade constructed from sheet metal that reduced the amount of sunlight. When plants experienced wilting, we administered an extra 150 ml of tap water at the time we noticed plant stress to all plants regardless of treatment.

Trait measurements—

Phenology—We measured days from seedling emergence to appearance of first flower as an index of phenology because it could be tracked accurately and consistently. Previous experiments showed that this variable is a good representative of overall phenology (Alfaro and Marshall 2019).

Plant size—We counted the number of leaves at the time of appearance of the first flower, and measured rosette size at 30 d after first flowering by selecting the two largest leaves that were diametrically opposite and measured the length between the tips of the two leaves to the nearest 0.1 cm. We measured plant height at 30 d after first flowering to the nearest 0.1 cm using a meter stick. We also weighed the aboveground biomass for each plant. First, we excised the entire aboveground portion of the plant at the base under the rosette, and

then placed each plant in a weighed paper bag and dried it in an oven for one week at 65°C before weighing it to the nearest 0.1 g.

Branch architecture—Anecdotal field reports point to tumbleweed dispersal, anemogeochory, as the main seed dispersal mode of *B. tournefortii* in its invasive ranges. This dispersal mode is affected by the architecture of branches (Baker 2007, Borger et al. 2007), so we included branch length and number of branches as trait variables. At 30 days from the appearance of the first flower, we measured branch length for each plant by selecting the first lateral branch closest to the apical branch and measuring its length from the base to the tip with a metric ruler. To determine the number of branches for each plant, we counted the total number of branch tips per plant.

Leaf traits—

Leaf size—At 30 days from first flower, we sampled two basal leaves from each plant. We removed each leaf by excising its petiole from the basal stem region. We know that leaves that emerge during bolting are reduced in size, so we haphazardly sampled two basal leaves between leaf four and leaf ten. We measured leaf length to the nearest 0.1 cm from the base of the petiole to the tip of the leaf blade using a metric ruler. We measured leaf width using a metric ruler to the nearest 0.1 cm at the lengthwise midpoint of the leaf blade. For each plant, we calculated the mean leaf length and mean leaf width from the two sampled leaves and used them as trait values.

Leaf margin morphology—We included number of lobes per leaf because of its association with water-related leaf stress and leaf metabolism. We measured the mean number of lobes per leaf per plant as an index of leaf margin morphology. For each plant, we counted the number of lobes for each sampled leaf and then obtained the average for both

leaves.

Reproductive traits—

Total number of fruits per plant—To measure fecundity of each plant, we counted the total number of viable fruits per plant at 30 d after appearance of first flower.

Mean seed number per fruit—As a second variable for fecundity, we also included mean seed number per fruit for each plant. We selected three mature fruits located at the basal regions of haphazardly selected inflorescence branches and then counted the total number of seeds in each individual fruit.

Individual fruit mass—to measure individual fruit mass, we used the three fruits used for seed counts. We weighed each fruit to the nearest 0.0001 g using a Mettler-Toledo AG135 digital balance (Ohio, USA). We took fruit masses for all three fruits per plant (including seeds) and used the mean as trait value for each sample to have a value for the mean mass of individual fruits, which we used to calculate reproductive biomass and other derived traits.

Derived traits—

Reproductive biomass—This variable provided an overall index of reproductive output and can be used to calculate reproductive allocation. We calculated reproductive biomass as the product of total number of fruits per plant and individual fruit mass.

Percent reproduction—As a measurement of reproductive allocation, we included percent reproduction in our analyses, which we calculated by dividing the reproductive biomass by total aboveground biomass. We performed arcsine-square root transformation for percent reproduction as: arcsine √ (percent reproduction/100).

Vegetative biomass—We included vegetative biomass to provide an index of vegetative allocation for our experiment. We calculated vegetative biomass by subtracting

reproductive biomass from total aboveground biomass.

Relative fitness—We used relative fitness as an index of performance of each individual plant relative to our entire study population. We calculated relative fitness by dividing each plant's total number of fruits, by the maximum total number of fruits that we recorded in this experiment. This is stated in the next paragraph.

Statistical analysis—Before performing statistical tests, we first asked whether each of the traits fit assumptions for analysis of variance and regression analyses. Except for relative fitness and percent reproduction (both were arc-sin square root transformed), we performed box-cox transformations in R Studio (*MASS* package) for all our trait variables to improve normality. We did not include traits that were used to calculate derived traits, such as total aboveground biomass, that will result in multicollinearity. To reduce the number of variables, we performed principal components analyses (PCA) for two trait groups, vegetative and reproductive, using the *prncomp* function in R on our data set. We ran these tests using built-in functions in R Studio (R Core Team 2018).

We tested Hypothesis 1 using both analysis of covariance (ANOVA) and reaction norm approaches. To test whether varying soil moisture levels affect different composite traits among ranges, and to compare the amount of plasticity in each trait between ranges, we ran ANCOVA tests for each composite trait. We did not use automated variable or model selection, but ran several models with different variable combinations, then removed variables that were not significant across composite traits to conserve degrees of freedom. Specifically, we used the form: Trait value = Planting Date + Range + Soil Moisture Level + Block + Population within Range + Block × Range + Range × Soil Moisture Level + Planting Date × Soil Moisture Level + Planting date × Range. We used PROC GLM in SAS

9.4 for all ANCOVA models and Tukey HSD tests for pairwise comparisons among means (SAS Institute, Inc., Cary, NC). To compare the patterns of the effect of range and trait means among soil moisture treatments, we plotted reaction norms using the *ggplot2* package (Wickham 2016) in R Studio (R Core Team 2018). We visualized the effect of planting date as a covariate to the composite traits by invoking the base generalized additive modeling method in *ggplot2*.

To test Hypotheses 2 and 3, we selected the two most highly loaded trait variables from each of our composite variables, then calculated the coefficient of variation (CV = standard deviation/mean ×100) for each individual trait. These traits were percent reproduction, total number of fruits, rosette diameter, leaf length, height, and lateral branch length. After calculating population trait CVs, we performed type III ANOVA tests via PROC GLM in SAS 9.4 on each trait with range as a categorical variable. To compare the relationship of mean population plasticity CVs versus relative fitness among ranges (Hypotheses 3), we performed ANCOVA tests via PROC GLM in SAS 9.4 using the model: mean arcsine √ relative fitness = Range + Population Trait CV + Range × Population Trait CV. We plotted regression lines using the *ggplot2* package in R Studio (R Core Team 2018).

RESULTS

Composite traits—After performing PCA, we narrowed down our trait variables to three composite variables, Reproductive Trait PC1, Vegetative Trait PC1, and Vegetative Trait PC2. All the highly loaded trait variables (individual fruit mass and reproductive biomass) in the Reproductive Trait PC1 were negatively correlated with the rest of the trait variables and explained 32% of the variability in this group of traits. The second reproductive

trait PC axis explained 25% of variability with reproductive biomass and mean seed number per fruit as the highest loaded traits. However, when we analyzed box plots for this variable, we observed that outliers were more than 20% of the data, even though we had stringent procedures for transforming the raw data. So, we did not include this composite variable for our subsequent analyses. For vegetative traits, the first PC axis explained 31% of variation and was highly loaded for basal rosette diameter, leaf length, number of leaves, and which were both negatively correlated with all vegetative trait variables. The second PC axis for vegetative traits explained 26% of variation and was highly loaded for height and lateral branch length, which were both negatively correlated to all other vegetative traits. The loadings for PC axes' variables are listed on Table 1.

Hypothesis 1—We predicted that because of selection for yield stability, landrace populations, would have flat or asymptotic reaction norms, while native and invasive populations would have linear reaction norms. Landrace populations of *B. tournefortii* had asymptotic reaction norms for two (reproduction and branching) out of three composite traits (Fig. 4). Further, we also show that native and invasive populations had strong linear reaction norms in two (reproductive and branching) out of three composite traits (Fig. 4). Reproductive Trait PC1, mostly mean fruit mass and total reproductive biomass, did not vary among ranges or planting dates (Fig. 3a, Table 2). Reproductive Trait PC1 increased from low to moderate soil moisture for plants from all three population types (Fig. 4a). However, the changes from moderate to high soil moisture amounts tended to be different among population types. Reproductive trait PC1 continued to increase in plants from the native and invasive range but showed no further increases in plants from landrace populations (Fig. 4a). While differences in mean response of Reproductive Trait PC1 among soil moisture

treatments were statistically different, there were no statistically significant effects of Range-by-Treatment interactions or Planting Date (Table 2). Block effects were statistically significant suggesting a plastic response to an unidentified environmental variable (Table 2).

Vegetative Trait PC1, highly loaded for rosette diameter, leaf length, number of leaves, and number of leaf lobes (negative correlations), was significantly larger in invasive and native populations than in the landraces (Fig. 3b, Table 2). Plants grown in moderate and high moisture levels had larger basal rosettes and longer leaves (Fig. 4b). These differences approach significance (Table 2). The native and invasive populations had similar patterns of response, in that Vegetative Trait PC1 was small in low soil moisture but increased in size from low to moderate soil moisture (Fig. 4b). Rosette size in native and invasive ranges did not change much from moderate to high soil moisture, while the pattern of response was more linear in landrace populations. These differences in pattern were not statistically significant (Table 2). For Vegetative Trait PC1 there were statistically significant effects of Planting Date interactions between Planting Date and Range, and Planting Date and Soil Moisture Treatment (Table 2).

Plants in the native populations were significantly taller and had longer branches (Vegetative Trait PC2) than in invasive and landrace populations (Fig. 3c, Table 2). Vegetative Trait PC2 (height and branch length) increased linearly in native and invasive ranges from low to high soil moisture (Fig. 4d). While the invasive populations had on average shorter height and branch length plants in low moisture compared to native and landrace populations, they had the largest increase in size from low to high moisture compared to native and landrace populations (Fig. 4d). The landrace range increased in height and branch length from low to moderate moisture but mean Vegetative Trait PC2 did

not change from moderate to high soil moisture (Fig. 4d). The differences in phenotypic responses to Soil Moisture Levels, Planting Date, and Range were statistically significant; but the difference in the pattern of response to Soil Moisture among Ranges was not. Planting Date × Range and Planting Date × Soil Moisture Levels were significant interaction effects for Vegetative Trait PC2 (Table 2).

Leaf traits (Vegetative Trait PC1) were at their highest values in invasive and landrace populations when planted at 20 d from the last frost date; the invasive populations, however, had a higher peak value than the landrace populations (Fig. 5b). When compared via ANCOVA, these differences were significant ($P < 0.001$). For branch traits (Vegetative Trait PC2), landrace populations increased in height and branch length as seeds were started later relative to the last average frost date. The native showed a parabolic pattern that had minimum branch value at 20d after the last frost date; the invasive populations showed a J-shaped curve in that branch trait values stayed low from the first planting to 22d after the last frost date, followed by a sharp increase. These differences were statistically significant ($P < 0.001$).

Reproductive Trait PC1 showed peak reproduction for plants that germinated at 25d from the last frost. The high water treatment was parabolic having minimum reproduction for plants started at 25 d from the last frost. The low water treatment showed a weak parabolic/curvilinear pattern that had lower minimum reproduction values relative to the high treatment. The increase in reproduction after the minimum values for the medium and high water levels are parallel and visually had similar slopes. For Vegetative Trait PC1 (leaf traits), the moderate water level treatment had a strong hump-shaped pattern with a maximum leaf trait value for plants started at 23d after the last frost. Leaf trait values

declined with later planting dates for plants in the low and high water treatments, but the high treatment had a strong linear response compared to the low treatment. Branch traits (Vegetative Trait PC2) increased in value as planting dates became later relative to the last frost in plants that received low water levels. Plants in medium and high water levels both showed parallel curvilinear (parabolic) trends for branch traits, with a sharp increase in branch trait values for individuals planted before and after 20d relative to the last frost.

Hypothesis 2—We predicted that the amount of trait plasticity due to varying soil moisture levels would differ such that invasive > native > landrace populations. Average per population coefficients of variation in highly loaded traits tended to vary among population types, but the patterns of variation were different for the various traits (Fig. 6).

Hypothesis 3—For Hypothesis 3, we tested the strength of the relationship between branching architecture and leaf trait plasticity and fitness among the invasive, native, and landrace populations. For the native and invasive ranges, relative fitness decreased with increasing plasticity of lateral branch length (Fig. 7). For the landrace range relative fitness increased when the population CV of lateral branch length increased (Fig. 7). The main effect of the ANCOVA test approached significance (P = 0.0674, df = 2, [add denominator df])

DISCUSSION

To have a thorough understanding of plasticity, it is sensible to design an experiment with an array of population types and of sources of variation that represents total coverage of the study area. We used plants that were sourced from the native, invasive, and landrace ranges of *Brassica tournefortii*, and created artificial populations to examine phenotypic variability.

We determined that different amounts of soil moisture translates into different phenotypes in composite life history traits. Here in the discussion, we touch on possible reasons for phenotypic variation resulting from varying planting time. We then explain likely reasons for the two types of reaction norms that we observed, stable (asymptotic) and opportunistic (linear), for native, invasive, and landrace populations.

Phenotypic variation of composite traits (Hypothesis 1)—

Reaction norms—Plant populations that have undergone partial or intensive domestication require artificial selection to homogenize genetic and phenotypic variation. Farmers typically select for seeds from maternal plants with consistently high yield and consistently desirable size, so the resulting seed bank populations for future crops are expected to contain individuals with consistent phenotypic expression, or stability, in good field conditions (Connor et al. 2011). Although phenotypic values increase from low to medium soil water levels, the composites of reproductive and branching traits for our experimental landrace populations of *Brassica tournefortii* did not change in phenotypic response from medium to high soil moisture. The strong linear response of the composite leaf trait suggests an opportunistic strategy to increasing resources. The crop literature points to several reasons for this trend of increasing leaf trait values versus increasing water resources. Typically drought detrimentally affects leaves in *Brassica* crops including decreases in chlorophyll content (*B.* juncea, Sahoo et al. 2015) and inability to osmoregulate (*B. napus*, Good and Maclagan, 1993), which can alter nutrient uptake (Sahoo et al. 2015). One common response in *B. napus* is reduction in leaf area and number of leaves in drought conditions (Qaderi et al. 2012). When more water becomes available, increase in leaf, overall plant size and yield is common in *Brassica*. Increase in leaf size as a response to high soil

moisture in particular has been experimentally tested in *B. carinata* (Husen et al. 2014). *B. tournefortii* may respond similarly to these oilseed rape species (i.e. linear leaf growth) given that they have ancestral populations that were originally cultivated in similar arid environments.

The patterns of plastic response of composite traits across water levels in the native and invasive ranges of *B. tournefortii* are essentially the opposite of landrace reaction norms. That is, where landraces show stability in composite variable for reproductive and branching traits, the native and invasive populations show linear reaction norms. And, where landraces show a strong linear response in the leaf composite trait, the native and invasive populations show signs of phenotypic stability (asymptotic reaction norms). We can interpret our results with respect to leaf economy (Wright et al. 2004). When water is scarce, the return on investment of water may have been low in native and invasive populations *B. tournefortii*, so seedlings underwent development with a conservative strategy that efficiently allocates water to development of smaller leaves and smaller rosettes to ensure enough water allocation to reproduction. But, in the moderate (450 ml/day) and high (750 ml/day) treatments, when water was abundant, the return on investment of water was greater, so native and invasive plants can afford leaf and rosette size to be consistently large (asymptotic). Theoretically, this allowed sufficient water allocation for linear growth and linear reproductive output in water-rich environments.

Given these reasons we outlined above, we can briefly summarize the reaction norms with respect to stability. When there was abundant water available, landrace populations showed stability in agronomic traits associated with yield (branching and reproductive composite traits). On the other hand, invasive and native populations showed stability in

ecologically and metabolically important traits (leaf composite trait), but were opportunistic (linear) for fitness traits (branching and reproduction).

Other sources of variation and the effect of planting time—While not a planned treatment for the experiment, implementation of the design resulted in variation in planting date and that variation was similar among population types. Therefore, we evaluated the effects of planting date and considered what these effects might mean in the context of evolution of phenotypic plasticity. Reproduction and growth of *B. tournefortii* depend on the time of seedling germination relative to the last frost date. In *Brassica* species, the initial conditions during the early seedling stage can determine trait expression and plant performance in the later stages (Angadi et al. 2000), as we have observed in our plants. The geographic and/or genetic source of seeds can affect these observed differences, but the environmental features (i.e. soil water levels) that we created also affect trends of trait expression in this species. By planting seeds in different times, seedlings emerged in different daytime and nighttime temperatures throughout the experiment, which likely affected trait expression. These conditions resulted in different patterns of trait values across the planting period, and the reproductive, leaf, and branch composite traits all responded differently.

Reproductive trait values have a weak hump-shape that indicates maximum reproductive output when seedlings are started in low water and in the absence of cold or heat stress. This trend reversed when the soil mix was supplied with abundant water daily in that maximum reproductive output was produced by plants grown in the coldest and warmest seedling start dates (parabolic); the pattern for moderate water supply is intermediate of the low and high treatments. One reason is that seedlings experiencing drought near the last frost and in peak summer months will tend to develop poorly due to freezing and heat stress,

respectively. However, there were seedlings planted with abundant daily water supply that grew vigorously. There may be different levels of drought and stress tolerance induced by varying amounts of temperature and moisture.

While the patterns of planting time versus branch trait values are different among ranges (and among water level treatments), the plants that were grown at the later planting dates had the highest branch trait values in their respective groups (mostly branch length, plant height, and branch number). After the threat of frost was gone, plants with later start times for all treatment groups increased in branch trait values. Branch development in crop *Brassica* has been shown to suffer in temperatures that are 35°C or higher, but reaction to heat stress is complicated and can be improved by temperatures in earlier and later development phases (Angadi et al. 2000). In other words, gradual warming during the early seedling stage may have induced resistance to heat stress during the reproductive stage, and/or colder nighttime temperatures during the flowering period may have promoted lateral branch development and branch elongation that increased plant height.

Individual trait plasticity (Hypothesis 2)—Based on our previous study on phenotypic variation (Alfaro and Marshall 2019) and on a forthcoming treatment of molecular marker diversity in *B. tournefortii*, we have evidence that there are different amounts of genetic diversity among the population types. In particular, we found that the invasive range has significantly lower microsatellite diversity than the native and landrace ranges (Alfaro unpublished). This difference in genetic diversity can lead to different amounts of individual trait plasticity among ranges. We therefore hypothesized that the mean plasticity of focal traits in invasive populations would be greater than in landraces and native populations. Plasticity was highest in the invasive range for plant height ($P > 0.05$),

reproductive biomass ($P > 0.05$), and lateral branch length ($P = 0.023$).

The original environments of these invasive populations are in different deserts of North America, which have distinct precipitation patterns or monsoons. In populations from Arizona, the localities are affected by the Sonoran Desert's climate, which receives 6 to 400 mm of rain (Hijmans et al. 2005) that is divided between winter and summer rains. In the Mojave Desert and Great Basin regions, the populations experience very dry conditions in the summer months and receive 55 mm of rain in the winter. The populations that we sampled have likely been established or expanding for only 80 years (Trader 2006) relative to millennia in the landrace and native populations. For a young invasive range with poor genetic diversity in a habitat with locally and regionally variable climate, phenotypic plasticity in physiological (leaf traits), structural (branching architecture), and reproduction (reproductive biomass) is a viable strategy for range expansion. Plasticity in leaf traits, which can determine plant size, reproduction, and photosynthetic activity, can allow new populations to survive the erratic timing of spring frosts and summer heat waves in these deserts. Plasticity in branching architecture for invasive *B. tournefortii* can translate into a versatile dispersal mode. Individual branches in large plants can break from strong winds to disperse seeds in the population vicinity; small plants (< 0.05 m) with ripe siliques can detach from its main stem to roll and disperse (Alfaro pers. observation).

So far, we have identified and described the plastic response of composite and individual traits through our garden experiment. We also reasoned that provenance type can affect the amount of plasticity in invasive populations. With proximate and ecological explanations for composite and individual trait plasticity, we can begin to discuss the trend of fitness consequences and phenotypic evolution for each range by addressing the third

hypothesis.

Adaptive signals of lateral branch length plasticity (Hypothesis 3)— It has been hypothesized that *B. tournefortii* can disperse seeds as a tumbleweed (anemogeochory), which was the reason why we selected traits associated with branching architecture in our study. In Alfaro and Marshall (2019), branching architecture traits (such as branching length) were variable in trait means and in fitness functions, and in this study, we also observed variable branch length plasticity among ranges, with mixed trends that show association with adaptive variation. In general, the length and density of branches are associated to reproductive output in *Brassica* and in *B. tournefortii*, but there could be different functions depending on the population type. For native and invasive ranges, the lengths of branches may affect the distance for seed dispersal due to either tumbling or gravitational dispersal modes. For landraces, and in most *Brassica* seed crops, branch length and count is related to seed yield and efficient harvest.

It is common to predict that genetically invariant populations in the wild can benefit more from increased ecological breadth due to phenotypic plasticity than from local adaptation (e.g. Sexton et al. 2002). Therefore, we expected fitness to increase with higher plasticity from varying soil water levels in branching and leaf traits in invasive populations more than in the native and crop populations. While among-range differences in trait value CVs can be interpreted as signals for rapid evolution of trait plasticity (Davidson et al. 2011), most differences in individual trait plasticity in our study were neutral and most relationships of mean trait plasticity with fitness did not differ among ranges. One trait, lateral branch length, had among-range differences in fitness effects of plasticity that approached significance. Over a longer period evolutionary advantage may tilt towards locally adapted

genotypes instead of phenotypic plasticity if an optimal genotype becomes fixed in the population (Bradshaw 1965, Sexton et al. 2002) and if the cost of plasticity outweighs its benefits (Lande 2015). It may be that these populations were past their colonization stage and in their naturalization stage, when phenotypic plasticity becomes costly and therefore detrimental.

In contrast, the landrace populations showed an increasing adaptive trend in plasticity of branch length despite exhibiting low individual trait plasticity (i.e. stability). We know from our genetic study that the landrace population have ample genetic variability as in the native range. So, we may be observing a phenotypically stable, but genetically rich group of populations that experiences a low to zero fitness cost for plasticity that can homogenize phenotypes across different soil moisture conditions. Typically, growers and crop breeders breed for phenotypic stability to ensure efficient harvest and high and consistent yield (e.g. Connor et al. 2011). Yet recently, even large-scale growers are re-establishing heirloom varieties or backcrossing commercial lines to earlier generations to increase genetic variation that can allow adaptive crop evolution in changing local and global environments. Perhaps achieving crop stability with variable genotypes is a viable solution for development of future crops that can positively respond to increasing climate variation.

CONCLUSIONS

Perhaps the fitness associations of our study groups may have been clearer with even numbers of populations per range, which may have allowed us to detect adaptive plasticity in more than one trait. Nonetheless, the reaction norms and effects of planting time that we identified show that *Brassica tournefortii* can potentially thrive when in habitats that receive

moderate to high moisture in critical stages of development. Plant conservation and weed eradication programs should consider conducting experiments that determine plastic responses to key environmental factors. This will provide information on which native populations to select for propagation; on the other hand, an invasive weed control effort can focus on removing populations with high levels of phenotypic plasticity. Finally, crop breeding programs should scan and select for both stability and plasticity of ecological and agronomic traits, so growers can adopt promptly to environments that may be changing too rapidly for technological and genetic development.

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Table 1. Summary of composite trait variables via PCA.

Reproductive Trait PC1	
Trait	Correlation
individual fruit mass	**-0.56**
reproductive biomass	**-0.50**
mean seed number per fruit	-0.44
total number of fruits	-0.44
days to first flower	0.02

Vegetative Trait PC1	
Trait	Correlation
rosette diameter	**-0.47**
leaf length	**-0.44**
number of leaves	**-0.42**
number of leaf lobes	**-0.41**
vegetative biomass	-0.32
height	-0.26
lateral branch length	-0.20
total number of branches	-0.19

Vegetative Trait PC2	
Trait	Correlation
height	**-0.52**
lateral branch length	**-0.51**
total number of branches	-0.40
vegetative biomass	-0.26
number of leaves	0.08
rosette diameter	0.25
number of leaf lobes	0.26
leaf length	0.31

Table 2. Summary of ANCOVA results that tested the effect of soil moisture levels on eight life history traits associated with leaf morphology, plant size, phenology, and branching architecture (415 plants). Planting date was used as a covariate to control for differences in planting time.

	Reproductive Trait PC1 (R^2 = 0.30)			**Vegetative Trait PC1** (R^2 = 0.36)			**Vegetative Trait PC2** (R^2 = 0.31)		
Source	**df**	**F**	**P**	**df**	**F**	**P**	**df**	**F**	**P**
Planting Date	1	1.85	0.175	1	44.03	**<0.001**	1	33.5	**<0.001**
Range	2	1.65	0.192	2	13.96	**<.001**	2	12.11	**<0.001**
Soil Moisture Level	2	9.77	**<0.001**	2	2.32	0.099	2	6.02	**0.003**
Block	2	17.66	**<0.001**	2	2.67	0.070	2	5.94	**0.003**
Population within Range	11	1.63	0.088	11	1.45	0.149	11	1.81	**0.050**
Block × Range	4	0.79	0.533	4	1.66	0.158	4	1.81	0.125
Range × Soil Moisture Level	4	1.96	0.141	4	5.07	**0.006**	4	0.43	0.653
Planting Date × Soil Moisture Level	2	3.44	**0.033**	2	6.6	**0.001**	2	4.84	**0.008**
Planting Date × Range	2	1.85	0.175	2	44.03	**<0.001**	2	33.5	**<0.001**

Table 3. Summary of ANOVA results that tested the differences of coefficient of variations of eight life history traits associated with leaf morphology, plant size, phenology, and branching architecture (n = 14 populations per trait).

Trait type (PCA composite)	**Population trait CV**	**Source**	**df**	**F**	**P**	**R^2**
Reproductive (Rep PC1)	Relative fitness	Range	2	2.07	0.172	0.274
Reproductive (Rep PC1	Reproductive biomass	Range	2	0.64	0.547	0.104
Reproductive (Rep PC1	Fruit mass	Range	2	2.14	0.164	0.28
Leaf (Veg PC1)	Rosette diameter	Range	2	0.05	0.956	0.008
Leaf (Veg PC1)	Leaf length	Range	2	2.36	0.14	0.3
Branching (Veg PC2)	Height	Range	2	0.33	0.726	0.057
Branching (Veg PC2)	Lateral branch length	Range	2	5.39	**0.023**	0.495

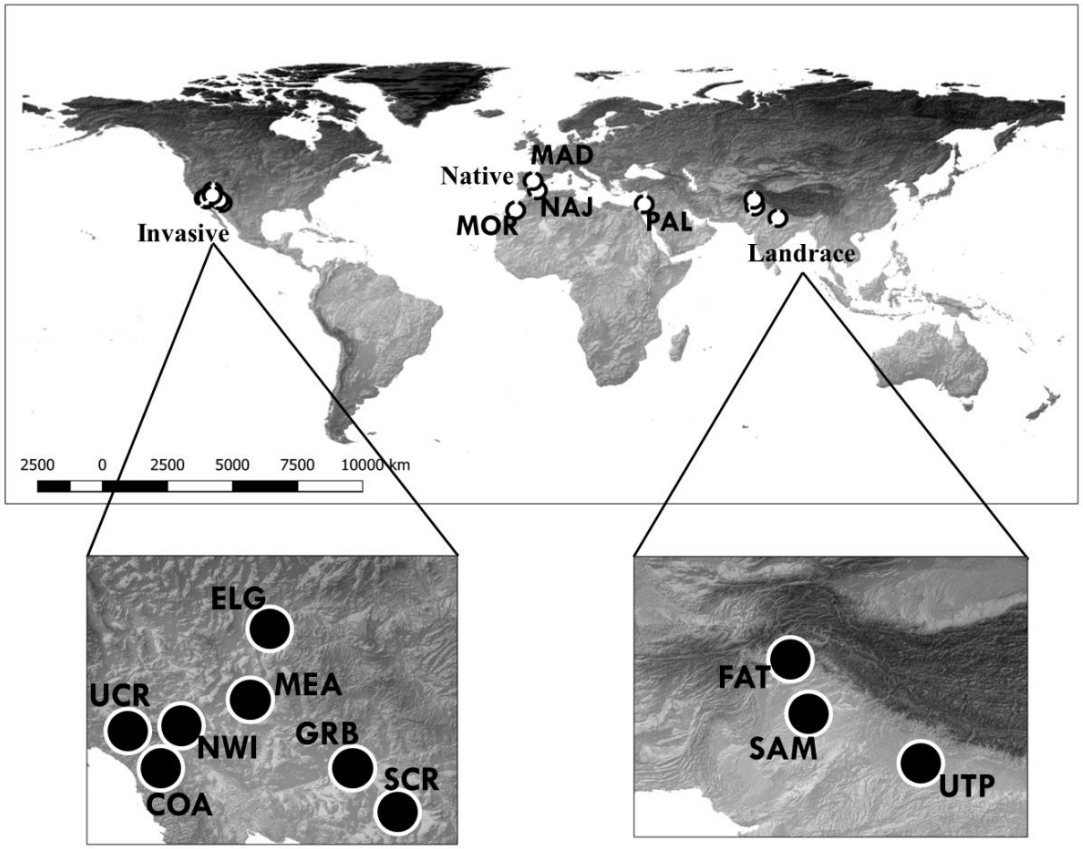

Figure 1. Map of original field collection sites for native and landrace seed accessions, and invasive populations.

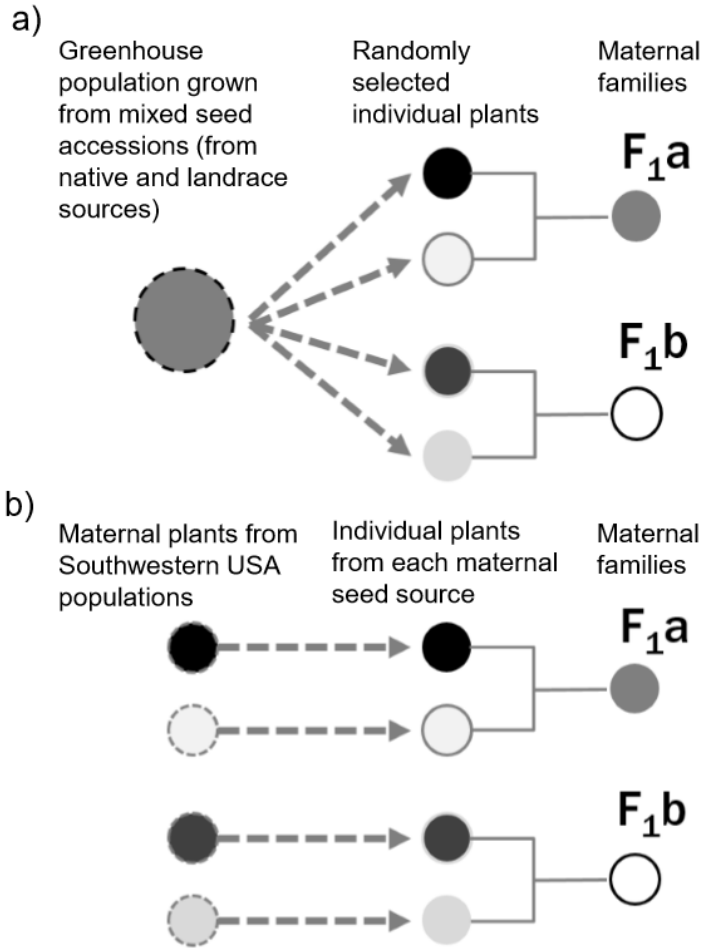

Figure 2. Summary diagram of artificial crosses for experimental parental populations of seed families.

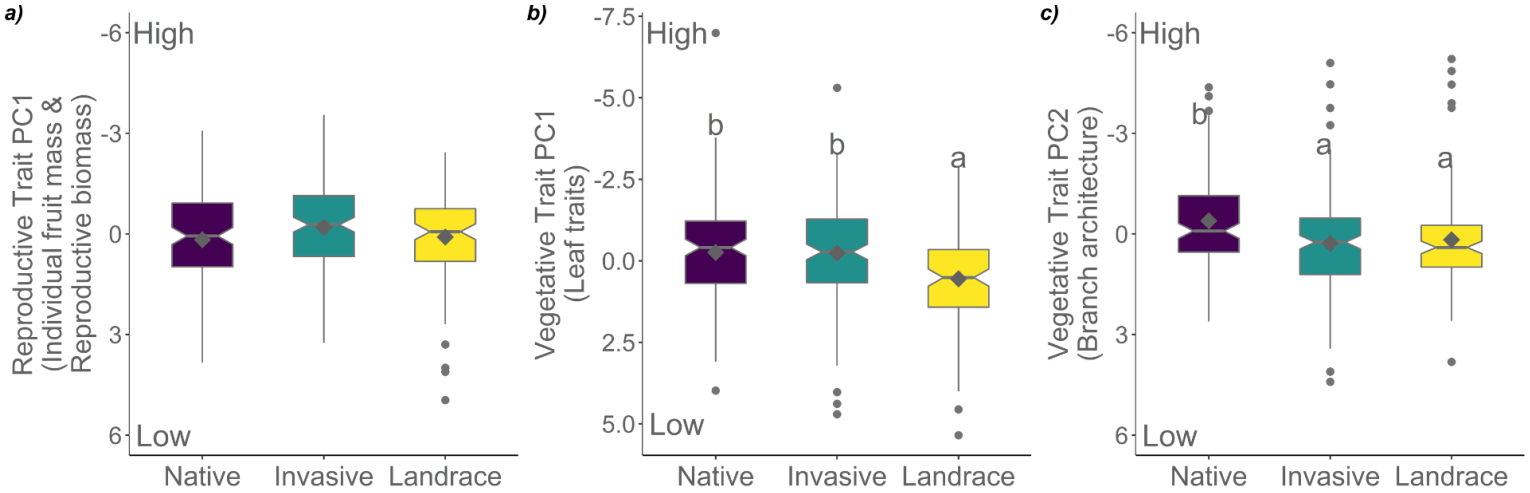

Figure 3. Box plots of trait principal components variables in native (*N*=139), landrace (*N*=109), and invasive (*N*=167) ranges. Box plots with notches (confidence intervals) that widely overlap are generally not statistically significant, while those with narrowly or non-overlapping notches are statistically significant. Significantly different pairwise comparisons are indicated with Tukey HSD letter superscripts.

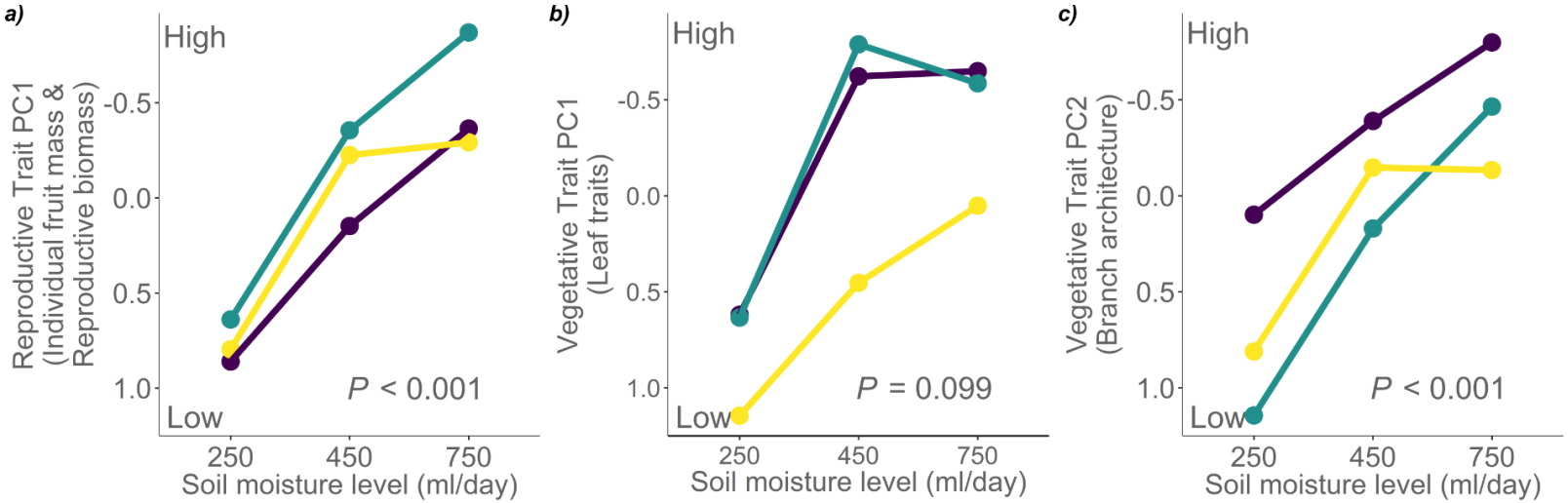

Figure 4. Reaction norms for three composite trait variables across three soil moisture treatments. ANCOVA P values are shown (Purple = Native, Green = Invasive, Yellow = Landraces)

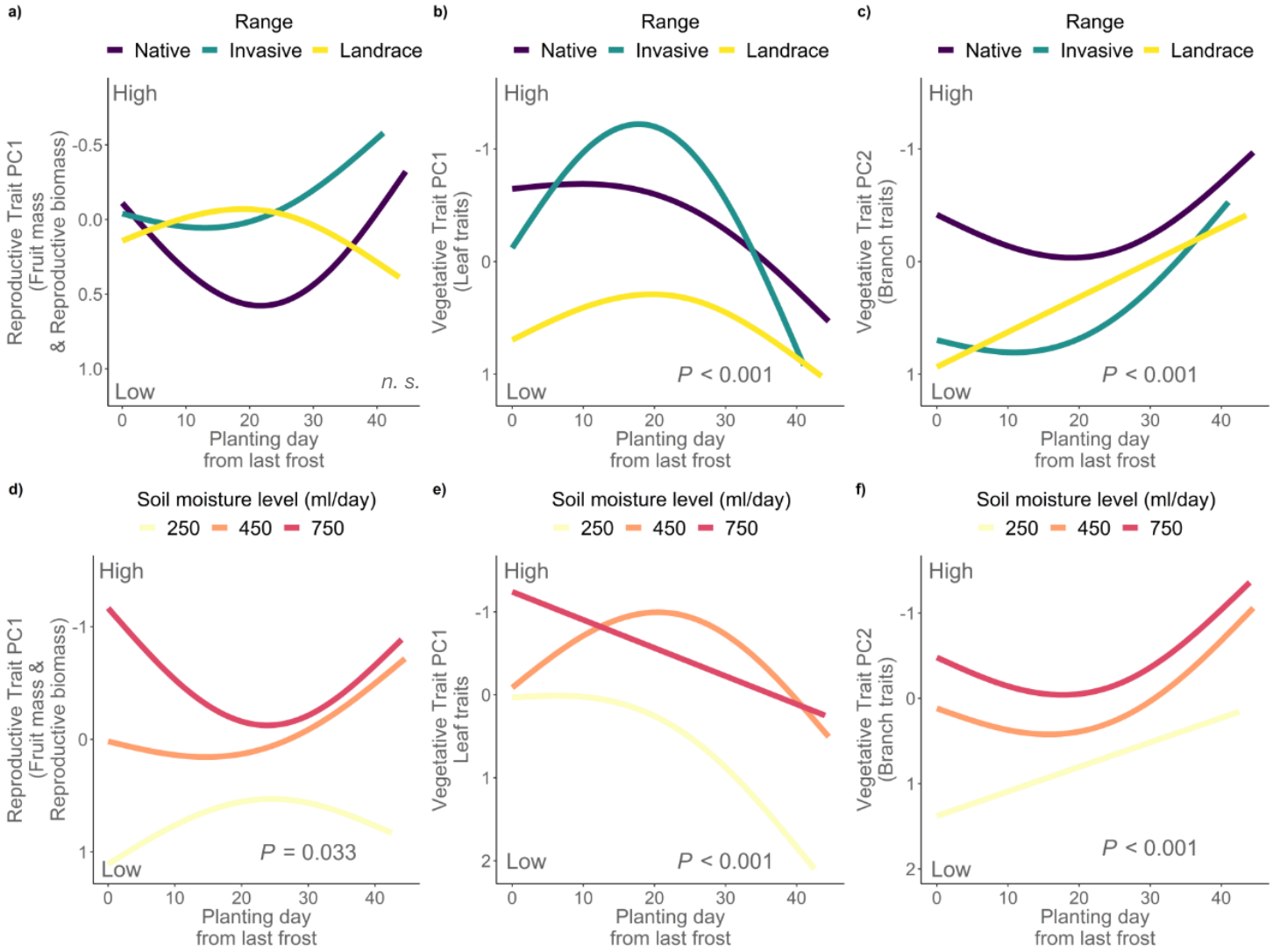

Figure 5. Regression lines via generalized additive modeling for three composite trait variables among three ranges (A to C), and among three soil moisture treatments (E to G). Significant ANCOVA P values are shown. Purple = Native, Green = Invasive, Yellow = Landraces.

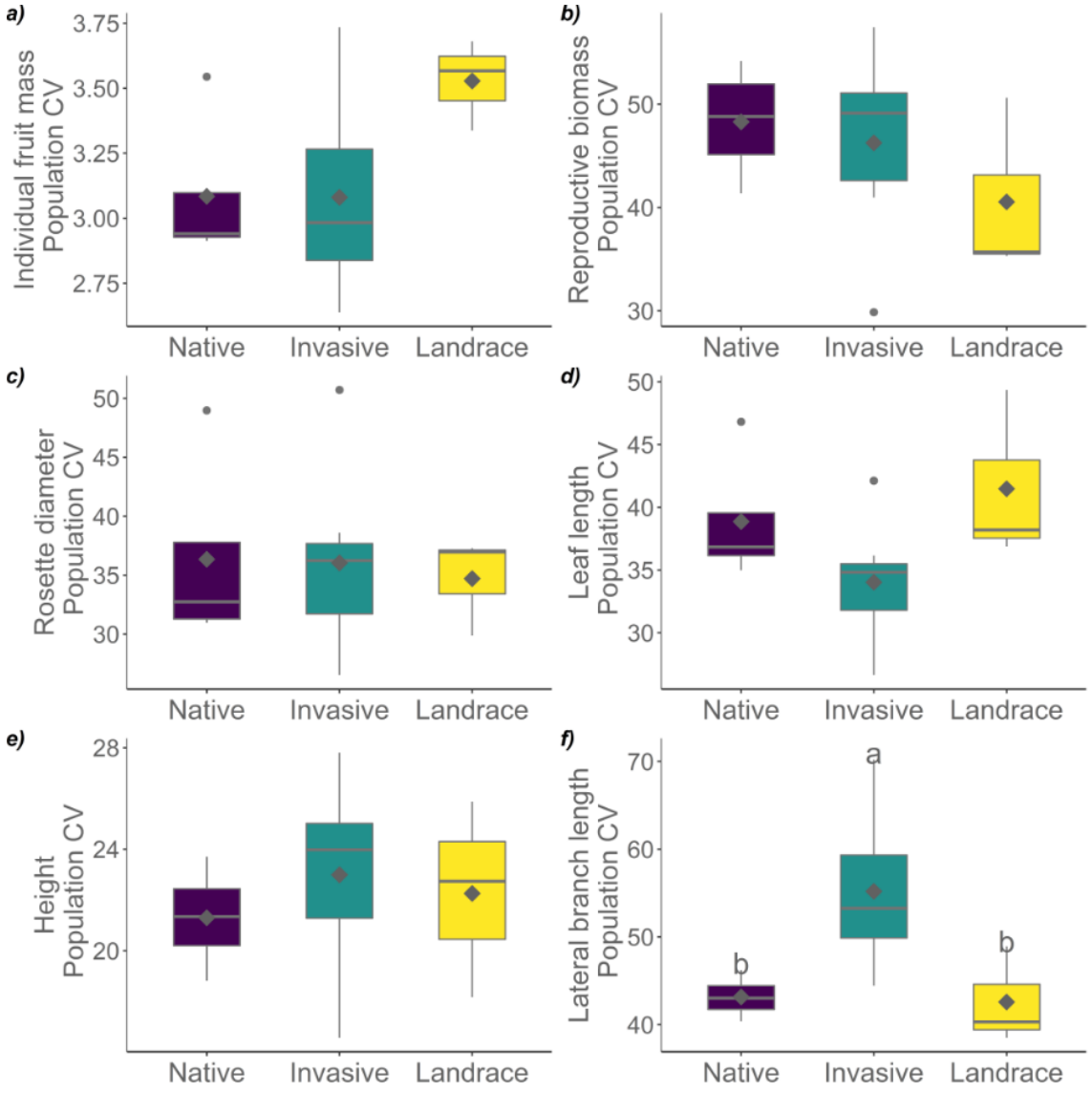

Figure 6. Box plot of mean population trait CVs (black circles) in native (*N*=4), landrace (*N*=3), and invasive (*N*=7) ranges. Pairwise comparisons of traits that have significantly different means are indicated by Tukey HSD superscripts.

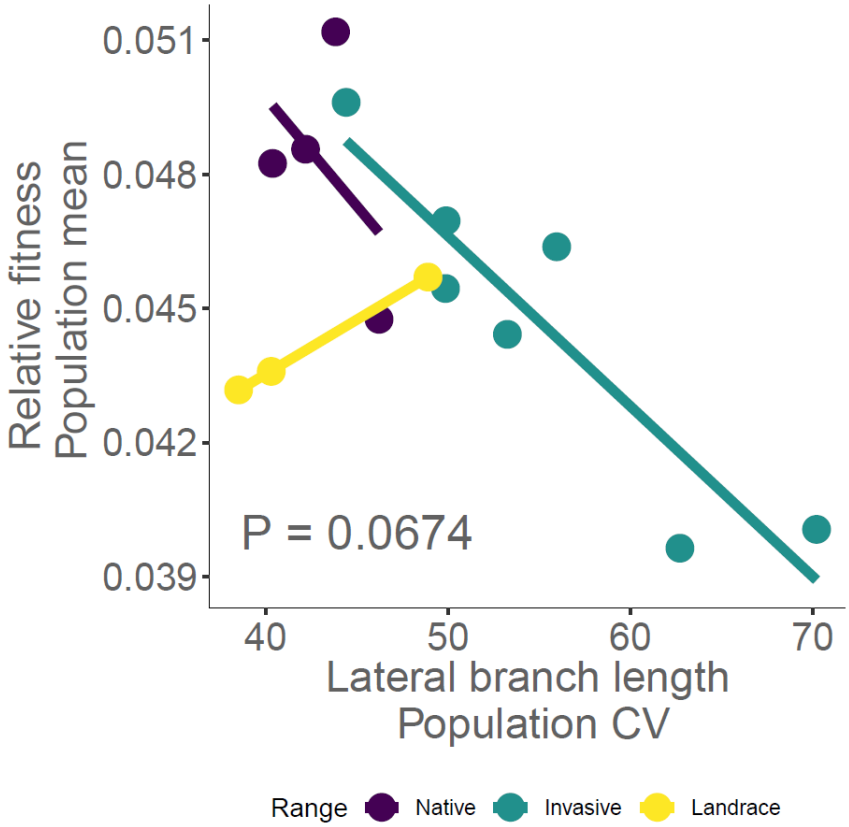

Figure 7. Interaction plots for the association of lateral branch length versus relative fitness in the native, invasive, and landrace ranges. To test if the relationship of branch length CV versus relative fitness differed among ranges, we performed ANCOVA with range as the categorical variable.

CHAPTER 3

Contemporary patterns of population divergence in

native, invasive, and landrace populations of *Brassica tournefortii*

(Formatted for submission to Plant Ecology

Alfaro, B, S.M. Witt, T.F. Turner, G.A. Fox, A. Sher, and D.L. Marshall 2021)

Abstract

Plant evolution in many species have been shaped by humans in different ways. Some species gain divergent populations via domestication, while other species can form new ranges by introduction of new populations assisted by human transport. Consequently, these different evolutionary histories can change the genetic composition that can lead to divergent adaptive evolution between population types. In this study, we tested if population types of *Brassica tournefortii,* a species that differs in history also genetically differed. We performed this comparison by genotyping individuals in native, invasive, and landrace ranges, using two variable microsatellite loci. Landraces had the highest estimates of genetic diversity compared to the native range, but the invasive range had much lower means of diversity estimators compared to native and landrace populations. In contrast, the invasive and native populations had high levels of gene flow in their respective genetic cluster assignments, while the landraces had moderate levels of migration. It is possible that these population genetic signatures are influenced by each ranges natural and anthropogenic histories.

Introduction

Though natural selection is evolution's primary force, the artificial change in genetic composition of populations due to anthropogenic activities can be equally important. This is because human-mediated activities can coincide with global changes that affect both human and natural environments (Thomann et al. 2015). For instance, advanced transportation of propagules in the last few centuries has made long-distance dispersal, range expansion, and non-native species introduction more frequent (Franks and Munshi-South 2014). We have also been intentionally sampling genotypes from natural plant populations, for millennia to produce domesticated crops (Lev-Yadun et al. 2000; Diamond 2002; Zeder 2008).

Artificial selection can enhance phenotypic value of heritable traits and homogenize genetic composition in a habitat (Bulmer 1971). Genetic diversity in crop populations is therefore expected to be lower compared to wild populations because crops undergo founder effects via domestication (Ladizinsky 1985, 1987). Seed and field populations of commercial crops are typically genetically homogeneous to produce consistent product, but landraces, which are traditionally bred and cultivated, can undergo bottlenecks but do not endure intensive selective breeding as commercial crops. Nonetheless, there are reasons why crops and landraces may have higher genetic diversity relative to invasive populations. In particular, Hamrick's review of isozyme patterns in crops and wild relatives suggest that there is plenty of variation across varieties or populations of crops (Hamrick and Godt 1997). Domesticated populations can serve as repositories for maladaptive alleles passed on by genetic hitchhiking (Burke et al. 2005) and by new mutations arising from inbreeding (Glémin and Ronfort 2013). If these events are followed by rapid population growth via cultivation, the effect of natural selection on crop fields can be dampened, potentially

increasing the frequency of unwanted alleles. These alternative evolutionary scenarios contribute to crop genetic diversity comparable to those in native populations (Meyer and Purugganan 2013).

Introduction of a species, whether intentional or accidental, can produce genetic variation that is different (e.g. Dlugosch and Parker 2008) or similar (e.g. Marrs et al. 2008) to native populations. Among populations within an invasive range, genetic diversity can vary due to local adaptation, multiple introductions, and serial founder effects. Many introduced populations have significantly lower genetic variation compared to their native sources due to bottlenecks (Marchini et al. 2016). On one hand, initial founder populations can have enough genetic variation to adapt to novel habitats; these preadapted populations can maintain similar amounts of genetic and phenotypic diversity as their native counterparts (Bossdorf et al. 2008; van Kleunen et al. 2011). On the other hand, strong selective pressures in the new environments can reduce genotypic diversity, but gene flow from multiple locations may rescue genetically depauperate localities with influx of migrant alleles, occasionally producing new genotypes (Dlugosch and Parker 2008). While introduced populations can rapidly evolve, the amount of genetic diversity depends on conditions and events that follow initial introduction.

The variety of population genetic outcomes that stem from human activities makes landraces and invasive populations important systems for assessing evolutionary potential in different or changing environments. To assess how genetic makeup of populations may differentiate as an indirect consequence of human activity, we used *Brassica tournefortii*, a desert annual that has native, invasive, and landrace populations with divergent histories. This species is native in the Middle East and North Africa, and has been cultivated as a seed

crop as early as 5,000 years BCE (Rao et al. 1996, Singh et al. 2015). *B. tournefortii* was introduced and has become invasive throughout Australia and western North America in less than 100 years (Boutsalis et al. 1999, Schiermeier 2005, Chauan et al. 2006, Trader 2006, Bangle et al. 2008, Barrows et al. 2009). We have examined trait variation in this system, and we have observed from greenhouse and garden experiments that *B. tournefortii* populations from these three range types vary phenotypically via local adaptation and plasticity (Alfaro and Marshall 2019). Thus, there is potential for differences in genotype diversity and differentiation among these populations. Examining genotype diversity in different population types in this species can address whether invasive and landrace populations have adaptive potential despite bottlenecks, or if they maintained similar levels of genetic variability compared to native populations.

In this study, we wanted asked whether disparate evolutionary histories have resulted in divergent *B. tournefortii* populations relative to the native populations. We first developed hypotheses on the contemporary evolutionary relationships of the native, landrace, and invasive populations of *B. tournefortii*. In our previous phenotypic analyses, we made assertions that the native, invasive, and landrace ranges, and the populations within them, were divergent in trait means as a result of their natural, domestic, and invasive environments and histories. Therefore, the patterns of genetic variation and diversity of native, invasive, and landrace ranges should reflect population divergence based on their histories.

After illustrating patterns of divergence and similarity among populations, we wanted to compared the amount of microsatellite diversity and differentiation among the regions and among populations so we could refine our interpretations. In our previous analyses, we observed that the three ranges varied phenotypically, and that the native populations had the

most diverse phenotypes of all three ranges, while domestication and invasion have reduced variation in the landraces and invasive populations (Chapters 1 and 2, Alfaro dissertation). We therefore asked whether the same patterns of differentiation occurred in microsatellite markers among native, invasive, and landrace populations. Before we analyzed genetic differentiation, we first examined allelic diversity and heterozygosity before comparing other population genetic estimators. Then, we compared microsatellite differentiation within ranges, among ranges, and among and within populations of native, invasive, and landrace ranges. We knew from pilot tests for molecular marker development that we had markers that were not in Hardy-Weinberg equilibrium for some locations. But, we estimated population rates of gene flow and/or migration nonetheless in order to better interpret and reconcile information on allelic diversity, genetic differentiation, and evolutionary histories of native, invasive, and landrace populations.

We predict the native populations to have high genetic diversity, the invasive populations to have low genetic diversity, and the landrace ranges to also have lower genetic diversity. Based on these predictions, we divided our analysis and interpretation in four parts:

1. First, we constructed a phylogenetic tree based on population pairwise genetic distances to infer patterns of genetic divergence and evolutionary relationships among our study populations of *B. tournefortii*.
2. We hypothesized that that the amounts of mean genetic diversity and mean heterozygosity would be: native >> landrace > invasive populations.
3. We hypothesized that focal microsatellites in our study populations will be differentiated as: native < landrace < invasive.

4. We hypothesized that our native accessions would show weak or no gene flow, while the landraces and invasive populations, would show higher rates of gene flow.

Methods

Study system

We analyzed microsatellite allelic diversity of *Brassica tournefortii* in native, landrace, and invasive populations. For native populations, we used seeds from plants that were regenerated from field-collections from Morocco (Tiznit), Spain (Madrid and Almeria), and Israel (Palmachim). These four source locations are in semi-arid to Mediterranean ecoregions where *B. tournefortii* is considered endemic. For landraces, we used seeds from plants that were regenerated from field-collections from India (Uttar Pradesh) and Pakistan (Chowk Azam, Sammundri, and Fateh Jang). Based on literature on crop development (Singh et al. 2015), our source localities in Pakistan and India are landraces. We used seeds from the invasive range in the warm desert ecoregions of western North America. Early records point to Mecca, California as the first introduction site for this species (Bangle et al. 2008), but there is anecdotal evidence that a ruderal form of *B. tournefortii* has been spreading across North America. Therefore, we collected from populations in Mecca and surrounding areas (invasion core), and from populations in Nevada, Arizona, and Texas (invasion front).

Sampling

Each accession from native and landrace ranges was first sampled from maternal plants in original sources by the USDA ARS-GRIN. The field collected seeds were planted into populations of 50 to 100 in plant cages at the USDA ARS-GRIN facility in Ames, IA

(USDA, Laura Marek personal communication). Seeds from each accession were regenerated periodically via selfing/open-pollination. Therefore, the landrace accessions have undergone an additional founder event, and the native accessions have experienced one founder event. For each native and landrace accession, we planted 10 to 20 individuals in the University of New Mexico (UNM) Research Greenhouse in 2013 for tissue collection and genotyping.

We collected leaf tissue in 2015 and 2016 within the vicinity of the first introduction site (Mecca, Coachella, Joshua Tree National Park, and nearby Mojave National Preserve); we directly genotyped seven to 10 plants from leaf or stem tissues that we sampled in each site. In 2009, professional biologists collected seeds in Arizona (lower reach of Gila River and Santa Cruz River), Nevada (Lake Mead National Recreation Area and Elgin Road) and Texas (El Paso); ten plants were sampled per site. We planted one individual to represent each sample plant per site in a greenhouse for tissue collection and genotyping.

Genotyping

Tissues collected from greenhouse propagations from native, landrace, and invasion front populations were transferred in cryogenic vials and stored at -20°C immediately after harvest and were kept in this condition until tissue preparation for DNA isolation. Field-collected tissues were stored in scintillation vials and fixed in 95% ethanol, or in coin envelopes with 5 g of silica beads, until tissue preparation for DNA isolation. In Dec 2018, we defrosted greenhouse collections and air-dried ethanol-fixed tissues before DNA isolation. After tissues were defrosted or air-dried, we measured 10 mg of tissue and transferred each sample to labeled 2 µl Eppendorf microcentrifuge tubes with 100 µl of grinding/extraction buffer and a glass bead. We homogenized plant tissue using an Eppendorf Tissue Lyser set at 30/s freq for

30 s or until plant material is visibly disrupted. To lyse the nuclei in each sample, we added 500 µl more of the extraction buffer to the homogenate, which we then incubated in a water bath for 1 hr at 65°C. After the lysis step, we added 3 µl of RNAse A and incubated the lysate in a heating block at 37°C for 15 minutes to denature RNA. We cooled the lysate to room temperature, and then added 200 µl of sodium acetate as our protein precipitation solution in the supernatant. Using a vortex, we resuspended plant material with the extraction buffer and protein precipitation solution, and then spun the mixture at maximum speed for 5 min. to isolate the supernatant from the plant debris and precipitate. We transferred 400 µl to 600 µl of the supernatant to a new tube containing ice cold absolute ethanol to precipitate DNA. To maximize DNA precipitation, we placed the supernatant in -20°C for 15 mins. Then, we spun the supernatant at maximum speed for 3 min to separate the DNA precipitate. We washed the DNA pellet further with 500 µl 75% ethanol, and then spun the tubes for 1 min. After samples were centrifuged, we decanted the ethanol from the tube, and air dried the pellets for 24 hours in a cardboard sample box. We rehydrated the DNA pellets in a solution of 1x TE buffer.

We diluted our DNA templates at a ratio of 1:10 before PCR. We used the Qiagen® Multiplex master mix with the following combination of reagents per sample: 2.6 µl of Millipore-filtered water, 5 µl of 2x master mix, 2.6 µl of 2 µM multiplex primer mix, 0.2 µl of 10 mg/ml bovine serum albumin, 0.2 µl of the proprietary Q solution, and 1 µl of diluted DNA template, a total reaction volume of 10 µl per sample. For the master mix, we used primers that were developed for *Brassica tournefortii* at Ecogenics (Switzerland); out of six primer pairs, the two that we selected showed moderate polymorphism for all ranges. We labeled the 5’ end of each forward primer with either HEX, 6-FAM, or TET fluorophores, for

capillary electrophoresis. Using 100 μM stock solutions, we prepared the 2 μM multiplex primer mixes as follows:

12 ul 6165-F (FAM-AAGGTTGGACGCGAGAAGAG)

12 ul 6165-R (GGAAGCAGCAAATCCTCCAG)

12 ul 5495-F (TET-TTCAGCTACTCAAACGCGAG)

12 ul 5495-R (GTCTCATCTGTTACTATGGATGATGG)

552 μl of Millipore-filtered water

The samples were randomly arranged on a 96-well plate with 1-2 negative controls. Our PCR profile was as follows:

1. Initial denaturation stage at 94°C at 30 s
2. Denaturation at 94°C at 30 s
3. Annealing at 55°C at 1.00 mins
4. Extension at 72°C at 1.00 mins
5. Repeat Steps 2-4 for 30 times
6. Final extension 72°C at 15.00 mins
7. Hold at 4°C

We determined the quality of PCR products by staining 1 μl of each sample with ethidium bromide to a final concentration of 0.5 1 μg/ml, and running them via 2% agarose gel electrophoresis on 0.5X TE buffer. Only products that produced intense bands were genotyped via capillary electrophoresis. We diluted each sample (1:4) for genotyping; we added 1 μl of the diluted PCR product to a cocktail of 8.5 μl HiDi formamide (Thermo

Fisher) and 0.5 µl of ROX-labeled molecular reference (Life Sciences). Prepared samples were denatured at 95°C on a heating block, then immediately quenched and stored at -20°C until capillary gel electrophoresis. We processed samples in an ABI 3130-XL Sequencer (Life Sciences). We used GeneMapper (v4 Thermo Fisher) to score microsatellite fragments. When sizing quality of a sample was detected as poor but raw data shows otherwise clean and or strong peaks, we manually adjusted the offset size references.

Analysis

Our sampled populations of *Brassica tournefortii* were widely distributed geographically across continents, and have potentially geographic and genetic clusters of populations (Table 1). While we know how these localities are arranged spatially, we do not have prior information on how our study populations are grouped based on genotypes or phylogenetic relationships. To understand the recent history of potential divergence and/or clustering among our populations, we first created a Nei's (1972) pairwise genetic distance matrix using GeneAlEx v. 6.5 (Peakall and Smouse 2012). Then, we used the *R* packages *ape* and *ggtree*, to create a phylogenetic tree via neighbor joining (R Core 2013; Guangchuang et al. 2017; Paradis et al. 2019).

We also statistically and graphically determined the number of genetic clusters using Bayesian inference via *STRUCTURE* (Pritchard et al. 2000). We have information on sampling locations, and we wanted to test the amount of gene flow, so we used the *LOCPRIOR* configuration of the *ADMIXTURE* algorithm. We ran models that ranged from $K = 1$ to $K = 12$ clusters for 5 iterations for each state at 20,000 burn-in length and over 20,000 MCMC repetitions. Then, we used the *STRUCTURE HARVESTER* application (Earl and

vonHoldt 2012) to plot number of genetic clusters predicted versus Δ*K* values to determine the peak slope in the log probability between consecutive *K* values (Evanno et al. 2005).

To compare genetic diversity between native, landrace, and invasive ranges, we calculated the $e^{\text{mean Shannon-Weaver index } (H')}$ (Jost 2006) and mean heterozygosity of the alleles of the two loci for each population using GeneAlEx. We included $e^{H'}$ among other estimators because it considers the total number of alleles in a population and how evenly alleles are distributed in a population. We performed ANOVA on the $e^{H'}$ and heterozygosity for the native, invasive, and landrace populations to determine differences in microsatellite diversity in *R*.

To determine whether the landrace and invasive populations are more differentiated than the native populations, we calculated analysis of molecular variance or AMOVA (Peakall et al. 1992) among ranges, among populations, and within populations via GenAlEx. In addition to AMOVA, we also used our *STRUCTURE* results to graphically interpret genetic differentiation for each range and population based on population structure. As we mentioned in the different parts of this study, we included estimates of gene flow rates to reconcile these different population genetic and evolutionary analyses with respect to history with human contact. In particular, we used *Q* values from the structure analysis, which are admixture indices calculated for each individual, to compare potential differences in gene flow among populations and among ranges. To compare differences in gene flow rates among the native, invasive, and landrace ranges, we performed ANOVA using range and population within ranges as categorical variables for comparing Q values.

Results

Recent evolutionary relationships

Phylogenetic tree

We observed two main groups or clades that are further separated into subgroups. One of the main groups or clades contained both the landrace and native population clusters, which were grouped into two separate sister clades. The putative native populations (Madrid, Almeria, Morocco, and Israel) were all grouped in one subclade that also included a population from Chowk Azam, PK, which was labeled as a landrace seed accession by the USDA ARS (Figure 2). Therefore, we reordered our groups so that Chowk Azam is included in the native populations. The landraces from Pakistan (Sammundri and Fateh Jang) and from Uttar Pradesh, India, were all grouped into a small clade that is sister to the native range (Figure 1). The invasive populations were in a divergent cluster from the native and landrace populations, and had two sister clades (Figure 1).

Structure and gene flow

Using the Evanno method via *STRUCTURE HARVESTER* application (Earl and vonHoldt 2012), we determined that the highest kD was at $k = 2$; therefore there were two potential genetic clusters in our samples that were distributed among the three ranges (Table 1, Figure 2). The invasive individuals were predominantly assigned to Cluster 1 and had high *Q* values for this cluster (Table 1, Figure 3). For Cluster 1, the invasive populations had high rate of admixture and therefore gene flow. Conversely, native individuals were predominantly assigned to Cluster 2 and had high *Q* values for this cluster; for Cluster 2, native populations have high gene flow (Table 1, Figure 3). Individuals in the landrace populations had mixed assignments for both clusters 1 and 2, and both clusters had relatively moderate amounts of

admixture or gene flow rates in these populations (Table 1, and Figure 3). These differences in *Q* values were significantly different for both clusters (Table 2).

Genetic differentiation

AMOVA

For the two loci, the ranges and populations of *B. tournefortii* show no genetic structuring, with 9 % of microsatellite variation coming from among the three ranges (Φ_{RT} = 0.086, *P* = 0.001, df = 2), and 4% of variation coming from among populations (Φ_{PR} = 0.049, *P* = 0.005, df = 14). Most of the allelic variation occurs within populations (87%, Φ_{PT} = 0.130, *P* = 0.001, df = 162).

Genetic diversity

Our limited study of microsatellite variation in *Brassica tournefortii* shows that three distinct population types or ranges have diverged into groups with different amounts of genetic diversity, heterozygosity, and genetic distance among populations. Two out of 17 populations were in Hardy-Weinberg equilibrium for locus 5495, and 11 out of 17 populations were in Hardy-Weinberg equilibrium for locus 6165. Locus 5495 was monomorphic in Marble Mountains, CA, and in Joshua Tree, CA. Locus 5495 had nine alleles, and locus 6165 had 6 alleles.

Our pooled comparison of populations among native, invasive, and landrace ranges shows that mean microsatellite e^H was lowest in the invasive populations (Figure 4). The native populations had the highest mean allelic diversity, and while the landrace populations also had high allelic diversity, it was slightly lower than the native populations (Figure 4).

These differences were statistically significant ($P < 0.021$). Mean heterozygosity was lowest in the invasive populations (Figure 3). Native and landrace populations had higher heterozygosity than the invasive populations (Figure 3). These differences were statistically significant ($P < 0.053$).

Discussion

Information on genetic divergence is critical for painting a clear picture of contemporary evolutionary relationships, especially for cosmopolitan species that have different histories. Thus, we conducted this study to provide information on genetic and evolutionary relationships that supports results from our phenotypic studies of native, invasive, and landrace populations of *Brassica tournefortii*. While two focal loci may seem low, these two markers had enough variability compared to other analyses (e.g. Winkler et al. 2019) to be able to describe baseline population genetic variables that make sense of previous and new findings. The native, invasive, and landrace populations varied in genetic diversity, genetic structure, and were grouped into two different clusters that were similar to the groupings in our experimental studies on adaptive phenotypic variation. We further discuss possible micro-evolutionary processes that shape the patterns of divergence that we observed.

Recent evolutionary relationships

Even though our putative native populations are distant, and even though they showed high within-population allelic diversity and heterozygosity, their genotypic identities were closely matched and were grouped in a small clade. It is therefore likely that these localities in Spain, Morocco, and Israel have populations that shared an ancestral origin. Another population,

Chowk Azam from Pakistan, was grouped in the same clade. We initially classified this accession as a landrace based on the collection notes. However, when we started a pilot garden study for the purpose of tissue collection, we did note that this accession had individuals that phenotypically did not show any evidence of domestication. In particular, it had open branch angles, small fruits and relatively small seeds that are brick red in color (Alfaro personal observation). The landrace populations in Sammundri, Fateh Jang, and Uttar Pradesh, which are clustered in a smaller group, had large seeds that had some yellowing and appressed branching typical of domesticated seed crops (Alfaro and Marshall 2019). Perhaps these accessions from India and Pakistan were derived from the wild populations near South Asia.

Invasive populations of *B. tournefortii w*ere grouped in the same clade likely because the populations in the southwestern United States have high genetic relatedness. Based on our observations of invasive phenotypes in the wild and in experimental populations, they do have similarities in the shape of the plant (Alfaro personal obs.). The invasive clade is split in two subgroups that are within two areas that are adjacent, the Great Basin region in Nevada, and in the Sonoran Desert in Arizona (Figure 1). These locations differed from the rest of the invasive populations in that they are not from roadside habitats, but from habitats close to aquatic waterways. However, we did not have high marker resolution that can show how these populations may have spread in this region or which landrace or native population(s) are most related to the invasive range.

Genetic diversity, structure, and gene flow

The native range on average had high gene flow. However, based on the identity of our seed sources from the native and landrace areas, we know that the possibility of gene flow from those exact accessions is low because they were collected at different years, and they were also geographically distant. Some collection sites for native accessions likely have ephemeral and therefore unstable habitats like desert washes and sand dunes. For example, the population from Israel is located on Palmachim, an area that is adjacent to the sandy coastline of the Gaza Strip. Populations in these habitats typically experience frequent extinction and recolonization that can increase the effect of drift (Kirkpatrick and Barton 1997) and/or genetic rescue (Brown and Kodric-Brown 1977) if the locality is within a metapopulation (Hanski 1998). Even with low gene flow, these mechanisms can reduce population structure.

Diverse mixes of genotypes can be more common in landraces than in invasive and native populations because seed banks or populations do not undergo extensive purging of deleterious alleles. *B. tournefortii* is traditionally cultivated and concentrated in South Asia, and it is not globally produced like commercial *Brassica*. Unlike commercial seed production facilities that breed for uniform phenotypes, traditional farmers collect seeds for subsequent growing seasons, which can allow diverse seed bank populations to survive multiple generations. This process can allow neutral or maladaptive alleles to proliferate because continuous cultivation can force the establishment of seeds/plants regardless of their genotypes (Meyer and Purruganan 2013). The more likely reason is that growers are exchanging seeds among crop fields (Meyer and Purruganan 2013). These conditions can weaken the effect of selection on neutral and/or maladaptive alleles and artificially increase genetic diversity, and therefore adaptive potential, of a crop population.

Our results matched our prediction that invasive populations would have lower genetic diversity than native and landrace populations, which we interpret as regional divergence. In many scenarios, initial introductions suffer losses in variability due to founder and bottleneck effects (Sexton et al. 2002). Given that *B. tournefortii* experienced a 40-year time lag before its first population boom (Winkler, 2019), the putative invasion epicenters in California may have been nearly homogenized before its first pulse of expansion. In some environments, landscape features that promote dispersal can link distant localities into metapopulations. Artificial structures, such as roads, can serve as conduits for population connectivity (reviewed by Holderegger and Di Giulo 2010). The invasive *B. tournefortii* populations were from separate ecoregions, but it is well-documented in North America that seeds can be mobile by sticking to vehicles during the rainy season (Trader et al. 2006). This potential for frequent and long-distance dispersal can be a factor for genetic homogenization in roadside populations of *B. tournefortii* in the southwestern United States. Some have also observed *B. tournefortii* to occur on the edges of lakes and riparian waterways and have reported fruits and entire plants floating and moving with the current (Bangle et al. 2008). Incidentally, our study populations in the southwest were sampled in roadsides and aquatic waterways. This potential connectivity is perhaps one reason why gene flow rates are high in the invasive range because landscape features associated with transportation can assist in long-range dispersal.

Summary for native, invasive, and landrace populations

Native

The native populations we studied have high genetic diversity. The resulting phylogenetic tree grouped all native locations in the same cluster, indicating that while they have relatively high genetic diversity for the two microsatellite loci that we analyzed, they also shared similar genotypes.

Invasive

Based on high amounts of genetic diversity from the native and landrace ranges, the invasive populations likely experienced genetic bottlenecks at the initial stage of introduction. These populations spread across the southwestern United States into two overlapping populations that are divergent potentially because of their landscape features.

Landraces

The genetic fate of landrace populations is in the hands of farmers, but traditional farming can maintain or even increase genetic variation in these populations. However, in addition to desired genotypes, it is highly likely that maladaptive alleles can be inadvertently introduced in a seed mix if there is no purging performed via more structured artificial selection.

Conclusion

Some species, such as *B. tournefortii*, have diverse histories with humans, and sometimes their evolution can reflect how we have changed our lifestyles (i.e. agriculture) and environments (i.e. infrastructures). In this study, we showed some evidence that landrace and invasive *B. tournefortii* has genetic signatures indicative of divergence from the native range. In a rapidly changing world, it is wise to recognize what mechanisms drive changes in the raw material for evolution. A fundamental step in detecting variability is to test genotypic composition among populations and regions. In some cases, this is important information to

consider before making permanent and long-term decisions on how to manage these organisms.

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Table 1. Summary of locations and population genetic estimators.

Location	Latitude	Longitude	Range	N	H_o	H_e	Mean $e^{H'}$	Mean Q_1	Mean Q_2
Santa Cruz River, Arizona	32.4	-111.15	Invasive	12	0.08	0.5	2.51	0.89	0.11
Upland Sonoran Desert, Arizona	33.33	-112.13	Invasive	3	0.5	0.36	1.72	0.98	0.02
Gila River, Arizona	33.39	-112.25	Invasive	8	0.56	0.54	2.47	1	0
Joshua Tree, California	33.72	-115.81	Invasive	5	0.1	0.09	1.18	0.99	0.01
Marble Mt., California	34.67	-115.71	Invasive	3	0.17	0.53	2.28	0.93	0.07
Mecca, California	33.57	-116.08	Invasive	10	0.6	0.51	2.51	0.74	0.26
Lake Mead, Nevada	36.74	-114.44	Invasive	9	0.33	0.69	3.47	0.78	0.22
Elgin Rd., Nevada	35.2	-114.57	Invasive	4	0.38	0.58	2.64	0.98	0.02
El Paso, Texas	31.85	-106.54	Invasive	11	0.05	0.38	1.74	1	0
Sammundri, Pakistan	31.06	72.95	Landrace	9	0.67	0.74	4.09	0.63	0.37
Fateh Jang, Pakistan	33.57	72.6	Landrace	13	0.46	0.69	3.47	0.67	0.33
Uttar Pradesh, India	26.85	80.91	Landrace	12	0.33	0.71	3.79	0.71	0.29
Almeria, Spain	36.97	-2.2	Native	9	0.16	0.67	3.75	0.6	0.4
Madrid, Spain	40.4	-3.68	Native	20	0.08	0.68	3.41	0.54	0.46
Tiznit, Morocco	29.72	-9.72	Native	20	0.38	0.75	4.44	0.45	0.55
Palmachim, Israel	31.93	34.71	Native	11	0.17	0.62	3.02	0.47	0.53
Chowk Azam, Pakistan	30.97	71.21	Native	9	0.17	0.36	1.95	0.13	0.87

Table 2. Summary of ANOVA results for admixture coefficients (*Q*) for two inferred clusters via *STRUCTURE*

	Source	df	*F*	*P*
Q_1	Range	2	26.63	< 0.0001
	Population within range	14	1.14	0.32
Q_2	Range	2	26.63	< 0.0001
	Population within range	14	1.14	0.32

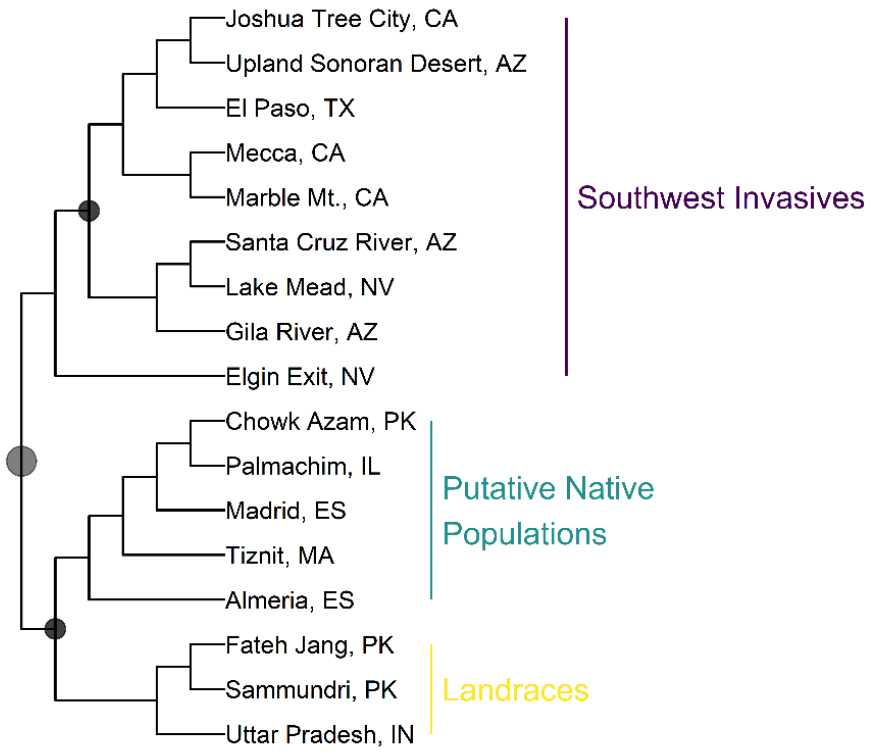

Figure 1. Neighbor joining tree of 17 study populations of *Brassica tournefortii*. To construct the dendrogram, a Nei's genetic distance matrix was created for the 17 populations.

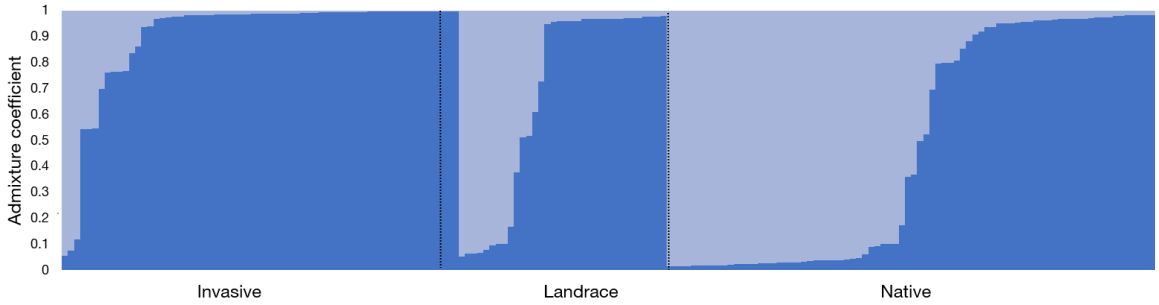

Figure 2. *STRUCTURE* bar plots for $K = 2$ cluster assignments of *B. tournefortii* grouped by invasive, landrace, and native populations.

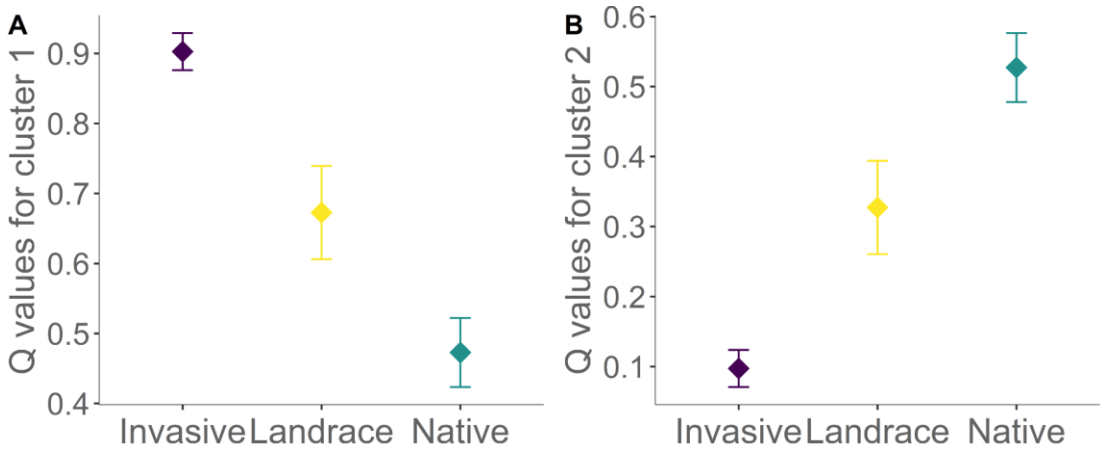

Figure 3. Box plots of mean Q for the two inferred clusters for two microsatellite loci for native, invasive, and landrace populations of *Brassica tournefortii*.

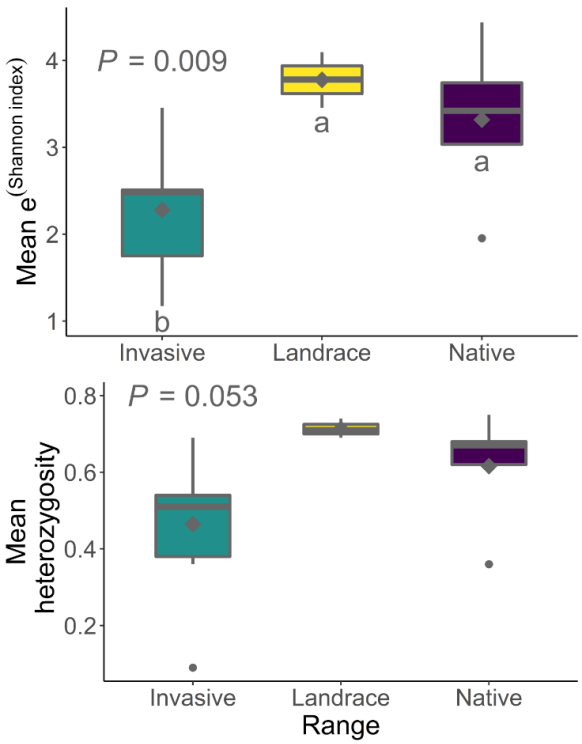

Figure 4. Box plots of mean $e^{Shannon\text{-}Weaver}$ diversity index means (top) and heterozygosity means (bottom) for two microsatellite loci for native, invasive, and landrace populations of *Brassica tournefortii*. Significant pairwise comparisons are labeled with Tukey letters. Mean values are indicated by gray diamonds, median values are shown by bold lines.

CHAPTER 4

Brief synthesis: relationship of heterozygosity and total plasticity

Background

Population genetic variables can affect the strength of plasticity. In particular, the number of genotypes in a population can set up initial conditions that can lead to increased (or decreased) plasticity (Lande 2015). Populations that have reduced genetic variability either from inbreeding depression and/or genetic bottlenecks can benefit from increased plasticity that can potentially increase phenotypic variation despite being genetically depauperate. On the other hand, populations that have high heterozygosity may have enough variation that can provide adaptive potential for selection of multiple locally adapted phenotypes (Sexton et al. 2002).

For this brief chapter, I used my greenhouse and molecular experimental results from Chapter 2 and 3 of my dissertation (Alfaro unpublished) to test if population means of heterozygosity affect population means of overall or composite estimates of phenotypic plasticity. I used my study system, *Brassica tournefortii,* because I have already studied phenotypic and genetic variation of this species in my first three dissertation chapters. More importantly the study groups in my experiment include native, invasive, and landrace populations of *B. tournefortii,* which have been shown to have diverse patterns of phenotypic and genetic variation (Alfaro and Marshall 2019), which can be the result of genetic variation via heterozygosity and/or phenotypic plasticity.

Introduced invasive populations have been shown to increase plasticity relative to their origin because they can benefit from the phenotypic variation from plasticity, but there are also mature invasive populations that have the same amount of plasticity as their original source (Matzek 2012). These mature populations may have gained genetic variability that

allowed them to adapt locally and reduce the fitness cost of plasticity (DeWitt et al. 1998). I hypothesized that the trend of heterozygosity versus total trait plasticity would decrease in the invasive range because it is a group of populations that has relatively lower mean heterozygosity than the native and landrace ranges (Chapter 3, Alfaro dissertation), and it is known to have suffered multiple genetic bottlenecks (Li et al. 2015).

There is molecular evidence that populations, including the ones I used in this study, can show high genetic variation (Hamrick and Godt 1997, Alfaro dissertation). But, there is the possibility that growers of this seed crop have selected for diverse genotypes with stable phenotypic expression despite varying environmental conditions. (Bradshaw 1965). I therefore hypothesized that the amount of total plasticity would be constant with increasing heterozygosity in the landraces.

Methods

For the native populations, I used microsatellite and plasticity data from Israel, Morocco, and Spain populations (n = 3); for the invasive populations, I used data collected from Arizona and Nevada localities (n = 4); for the landrace populations, I used molecular and phenotypic data from India and Pakistan accessions (n = 3). I obtained the molecular data from two microsatellite loci for *Brassica tournefortii* that was analyzed in Chapter 3; the laboratory methods used to acquire this data set is described in detail in Chapter 3. I used GenAlEx (Peakall and Smouse 2012) to calculate unbiased estimates of heterozygosity (Nei 1972). I obtained the plasticity data from Chapter 2 from a garden study that examined trait variation resulting from variable soil moisture treatments. I first calculated total plasticity for each population by averaging individual trait coefficient of variations (CVs) in Chapter 2 for

rosette diameter, plant height, leaf length, branch length, seed number, relative fitness, reproductive biomass, and fruit mass. Then, I added to the data set heterozygosity values for each population (Chapter 3). I tested the simple model total plasticity = range + heterozygosity + range × heterozygosity via Type 3 ANCOVA (*lm* function, R Core Team 2018) to 1. determine if amount of total plasticity of all individual traits analyzed in Chapter 2 varies with heterozygosity, and 2. if any association is variable among the three ranges. I plotted population mean heterozygosity versus total amount of plasticity using the *ggplot2* package in *R* (Wickham 2016) for native, invasive, and landrace ranges. I performed this graphical visualization to see if allelic diversity in my focal loci resulted in association with a component of phenotypic evolution that I analyzed for my dissertation.

Results and Discussion

With increasing heterozygosity (Fig 1), native populations showed a weak decline in amount of composite plasticity. Theoretical models and empirical information show that the decrease in frequency and adaptive potential of plastic individuals and/or populations can be due to localities that have turned mature and evolutionarily stable (Sexton et al. 2002). As a population gets stabilized, its genetic diversity can decrease due to local adaptation (Bulmer 1971). At that point, when a population reaches maturity, the fitness costs of phenotypic plasticity outweighs its benefit (Bossdorf et al. 2004). A possible scenario could be that native populations I analyzed in this study are ancient localities that have evolved different locally adapted genotypes as their source of phenotypic variation instead of environmentally induced phenotypes.

My results contradict the prediction that total plasticity will have a decreasing trend versus heterozygosity of the two microsatellite loci in the invasive range. A plausible explanation for this pattern is that the two loci I used in my experiment are associated with genomic regions that are used for regulating gene expression (Schlichting and Pigliucci 1993). If this is the case, then increasing heterozygosity can potentially increase the amount of phenotypic plasticity, as I have observed in the invasive range. Further, this means that there is genetic variability associated with evolutionary potential of plasticity in these populations.

My result for landrace *B. tournefortii* is the opposite of my prediction that the trendline of heterozygosity versus overall plasticity will have a zero slope. Instead, I observed an increase in composite plasticity with increasing heterozygosity (Figure 1). It is common for growers and breeders to reduce or eliminate plasticity, but some growers include phenotypic plasticity of agronomic traits in their breeding programs to increase their yields (Peltonen-Saino et al. 2011, Kusmec et al. 2018).

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Table 1. Summary of ANCOVA results

Source of variation	*SS*	df	*F*	*P*
heterozygosity	57.44	1	25.93	0.0038
range	205.31	2	46.33	< 0.001
heterozygosity × range	46.18	2	10.42	0.016

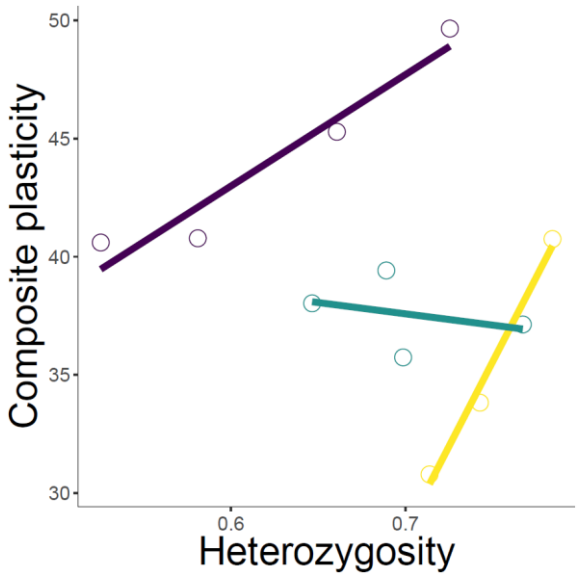

Figure 1. Trendlines of heterozygosity versus total plasticity significantly varied among ranges ($P < 0.001$). Purple = invasive, teal = native, yellow = landrace.

SUMMARY

Before I can identify patterns of phenotypic evolution, the type of traits that are ecologically important and have genetic bases first needed to be identified. Therefore, the impetus for a large portion of my work was to identify these characters experimentally via common greenhouse and garden studies. It was also critical for me to determine how my study species, *Brassica tournefortii*, might respond to environmental variability. So, I used both geographic analysis and experimental data to analyze how aridity and soil moisture affects phenotypic variation, respectively. Next, I also estimated allelic diversity of microsatellites to determine if geographical location that shape phenotypic variability also affects molecular variation. Using these indicators, I was able to answer proximate biological questions, but I also examined evolutionary questions by analyzing how fitness components are affected by phenotypic means and plasticity indices. In addition to quantifying and comparing allelic diversity and differentiation, I also derived migration/gene flow rates from admixture coefficients, to assess if the similarities or divergence of populations are due to connectivity or via local adaptation. Further, I constructed a phylogenetic tree of genetic distances among populations, so I can delineate and better explain possible reasons for divergence or similarities among populations of *B. tournefortii*. In the last chapter, I examined the relationship between heterozygosity and total plasticity to determine if genetic diversity is associated with plastic response. Outlined below are the answers to the main questions for each dissertation chapter.

List of questions and hypotheses

Chapter 1

1. Question: Do the suites of phenology, leaf morphology, branch architecture, size, and reproductive traits vary among ranges, among population nested within ranges, and among maternal families nested within populations within ranges? **Yes.**
2. Question: Which traits have significant fitness functions, and do these fitness functions vary among the native, invasive, and landrace ranges? **Five of the seven composite traits had at least the linear or non-linear trait values associate with fitness, all of the fitness functions significantly varied among ranges.**
3. Question: Do composite trait means (mean factor scores) vary along climate gradients to form clines, and do these potential clines vary among native, invasive, and landrace ranges? **Only for Phenology PC1.**
4. Question: Do composite traits vary in strength of selection among populations along climate gradients, and do regression lines of environmental variation versus selection strength differ among native, invasive, and landrace ranges? **For Phenology PC1, regression lines varied among ranges; Leaf PC2 varied with aridity, but there was no differences in associations between the three ranges.**

Chapter 2

1. Hypothesis: Because of selection for yield stability in domesticated populations, the amount of plasticity due to varying soil moisture in traits related to reproduction, leaf morphology, plant size, and branching architecture, will differ such that native and invasive populations will have linear reaction norms, while landrace populations will have flat or asymptotic reaction norms.

The reaction norms have the following patterns:

Table 1 – Summary of reaction norm patterns

Range	Fruit mass/Reproductive biomass	Leaf traits	Branch architecture
Native	Linear	Asymptotic	Linear
Invasive	Linear	Asymptotic	Linear
Landraces	Asymptotic	Linear	Asymptotic

2. Hypothesis: Because there are likely different levels of genetic diversity among the population types leading to different fitness consequences of plasticity, a) the amount of plasticity due to varying soil moisture in traits related to reproduction, leaf morphology, plant size, and branching architecture, would differ such that invasive > native > landrace populations. **Only lateral branch length varied in amount of plasticity, and invasives had the most plasticity.**
3. Hypothesis: Because of likely lower genetic diversity such that response to environmental variation required plasticity, fitness will increase in value with increased plasticity due to varying soil moisture in branching architecture and leaf traits in the invasive populations more than the native and crop populations. **Only lateral branch length showed association between amount of plasticity and fitness. The differences in the main effects approached significance. Native and invasive populations had declining trends, while landraces increased in fitness with more plastic populations.**

Chapter 3

1. Phylogenetic tree based on population pairwise genetic distances to infer patterns of genetic divergence and evolutionary relationships among study populations of *B. tournefortii*. **Invasive populations were grouped in a large cluster; the other**

cluster contained the native and landrace populations, which were both grouped in two separate clusters or subclades.

2. Hypothesis: The amounts of mean genetic diversity and mean heterozygosity will be in this particular order: native >> landrace > invasive populations. **The amounts of mean genetic diversity and mean heterozygosity were ordered as landrace > native >> invasive populations.**
3. Hypothesis: Focal microsatellites in study populations will be differentiated in this particular order: native < landrace < invasive. **The pattern of microsatellite differentiation and structure showed the pattern: native < landrace < invasive.**
4. Hypothesis: Native accessions will show weak or no gene flow, while the landraces and invasive populations, which are in their same respective regions, will show higher rates of gene flow. **There was a pattern of gene flow that showed native << invasive < landrace, but this was not significant.**

Chapter 4

1. Hypothesis: Microsatellite genetic variation will be associated with overall phenotypic plasticity. **Microsatellite genetic variation was associated with overall phenotypic plasticity.**
2. Hypothesis: The association of microsatellite genetic variation will have different trends among the native, invasive, and landrace ranges. **For the two loci that I analyzed, total trait plasticity increased with increasing genetic diversity in the invasive and native ranges, but plasticity was constant in the landraces. These differences were significant.**

CONCLUSION

While I expected the different *Brassica tournefortii* populations that I studied to genetically and phenotypically vary based on their geographical origins, there were sources of variation that were not straightforward to predict. The possibility for mixed results was inevitable, so in addition to having multiple factors to determine variable phenotypic means, I analyzed and interpreted the patterns of adaptation, the effect of confounding variables, and the relationship of molecular data with phenotypic variation, among the native, invasive, and landrace ranges of my study species. As I expected, the results were complex and multi-faceted, and can be applied to other *Brassica* species that have both wild and domesticated populations. Nonetheless, the populations of *B. tournefortii* that I studied showed both adaptive and maladaptive evolutionary potential.