ADAPTATION TO AGRICULTURE IN A SERIOUS CROP WEED, WEEDY RADISH (*RAPHANUS RAPHANISTRUM*)

By

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PUBLIC ABSTRACT

ADAPTATION TO AGRICULTURE IN A SERIOUS CROP WEED,
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Colonizing new environments requires organisms to shift their traits in response to different environmental conditions in order to survive and persist. This response can be done through phenotypic plasticity (a trait shift in response to the environment), adaptation (a trait shift due to genetic change), or both strategies can be used together, with plasticity "buying time" for adaptation to occur. The colonization of novel environments is especially important to the establishment of agricultural weeds worldwide, which thrive in these extreme environments of intense competition and frequent soil disturbance. In this dissertation, I address the establishment and evolution of a harmful agricultural weed, weedy radish (*Raphanus raphanistrum*) through its divergence from a wild relative of the same species. We found that weedy radish has adapted to the agricultural environment thanks to key trait shifts like faster flowering, taller flowers, and fewer rosette leaves. Additionally, phenotypic plasticity in these traits upon colonizing agricultural fields may have enabled this adaptation to occur. Taken together, these findings work to piece together the history of weedy radish, providing insight into how it initially established in agricultural fields. This work also contributes to our overall understanding of rapid adaptation and phenotypic plasticity in the colonization of novel environments, in agricultural weeds and beyond.

ABSTRACT

ADAPTATION TO AGRICULTURE IN A SERIOUS CROP WEED,
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The colonization of novel environments requires organisms to shift their trait means in response to differing abiotic and biotic conditions in order to survive and persist. This response can be done via phenotypic plasticity (a trait shift in response to the environment), adaptation (a trait shift due to genetic change), or both strategies can be used together, with plasticity "buying time" for adaptation to occur. The colonization of novel environments is especially important to the establishment of agricultural weeds worldwide, which thrive in these extreme environments of intense competition and frequent disturbance. In this dissertation, I address the establishment and evolution of a harmful agricultural weed, weedy radish (*Raphanus raphanistrum*), as well as its divergence from a wild relative of the same species, the native radish ecotype.

I first investigated the hypothesis of phenotypic plasticity "buying time" for adaptation to agricultural fields in weedy radish. Using growth chambers to simulate the ancestral (native) and derived (weedy) environments of weedy radish, I performed a reciprocal transplant with the weedy and native radish ecotypes. I found phenotypic plasticity between environments and genetic divergence between ecotypes to be equally common among traits, suggesting similar importance of plasticity and adaptation in weedy radish establishment. Further, in the majority of traits that were both plastic and differentiated between ecotypes, the direction of change matched, with the weedy environment producing phenotypic shifts in the direction of the weedy ecotype mean. This suggests plasticity in these traits may have enabled the subsequent adaptation and ecotype differentiation, supporting the buying-time hypothesis.

Next, I explored the role of the plant hormone Gibberellic Acid (GA) in the evolution of weedy radish. Using exogenous application of GA both in the greenhouse and in weedy and native growth chamber environments, I found evidence that there has been an evolutionary change in the role of GA in trait expression between the two ecotypes. Namely, weedy radish is less responsive to GA application than native radish, suggesting either upregulation in GA production in weeds, or a lower level of GA required to enable gene expression in the weedy ecotype. This change in gene regulation by GA may have been important in the evolution of weedy radish in the agricultural field.

Finally, I assessed the likelihood of weedy radish diverging from a native ancestor via adaptive evolution. I found that adaptive evolution was likely in the establishment of weedy radish due to increased fitness of the weedy ecotype compared to the native ecotype in the agricultural field. I also found traits under directional selection in the native ecotype, with the key takeaway that faster flowering is adaptive in the agricultural fields. I finally looked at the ability of weedy radish to evolve advanced flowering in the agricultural field via standing genetic variance by artificially selecting for early flowering in native radish. I found that in only two generations of selection, native populations significantly advanced their flowering time, supporting the notion of weedy radish rapidly adapting to agricultural conditions via standing genetic variation alone.

Taken together, these findings work to piece together the evolutionary history of weedy radish, providing insight into its mechanisms of establishment. This work also contributes to our overall understanding of rapid evolution and phenotypic plasticity in the colonization of novel environments, in agricultural weeds and beyond.

Dedicated to plants that grow out of cracks in the concrete

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KEY TO ABBREVIATIONS

GA	Gibberellic Acid
S	Total selection measured via selection differential
β	Direct selection measured via selection gradient
AFFR	Fontfroide Abbey, France (weedy radish population)
AUFI	Aura, Finland (weedy radish population)
AZES	Aznalcázar, Seville, Spain (native radish population)
BINY	Binghamton, New York (weedy radish population)
CLNC	Clayton, North Carolina (weedy radish population)
CNES	Castillo de Calatrava de Nueva, Spain (native radish population)
DAES	Dehesa de Abajo Natural Reserve, Seville, Spain (native radish population)
DEES	Despeñaperros Natural Park, Spain (native radish population)
HCES	Location near Seville, Spain (native radish population)
MAES	Madrid, Spain (native radish population)
NAAU	Naracoorte, Australia (weedy radish population)

INTRODUCTION

Populations colonizing a new environment must respond to novel abiotic and biotic conditions in order to establish and persist. The more novel the environment that they face and the more extreme the selection pressures, the more important it is to respond quickly for the population to persist. There are two, mutually non-exclusive ways for populations to remain in these novel environments: phenotypic plasticity and rapid adaptation. Phenotypic plasticity is the ability of a single genotype to express different phenotypes in different environments (Bradshaw 1965; West-Eberhard 1989), and it alone can be enough for populations to persist in new environments if plasticity is great enough for traits to approach their fitness optima (Gibert, Debat, and Ghalambor 2019). Adaptation, on the other hand, is the result of genetic change that moves traits toward their fitness optima through selection either standing genetic variation or new mutations (Barrett and Schluter 2008). While evolution was formerly thought to only occur over long timescales (Livezey and Darwin 1953), rapid adaptation to novel environments has been clearly demonstrated (Hendry and Kinnison 1999; Reznick and Ghalambor 2001). Being mutually non-exclusive, phenotypic plasticity can enable subsequent adaptation by reducing the probability of extinction in the colonizing population and thereby "buying time" for adaptive evolution to occur (Pfennig 2021; Diamond and Martin 2021). However, how often phenotypic plasticity, rapid adaptation, or both are mechanisms for colonizing novel environments is not clear.

Human-modified landscapes such as agricultural fields are models for evolution over short timescales, and in particular the roles of phenotypic plasticity and adaptation in colonizing populations. Agricultural fields are widespread, with row crops covering 12% of earth's land

(FAO 2003) and are home to selection pressures not found in natural areas. These pressures include herbicides, intense competition from rapidly and uniformly growing crops, and regular, complete aboveground disturbance from tilling and harvesting. Despite these selection pressures, numerous weed species (i.e., unwanted plant species) have nonetheless successfully colonized agricultural fields for as long as agriculture has been a practice (Dekker 1997). The ubiquity of agricultural weeds, along with the fact that these field conditions are similar around the globe, makes agricultural weeds excellent models for studying how plants colonize and adapt to novel environments.

In addition to their value to studying evolution, agricultural weeds are also important to study because of their economic impact and risk to global food security. In the United States alone, the annual cost of agricultural weeds has been estimated at $26.4 billion, with the majority due to crop losses and damage by weeds (Pimentel et al. 2000). Weed control is also costly, with over $10 billion spent on herbicides in 2004. More troubling is the risk to global food security, with food price crises already resulting from yield loss due to weeds, and the threat of more to come (Yaduraju and Rao 2013). Once established, agricultural weeds are exceedingly difficult to manage due to their ability to adapt to control; with over 220 weed species already resistant to at least one herbicide (Heap 2014) and non-chemical control resulting in adaptations like seed-mimicry (Barrett 1983). We have a good understanding of how weeds adapt to human control (De Wet and Harlan 1975; Neve, Vila-Aiub, and Roux 2009; Ellstrand et al. 2010; Moyle and Muir 2010), but far less understanding of how weeds first establish in agricultural fields to become crop competitors.

Agricultural weeds have three general origins that affect their ability to establish in crop fields. Weeds can result from crops that go feral (crop-to-weed route), crops hybridizing with

wild plants (hybrid-to-weed route), or directly from wild plants that colonize agricultural fields (wild-to-weed route; De Wet and Harlan 1975). In weeds with crop origins, the establishing weeds already possess traits that are adaptive in agricultural fields which likely eases this establishment process. The process of establishment in the wild-to-weed route is less clear, especially because the wild ancestors of these weeds tend to be understudied (Vigueira, Olsen, and Caicedo 2013). In particular, it is not clear how often the novel agricultural environment necessitates rapid adaptation for wild populations to persist, as opposed to when wild plants are able to establish via plasticity alone. Therefore, agricultural weeds from wild origins are particularly valuable for studying methods of adaptation to novel environments.

The roles of phenotypic plasticity and adaptation in colonization of novel environments is of particular importance in agricultural weeds. Decades ago, Baker (1974) described the "ideal weed" as having tolerance to a wide range of environmental conditions and phenotypic plasticity, among other traits. Since then, the question of whether adaptation is necessary given generalist nature of weeds has been explored (Neve and Caicedo 2022). Growing evidence suggests that, even in generalist species, adaptation to agricultural fields may still be necessary for population establishment (Clements et al. 2004; Weinig 2005; Kane and Rieseberg 2008). When adaptation is required, these establishing populations need adequate additive genetic variance to respond to selection in the field, or else wait for beneficial mutations to appear (Barrett and Schluter 2008). In these cases, plasticity may be especially crucial for population persistence in the time it takes for plants to adapt. However, the relative importance of phenotypic plasticity and adaptive evolution in agricultural weeds is still not known.

To further explore this topic, I used the agricultural weed *Raphanus raphanistrum ssp. raphanistrum* as my model species for investigating weed evolution. Weedy radish is a model

system in plant ecology and evolution, pollination and herbivory studies in particular (Stanton, Snow, and Handel 1986; Conner and Via 1993; Strauss, Conner, and Rush 1996; Agrawal et al. 2002; Irwin and Strauss 2005). It is a damaging weed in small grain crops, (Schroeder 1989; Hashem and Wilkins 2002) as it has been found to decrease crop yield in wheat by up to 55% (Eslami et al. 2006), has evolved resistance to multiple herbicides (Walsh et al. 2004; Walsh, Owen, and Powles 2007), and is found on every continent except Antarctica (Holm 1997). Weedy radish may also be an ideal model for studying the wild-to-weedy route of agricultural weeds, as according to both cDNA phylogeny (Shen et al. 2013) and molecular marker analysis (Charbonneau et al. 2018), its closest relative is a plant in the same subspecies that is native to the Mediterranean (hereafter "native radish"). As the closest living relative of weedy radish, this native ecotype is a useful proxy of a wild ancestor to weedy radish that can uncover insights in its establishment as an agricultural weed.

In my dissertation research, I have sought to explore evolution in agricultural weeds from wild origins using weedy radish as my study system. In my first chapter I investigate using reciprocal transplants the roles of plasticity and genetic differentiation in the establishment of weedy radish in agricultural fields. In my second chapter, I explore the role of a plant hormone in the evolution of weedy radish. In my third chapter, I look for evidence of adaptive evolution and traits under selection in weedy in active agricultural fields. Finally, in my fourth chapter I assess the standing genetic variance in the native radish ecotype and its potential as a weed predecessor, as well as traits that may have diverged between the weedy and native ecotype via genetic correlations with flowering time. My findings work to piece together the evolutionary history of a damaging agricultural weed and contributes to our overall understanding of the role of rapid evolution and phenotypic plasticity in the colonization of novel environments.

CHAPTER 1:
PLASTICITY-ENABLED COLONIZATION AND SUBSEQUENT LOCAL ADAPTATION IN A SERIOUS AGRICULTURAL WEED

Introduction

Baldwin (1896) first suggested that phenotypic plasticity can be crucial for a population's establishment in a novel environment. Defined as the ability of a single genotype to express multiple phenotypes in different environments (Bradshaw 1965; West-Eberhard 1989), plasticity can reduce the probability of extinction while the colonizing population undergoes adaptive evolution ("buying time" sensu Pfennig 2021 and Diamond and Martin 2021; e.g., Yeh and Price 2004, Price, Yeh, and Harr 2008, Handelsman et al. 2013). However, it is not clear how often this phenomenon of plasticity enabling adaptive evolution occurs, as opposed to when plasticity weakens selection by moving a trait closer to its optimum (Hendry 2016; Gibert, Debat, and Ghalambor 2019). It is therefore important to be able to identify instances where phenotypic plasticity may have played a role in adaptation to a novel environment.

Clear examples of buying time for evolution in nature are limited because demonstrating both that the population in question was exposed to a novel environment and that the population persists in that new environment as a direct consequence of plasticity can be difficult (Diamond and Martin 2021). However, studies that are able to show adaptive plasticity in a novel environment provide good evidence for plasticity buying time. One such method is performing a reciprocal transplant of populations across the ancestral and derived environments and testing for trait plasticity and genetic differentiation in the same direction (Pigliucci and Murren 2003). When the direction of phenotypic plasticity of the population from the ancestral environment matches the direction of trait differentiation in the derived environment, this suggests that plasticity in the derived environment was adaptive (Fig. 1-1). Note that this assumes locally

adapted differentiation in the current derived environment. Examples of this are limited, and a recent meta-analysis of local adaptation studies from Radersma et al. (2020) found that in most cases genetic differentiation did not match phenotypic plasticity. However, a notable exception is in *Prunella vulgaris*, where plasticity for plant height and specific leaf area may have facilitated the colonization of and subsequent adaptation to shady environments (Godoy et al. 2011). Similar examples, albeit where the ancestral vs. derived states are not clear, include plasticity for hindlimb length facilitating adaptation to different habitats in *Anolis* lizards (Losos et al. 2000), and plasticity in the growth habit of *Plantago lanceolata* aiding in the adaptation to both pastures and hayfields (Van Tienderen 1990).

As in the (Van Tienderen 1990) study, agricultural fields are an ideal environment for studying plasticity buying time for evolution. Agricultural fields are a widespread and novel environment, with row crops covering approximately 12% of the earth's land (FAO 2003) and with selective forces not found in nature, such as synthetic fertilizer, herbicides, intense competition from rapidly and uniformly growing crops, and regular, complete aboveground disturbance (e.g., tilling and harvesting). These strong and similar selective pressures in fields worldwide mean that agricultural weeds are excellent models for studying convergent adaptation to novel environments (e.g., Thurber et al. 2013).

Agricultural weeds are also valuable to study because of their economic impact, costing billions of dollars annually due to yield loss and herbicide use (over $10 billion in global herbicide sales in 2004; Oerke 2006). Management practices repeatedly fail in the worst weeds, as the weeds adapt to the selection that is imposed in an attempt to control them. For example, at least 220 weed species have evolved resistance to at least one herbicide type (Heap 2014). Non-chemical control methods can also lead to evolution in weeds, like crop mimicry evolving in

response to hand removal (Barrett 1983). While we understand much about how weeds adapt to human control (Ellstrand et al. 2010; Moyle and Muir 2010; De Wet and Harlan 1975; Neve, Vila-Aiub, and Roux 2009), these control methods are only necessary after weeds have become well-adapted to the agricultural habitat. We have far less information on the mechanisms by which weeds become so well-adapted to the agricultural setting that control becomes necessary.

Weeds have three origins: crops that go feral (crop-to-weed route), crops hybridizing with wild plants (hybrid-to-weed route), and directly from wild plants (wild-to-weed route; De Wet and Harlan 1975). In the first two instances, the weed will already possess traits that are adaptive in agricultural fields from the crop parent, perhaps easing the crucial step of population establishment on the path to becoming a weed. In the case of the wild-to-weed route, even when the plants are colonizers employing a generalist weedy life history strategy (Kane and Rieseberg 2008), they still must adapt rapidly to the extremely novel environment in agricultural fields in order for their population to persist and thrive. Additionally, the mechanisms underlying the wild-to-weedy route are poorly understood because the wild ancestors of these weeds tend to be understudied (Vigueira, Olsen, and Caicedo 2013). Thus, agricultural weeds that developed through this route are an interesting model to study rapid adaptation to a novel environment.

Decades ago, Baker (1965; 1974) described traits he thought to be important for the "ideal weed", which included rapid growth and flowering, self-compatibility, high seed output, tolerance to a wide range of environmental conditions, phenotypic plasticity, short- and long-distance dispersal, and special means of interspecific competition. Since then, few studies have directly addressed the initial adaptations that make agricultural weeds successful, and most that do investigate the crop-to-weed route (Ellstrand et al. 2010). In feral rye (*Secale cereale L*), traits that had diverged from its domesticated predecessor were identified, and included smaller seeds

and delayed flowering relative to cultivars (Burger, Holt, and Ellstrand 2007). Most evidence of adaptive evolution in weeds are in traits that evolved in response to human control, like crop mimicry in barnyardgrass (Barrett and Wilson 1983) and herbicide resistance in weedy *Helianthus annuus* (Kane and Rieseberg 2008), so information on adaptations that are important when weeds from wild ancestors first colonize new environments is still scarce.

In this study, we used *Raphanus raphanistrum* to investigate the mechanisms underlying the rapid adaptation of a plant to the agricultural field habitat. Weedy wild radish (*Raphanus raphanistrum ssp. raphanistrum*) is a model system in plant ecology and evolution, especially in pollination and herbivory studies (Stanton, Snow, and Handel 1986; Conner and Via 1993; Strauss, Conner, and Rush 1996; Agrawal et al. 2002; Irwin and Strauss 2005). Weedy radish also hybridized with crop radish to form the invasive California wild radish (Panetsos and Baker 1967; Hegde et al. 2006). Weedy radish is a damaging weed in small grain crops, especially those in winter annual fields (Schroeder 1989; Hashem and Wilkins 2002), that can decrease crop yield in wheat by up to 55% (Eslami et al. 2006), has evolved resistance to multiple herbicides (Walsh et al. 2004; Walsh, Owen, and Powles 2007), and is found on every continent except Antarctica (Holm 1997). There is evidence of a wild origin for weedy radish, and thus weedy radish could be a model for describing the poorly-understood wild-to-weedy route of agricultural pests. According to a cDNA phylogeny of the *Raphanus* genus (Shen et al. 2013), the closest relative to weedy radish is a plant in the same subspecies that is native to the Mediterranean (hereafter "native radish"). Molecular marker analyses including large numbers of populations also suggest that native radish are most closely related to the weeds, with possible introgression from other members of the *Raphanus* genus (Charbonneau et al. 2018).

Comparisons to the closest living relative of weedy radish will help identify weedy traits that are adaptations to agriculture.

Previous work has identified important differences between the weedy and native ecotype, with Sahli et al. (2008) using Fst/Qst comparisons to provide evidence that more rapid flowering, taller flowers, and fewer rosette leaves were adaptive traits in weedy radish. While native radish overwinters in its native range, often requiring vernalization (cold temperatures) to flower, weedy radish can flower in as few as 20 days from emergence and has no vernalization requirement (Charbonneau et al. 2018) despite commonly (but not exclusively) infesting winter annual crops. Both native and weedy radish respond rapidly to artificial selection for early flowering (Ashworth et al. 2016). Growing primarily in fields with small-grain crops, faster flowering in weedy radish is necessary for it to grow and reproduce in the time between tilling and harvesting. Taller flowering stalks and perhaps larger petals (Sahli et al. 2008) likely evolved in crop fields to increase visibility to pollinators; radish is self-incompatible and thus relies on insect pollinators. Finally, a loss of rosette leaves may have resulted from greater resource allocation to reproduction, and more upright rosette leaves may increase light capture in competition with rapidly-growing crops. This is in contrast to native radish, which grows in natural to disturbed habitats with little surrounding vegetation (Arroyo and Gómez, pers. comm.) and produces overwintering rosettes with many leaves close to the ground.

These trait differences reflect a difference in life history strategy between the ecotypes (Adler et al. 2014), where native plants invest in vegetative growth and flower later, whereas weeds switch their investment to reproduction earlier to flower faster and produce fewer leaves overall. However, genetic differentiation in traits other than flowering time has only been quantified using one population of native radish, so it is unclear how general these differences

are, or if there are other traits of importance that were missed. Additionally, studies up until this point have been in common gardens, so we do not have information on whether plasticity has enabled the colonization of agricultural fields.

Here we use a growth-chamber reciprocal transplant experiment to quantify the relative contributions of genetic differentiation and phenotypic plasticity to trait differences between weedy and native radish. When plasticity and differentiation are in the same direction we interpret this as evidence for adaptive plasticity. Native and weedy plants were grown in conditions simulating each of their home environments. A winter annual growth cycle represented the native home environment, as all *R. r. raphanistrum* are winter annuals in their native Mediterranean habitats. A spring annual growth cycle represented the weedy home environment, although many weedy populations infest winter annual crops, including in the Mediterranean region. The earliest cereal crops were likely winter annuals (Flannery 1973), so initial invasion of wild radish into farmer's fields may well have been as winter annuals; our experiment mimics the subsequent spread of weedy radish into spring annual crops. We found significant genetic differentiation and phenotypic plasticity for flowering time, rosette number, rosette height, leaf length and width, flower height, and ovule number, and flower size; plasticity and differentiation were in the same direction for all but ovule number and flower size, suggesting that plasticity could have aided initial establishment in agricultural fields.

Methods

We chose six populations of *R. raphanistrum* ssp. *raphanistrum* for our study: three of the native winter annual ecotype collected outside active agricultural fields, and three of the weedy ecotype collected infesting crops in both winter and spring annual agricultural fields (Table 1-1). We planted five seeds from each population in each of forty 7.5cm pots, for a total

of 1200 seeds in 240 pots. Growth chambers were used to mimic the temperature and daylength environments of Grenada, Spain (LEGR station) beginning October 1st, and Battle Creek, Michigan (KBTL station) beginning April 1st, the approximate dates that native plants in Spain and weeds in Michigan would germinate. These represent the winter annual native conditions and the spring annual northern temperate weedy conditions respectively. Temperature and day length information was collected from www.wunderground.com, using average maximum and minimum temperatures for the day and night, respectively.

Each environment was replicated across two chambers with 10 plants from each population grown in each chamber. The plants were randomly arranged in four flats per chamber (two per shelf), well-spaced to avoid competition for light. The position of the flats was rotated every Monday, Wednesday, and Friday to reduce the effects of environmental differences within the chamber. After six months all but one CLNC and eight DEES plants growing in spring annual conditions had flowered; at that time these nine plants were combined into a single growth chamber.

The date of emergence from the soil was recorded for each germinant, and pots with no germinants received transplants from pots with excess germinants in the same population and chamber. Seedlings were then haphazardly thinned to one per pot. The date of first flower was also recorded, with flowering time defined as the number of days from emergence to first flower. At the time of bolting (first appearance of buds at the base of the plant), top- and side-view photos of the rosette were taken, the number of leaves in the basal rosette was counted, and the height of the highest point of the rosette from the soil surface was measured. Using rosette photographs, the length and width of the largest leaf from each rosette was measured using ImageJ (Schneider, Rasband, and Eliceiri 2012). The height of the first flower from the soil to

the point of attachment of the pedicel to the stalk was measured on the day it opened. After the third flower opened, the most recently opened flower was photographed from the top and side and the number of ovules was counted under a dissecting scope. For the side photo, the sepal in front of a short stamen was removed, as were the petal to the left and one long stamen to the left in order to get a clear view of the inner floral parts. These pictures were used to measure petal (limb) length and width, corolla tube (claw) length, pistil length, and the lengths of both the anther and filament of one short and one long stamen using ImageJ. These measurements were then used to calculate three composite traits: anther exsertion (long filament length minus corolla tube length; Conner et al 2011), anther separation (long filament length minus short filament length; Sahli and Conner 2011), and flower size (the first principal component of the eight traits mentioned above).

Data were analyzed using ANOVA (JMP, Version 15. SAS Institute Inc., Cary, NC, 1989-2021), with the plant traits as separate response variables and ecotype, environment, their interaction, population (nested within ecotype) and its interaction with environment, and chamber (nested within environment) as predictor variables. Additionally, Tukey's HSD comparisons were made for both ecotypes in both environments. Correlations among response variables were calculated within populations and environments, and then averaged to create an overall correlation table (Table S1-1). Average correlations ranged from -0.6 to 0.71, but most were less than ± 0.2, and only the correlation between leaf length and width was significant based on averaged correlation probability as calculated by JMP.

Results

As expected from earlier studies (Sahli et al. 2008; Charbonneau et al. 2018), most traits measured showed significant genetic differentiation between weedy and native radish ecotypes

(Table 1-2). Averaged across both environments, weedy radish flowered 51.5 days sooner, placed first flowers 4.1cm higher, and made 13.4 fewer rosette leaves that were 1.1cm taller, 2.1cm longer and 1.7cm wider than native radish (Fig. 1-2A-F). The weedy ecotype also produced larger flowers with 1.7 fewer ovules and a smaller anther separation (Fig. 1-2G&H, Fig. S1-2A). The ecotypes did not differ significantly in days to emergence or anther exsertion (Fig. 1-2I & Fig. S1-2B).

There was also significant plasticity for all but the two anther position traits in response to the two chamber environments. Plants emerged 6.0 days later but flowered 37.6 days earlier on average under the spring annual environment than the winter annual environment (Fig 1-2A&I). Plants in the spring annual environment made smaller flowers that were 5.6cm higher and rosettes 1.7cm taller in height but 2.1cm shorter in length and 0.8cm narrower with 2.0 fewer rosette leaves, and 1.7 more ovules (Fig. 1-2C-H). With the exception of ovule number and flower size, for all traits that are differentiated by ecotype, their phenotypic plasticity matched the direction of genetic differentiation (i.e., traits that are higher in the weedy ecotype are also higher in the "weedy" spring annual environment and vice versa).

There were significant ecotype by environment interactions for only two traits, first flower height and leaf length (Table 1-2). The difference in first flower height between weedy and native populations was much more pronounced in the spring annual environment, where weedy plants produced flowers 6.7cm higher than the natives, as opposed to in the winter annual environment where weedy flowers were no longer significantly higher (Fig. 1-2B; Tukey's HSD). The opposite pattern is true for leaf length, with weedy radish only producing significantly larger leaves in the winter annual environment (3.1cm on average), and that

difference shrinks to a non-significant 1.1cm in the spring annual environment (Fig. 1-2E; Tukey's HSD).

Overall, the phenotypic differences were not clearly the result of either plasticity or genetic differentiation, but in most cases were due to a mix of both. The number of rosette leaves does not appear to be a highly plastic trait, and the magnitude of change was much greater between ecotypes than between environments. Days to emergence was the only trait to differ significantly by environment but not by ecotype. Conversely, anther separation showed the opposite pattern and was the only trait to differ significantly by ecotype but not by environment.

Individual populations of wild radish varied significantly within their ecotypes for all traits except flower size and anther separation (Table 1-2, Fig. 1-3, & Fig. S1-3). Populations clustered within ecotypes in these two traits and in days to first flower, number of rosette leaves, and number of ovules. However, populations from different ecotypes were sometimes mixed in the other traits.

The native population DEES had the most extreme native phenotype, flowering the slowest of all populations and with the shortest flowers and the lowest, smallest, most abundant rosette leaves (Fig. 1-3). One notable deviation from the ecotype trend is that DEES actually took longer to flower in the spring annual environment than in winter annual due to some plants requiring vernalization to flower (Fig. S1-1); vernalization started at the beginning of the winter annual treatment but not until 6 months after the start of the spring annual treatment. The native population MAES was slower to flower than any weedy population, but did not require vernalization to flower and is intermediate among native plants for first flower height, rosette number, rosette height, and leaf size. HCES was least like the native ecotype average, flowering almost as fast as any of the weedy populations in the spring annual environment, and having the

tallest flowers (taller than all but BINY) as well as the tallest, largest, least abundant rosette leaves among the native plants, more similar to the weedy populations.

Among the weeds, BINY was the population with the most extreme phenotype as it was on average the fastest to flower with the tallest flowers and the tallest, largest, least abundant rosette leaves. The other two weedy populations, the winter annuals AFFR and CLNC, had similar but less extreme population averages for those traits, with a notable exception in rosette height where both populations' averages are close to the native ecotype mean, and very similar to MAES. In fact, the ecotypic differentiation in rosette height was largely driven by BINY among the weeds, and DEES among the native plants.

Days to emergence and anther exsertion were the only traits for which there were population-level differences, but no overall difference between ecotypes. Days to emergence are similar among all populations in the winter annual environment, but differ in the spring annual environment, with a native population (DEES at 11.8 days) and a weedy population (BINY at 10.4 days) taking the longest to emerge (Fig. 1-3I). Similarly, the population-level differences in anther exsertion are driven by both a weedy and a native population, with AFFR and MAES producing filaments that are shorter than the floral tube, while all other populations have exserted anthers (Fig. S1-3B).

Discussion

The initial colonization and adaptation of wild radish to agricultural fields may have occurred thousands of years ago in the Mediterranean region, with subsequent colonization and local adaptation to cultivated fields worldwide following the spread of agricultural practices. Using native radish to represent the ancestral phenotype, we were able to find evidence that plasticity in many wild radish traits may have aided in its establishment in spring annual

agricultural crop fields. While we do not have fitness data from this study to confirm that phenotypic plasticity was shifting toward the fitness optimum in each environment, a consistent plastic shift toward the derived phenotype in a novel (spring annual) environment suggests that the plasticity may be adaptive. Additionally, earlier flowering time in radish has been shown to be adaptive in agricultural fields (Garrison Chapter 3). Therefore, in traits where phenotypic plasticity and genetic differentiation are in the same direction (Fig. 1-2), plasticity may have aided in population establishment and subsequent adaptation in the novel spring annual environment.

Plasticity in some traits would have been crucial for survival and initial adaptation in agricultural fields. For instance, plasticity in flowering time likely was extremely important for population persistence in agricultural fields so that plants could grow and set seed in the time between when fields were cleared for planting and harvesting (Baker 1974); this time is typically much shorter in spring annual as compared to winter annual crops. Since wild radish is an obligate outcrosser, plasticity for higher flowers may have also aided in initial establishment by enabling radish flowers to be more easily seen by pollinators (e.g., Lortie and Aarssen 1999), especially among rapidly growing crops. Weedy radish has also evolved an increased flower height plasticity (significant ecotype*environment interaction, Fig 1-2B), perhaps as adaptation to variable crop growth in different years or infesting crops of different heights. In the agricultural fields taller and larger rosette leaves may have been important in competing for sunlight among fast-growing crop plants. In each case, plasticity matches genetic differentiation between ecotypes, also suggesting that plasticity for these traits is adaptive.

After phenotypic plasticity potentially enabled establishment in agricultural fields, adaptation in key traits led to genetic differentiation between weedy and native radish. For

instance, in spring annual environments, reducing investment in an unnecessary overwintering rosette by instead producing fewer, larger leaves may have allowed for resources to be allocated to growth and reproduction (Adler et al. 2014). Additionally, the strong ecotypic differentiation for flowering time suggests that plasticity alone was not enough to reach the new fitness optimum. Native radish has standing additive variance for flowering time (Garrison Chapter 3), so selection on this trait, and possibly others, could have resulted in an evolutionary response immediately upon introduction to the novel agricultural environment.

While plasticity matched ecotype differentiation in each of the cases above, there was some variation in how well each population followed the overall ecotype trends. Rosette height was not strongly differentiated between the two ecotypes, and the significant ecotype difference in rosette height seems to be driven mainly by one weed population (BINY) and one native population (DEES). We would expect all the native populations to produce rosettes that are flush to the ground to aid in overwintering. However, given that two of the native populations (MAES and HCES; Table 1-1) were collected near an active agricultural field and in an abandoned orchard respectively, gene flow from weedy populations could be contributing to this more weedy phenotype. However, these populations show similar genetic differentiation from weedy radish populations as DEES at neutral marker loci (Charbonneau et al. 2018). Finally, the other two weed populations (AFFR and CLNC) were collected in winter annual agricultural fields, which might explain their lower rosette leaves.

In some traits, phenotypic plasticity and genetic differentiation did not match, suggesting either plasticity or evolution may not have been adaptive. The weedy ecotype produced larger flowers on average than the native ecotype, but there was phenotypic plasticity for larger flowers in the winter annual (ancestral) environment. As an obligate outcrosser, larger flowers should

benefit both weedy and native radish, so it is not clear what is driving the ecotype difference. However, there was a significant effect of growth chamber on flower size, with the smallest flowers overall produced in one of the spring annual chambers, which may be largely driving the environmental difference. Additionally, ovule number was significantly lower in weedy plants but higher in the spring annual (novel) environment. It is possible that plasticity for more ovules was useful for initial population persistence, while subsequent evolution shifted to a strategy favoring more flowers and fewer ovules (Burd 1999). However, corresponding fitness data is needed to provide further support for this hypothesis.

Our use of growth chambers in this study gave us the opportunity to investigate the trait response to temperature and photoperiod differences in two very different study environments. This excluded all other environmental variables that may have influenced the adaptation of weedy radish, such as competition or nutrient differences. Additionally, our growth chamber conditions limited us to investigating adaptation to the spring annual environment rather than the winter annual crop environment where the initial adaptation of weedy radish likely occurred. A similar reciprocal transplant in agricultural and natural habitats in the Mediterranean region would address these limitations. Still, our results from the growth chambers provide important insight into the colonization and subsequent adaptation of a serious agricultural weed to a novel environment.

The nature of agricultural fields, with some nearly uniform selection pressures worldwide, has led agricultural weeds to converge on many of the same traits, including rapid growth and increased seed set. But studies investigating the ways in which weeds first adapt to agricultural fields are still scarce (Baker 1974; Burger, Holt, and Ellstrand 2007; Ellstrand et al. 2010; Qiu et al. 2017) compared to the number of studies on weed adaptation to modern control

(Heap 2014; Neve, Vila-Aiub, and Roux 2009; Ashley et al. 2003; Jasieniuk, Anita L. Brûlé-Babel, and Ian N. Morrison 1996; Mortimer 1997; Walsh et al. 2004; Roux, Paris, and Reboud 2008; Manalil et al. 2011; Harker 2013). Current weed management practices are unsustainable (Neve et al. 2018), and weed range expansions due to climate change may be severe (Clements and Ditommaso 2011), leading to increased establishment of weed species in agricultural fields worldwide. Therefore, understanding how weeds first adapt to agricultural fields is necessary for taking an evolutionarily-informed approach to weed management, both for controlling established weed populations and for preventing new weeds from emerging. With the ongoing struggle to produce efficient crop yields for a growing world population (Schmidhuber and Tubiello 2007), the importance of studying mechanisms of weed adaptation to novel environments cannot be understated.

In addition to the applied relevance of this study, it also provides insight on the fundamental role of phenotypic plasticity in adaptive evolution. Our study found that both genetic differentiation and phenotypic plasticity contribute to ecotype differences, contrasting with the ideas that either plasticity alone is sufficient for successful colonization of novel environments, or that genetic differentiation will often be hindered by phenotypic plasticity. A recent meta-analysis found that, on average, phenotypic plasticity was more important in explaining phenotype divergence between locally adapted populations (Stamp and Hadfield 2020), but also that the direction of plasticity and differentiation matched in 60% of the traits studied. A previous meta-analysis in plants found adaptive plasticity to be much less common, with only about 33% of the traits measured found to be adaptively plastic (Palacio-López et al. 2015). In each of these cases, however, the authors were not investigating this relationship in

ancestral vs. derived populations specifically, so we cannot extrapolate these findings to answer questions about plasticity buying time for evolution.

The idea that plasticity can actually facilitate adaptive evolution by enabling survival in a novel environment has been discussed for over a century (Baldwin 1896; West-Eberhard 1989; Levis and Pfennig 2016), but there are still limited examples of this phenomenon occurring in nature. A recent meta-analysis by Radersma at al. (2020) investigated the role of phenotypic plasticity in local adaptation by calculating the angle between phenotypic plasticity vectors and vectors of both phenotype divergence (phenotype differences between ecotypes in their home environments; Fig. 1-1) and evolutionary divergence (genetic differentiation between ecotypes in the novel environments). They found that on average phenotypic plasticity did match total phenotype divergence, but not genetic differentiation; note that phenotype divergence includes plasticity (Fig. 1-1). However, many of the studies used in the meta-analysis either used fitness metrics rather than traits that could be adaptively plastic, or did not have clearly ancestral vs. derived ecotypes, so it is still difficult to draw general conclusions about how often plasticity facilitates versus constrains evolution.

Of the 32 studies included in the Radersma et al. meta-analysis, only one found clear evidence of plasticity buying time for evolution following the criteria described here (Godoy et al. 2011). Studies outside the meta-analyses that show evidence of adaptive plasticity enabling evolution are similarly scarce, likely due to the difficulty of identifying ancestral versus derived populations within species. Examples in plants are mainly limited to invasive species colonizing areas outside of their native range or habitat (Williams, Auge, and Maron 2008; Godoy et al. 2011). In animals, examples include both behavioral changes (Yeh and Price 2004; Handelsman

et al. 2013) and morphological changes (Aubret and Shine 2009; Torres-Dowdall et al. 2012; Corl et al. 2018).

As more studies are done that explicitly investigate the relationship between phenotypic plasticity and genetic differentiation between ancestral and novel environments for potentially adaptive traits we can learn more about the extent to which plasticity helps or hinders adaptation in new and changing environments. Studies finding evidence for either instance (plasticity hindering evolution by shifting traits closer to the phenotypic optimum, or conversely buying time for evolution by allowing for population persistence in a novel environment) are rare. This study has shown that overall, both phenotypic plasticity and genetic differentiation underlie the native and weedy radish ecotype differences, and that plasticity was likely a crucial first step in their evolutionary divergence. It joins a small number of studies that find evidence for plasticity buying time in ecotypes that diverged previously, and we hope more studies of this nature follow.

APPENDIX

APPENDIX

Tables and Figures

Table 1-1. The six populations studied.

Population	Ecotype	Location	Location Description	Growth Cycle
DEES	Native	Despeñaperros Natural Park, Spain (38°23'25.18N, 3°29'39.88W)	Natural habitat in a montane park	Winter Annual
HCES	Native	Near Seville, Spain (37°18'12"N, 5° 58'04"W)	Abandoned field near industrial area	Winter Annual
MAES	Native	Near Madrid, Spain (40°39'37.5078"N, 3°46'14.1384"W)	Bordering a cereal field	Winter Annual
AFFR	Weedy	Fontfroide Abbey, France (43° 08'260" N, 2° 53'547" E)	Barley field	Winter Annual
BINY	Weedy	Near Binghamton, NY; USA (42°11'2.3994" N, 75°50'7.08" W)	Alfalfa field	Spring Annual
CLNC	Weedy	Clayton, NC; USA (35°39'54"N, 78°30'31"W)	Winter wheat experimental plots	Winter Annual

Table 1-2. ANOVAs for plant traits as response variables, with environment, ecotype, population (nested within ecotype), and chamber (nested within environment) as main effects, and interaction terms for Ecotype × Environment and Population (nested) × Environment.

Trait	Predictor	DF	Sum of Squares	F Ratio	Prob > F
Days to First Flower	**Ecotype**	**1**	**160164.05**	**96.59**	**<0.001**
	Environment	**1**	**81057.94**	**48.88**	**<0.001**
	Population[Ecotype]	**4**	**124735.89**	**18.81**	**<0.001**
	Ecotype*Environment	1	720.41	0.43	0.51
	Population[Ecotype]*Environment	**4**	**57093.05**	**8.61**	**<0.001**
	Chamber[Environment]	2	537.11	0.16	0.85
First Flower Height	**Ecotype**	**1**	**976.82**	**18.43**	**<0.001**
	Environment	**1**	**1894.84**	**35.76**	**<0.001**
	Population[Ecotype]	**4**	**4981.83**	**23.50**	**<0.001**
	Ecotype*Environment	**1**	**417.06**	**7.87**	**0.006**
	Population[Ecotype]*Environment	4	457.24	2.16	0.07
	Chamber[Environment]	2	1.82	0.02	0.98
No. Rosette Leaves	**Ecotype**	**1**	**10641.63**	**371.84**	**<0.001**
	Environment	**1**	**222.86**	**7.79**	**0.006**
	Population[Ecotype]	**4**	**6764.66**	**59.09**	**<0.001**
	Ecotype*Environment	1	26.66	0.93	0.34
	Population[Ecotype]*Environment	4	184.17	1.61	0.17
	Chamber[Environment]	**2**	**236.79**	**4.14**	**0.017**
Rosette Height	**Ecotype**	**1**	**69.62**	**16.54**	**<0.001**
	Environment	**1**	**165.65**	**39.35**	**<0.001**
	Population[Ecotype]	**4**	**625.56**	**37.15**	**<0.001**
	Ecotype*Environment	1	2.90	0.69	0.41
	Population[Ecotype]*Environment	**4**	**53.54**	**3.18**	**0.014**
	Chamber[Environment]	**2**	**26.97**	**3.20**	**0.042**

Table 1-2 (cont'd)

Trait	Predictor	DF	Sum of Squares	F Ratio	Prob > F
Leaf Length	**Ecotype**	**1**	**215.29**	**26.42**	**<0.001**
	Environment	**1**	**225.07**	**27.62**	**<0.001**
	Population[Ecotype]	**4**	**423.63**	**13.00**	**<0.001**
	Ecotype*Environment	**1**	**49.32**	**6.05**	**0.015**
	Population[Ecotype]*Environment	4	21.08	0.65	0.63
	Chamber[Environment]	2	12.20	0.75	0.47
Leaf Width	**Ecotype**	**1**	**147.18**	**164.32**	**<0.001**
	Environment	**1**	**31.71**	**35.40**	**<0.001**
	Population[Ecotype]	**4**	**91.75**	**25.61**	**<0.001**
	Ecotype*Environment	1	0.67	0.75	0.39
	Population[Ecotype]*Environment	4	7.31	2.04	0.09
	Chamber[Environment]	2	1.00	0.56	0.57
Flower Size	**Ecotype**	**1**	**35.62**	**10.66**	**0.001**
	Environment	**1**	**13.65**	**4.09**	**0.044**
	Population[Ecotype]	4	22.09	1.65	0.16
	Ecotype*Environment	1	0.10	0.03	0.86
	Population[Ecotype]*Environment	4	6.25	0.47	0.76
	Chamber[Environment]	**2**	**33.82**	**5.06**	**0.007**
Ovule Count	**Ecotype**	**1**	**159.84**	**58.12**	**<0.001**
	Environment	**1**	**164.71**	**59.9**	**<0.001**
	Population[Ecotype]	**4**	**63.77**	**5.8**	**<0.001**
	Ecotype*Environment	1	0.15	0.05	0.82
	Population[Ecotype]*Environment	4	2.95	0.27	0.9
	Chamber[Environment]	2	11.94	2.17	0.12
Days to Emergence	Ecotype	1	45.38	1.82	0.18
	Environment	**1**	**2192.73**	**88.02**	**<0.001**
	Population[Ecotype]	**4**	**1056.13**	**10.60**	**<0.001**
	Ecotype*Environment	1	28.33	1.14	0.29
	Population[Ecotype]*Environment	**4**	**255.12**	**2.56**	**0.04**
	Chamber[Environment]	2	65.00	1.30	0.27
Anther Height Difference	**Ecotype**	**1**	**8.24**	**8.38**	**0.004**
	Environment	1	1.81	1.84	0.18
	Population[Ecotype]	4	3.19	0.81	0.52
	Ecotype*Environment	1	0.67	0.69	0.41
	Population[Ecotype]*Environment	4	2.42	0.62	0.65
	Chamber[Environment]	2	1.17	0.59	0.55
Anther Exsertion	Ecotype	1	0.79	0.31	0.58
	Environment	1	4.24	1.66	0.20
	Population[Ecotype]	**4**	**28.20**	**2.76**	**0.03**
	Ecotype*Environment	1	1.82	0.71	0.40
	Population[Ecotype]*Environment	4	3.81	0.37	0.83
	Chamber[Environment]	2	4.69	0.92	0.40

**P< 0.05, **P<0.001, ***P<0.0001*

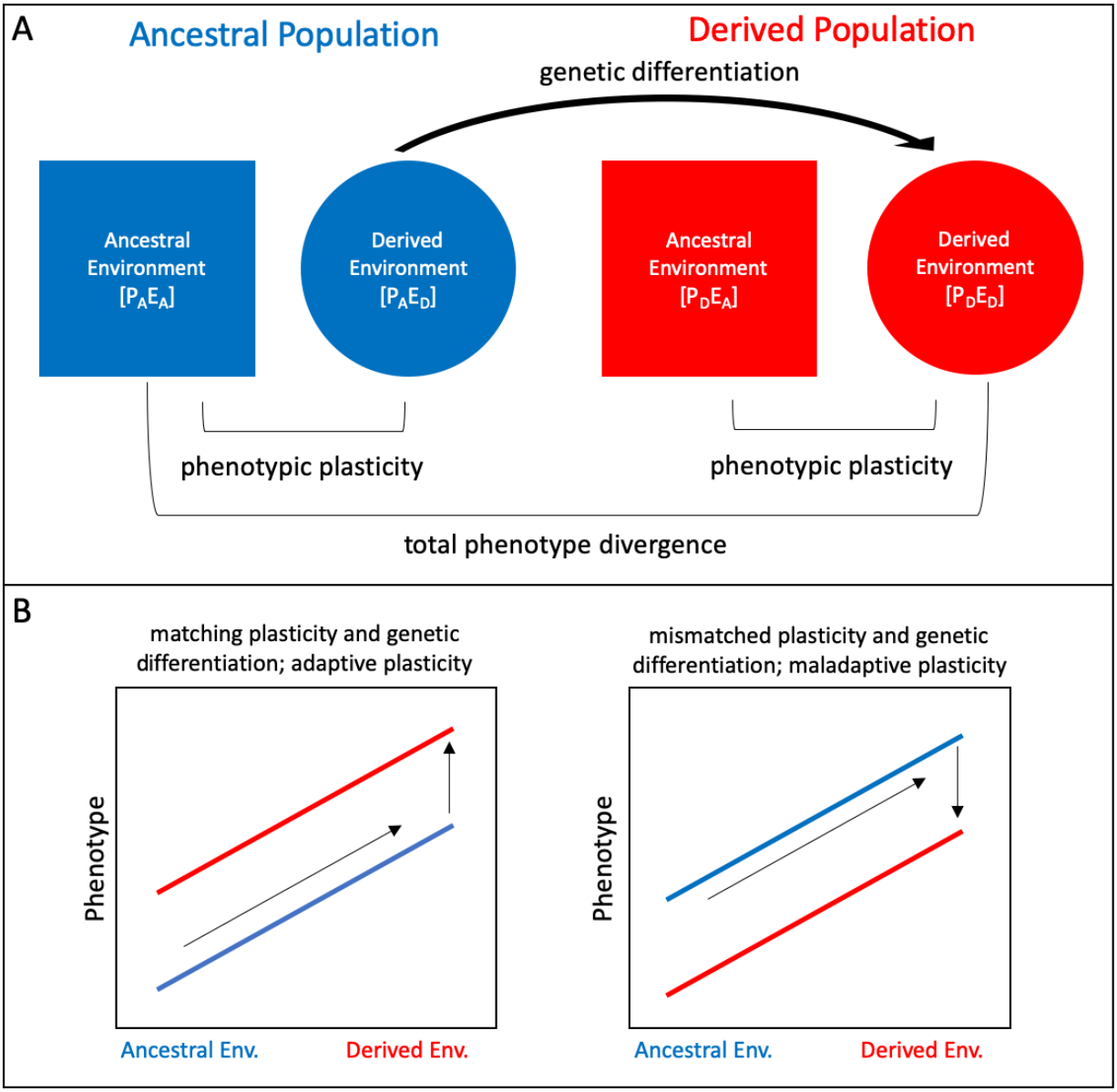

Figure 1-1. The different populations and environments used in a reciprocal transplant and how they can be used to determine whether plasticity was adaptive in the colonization of a new environment. A) Populations from the ancestral environment (E_A) are considered ancestral (P_A), and those from novel environment (E_D) derived (P_D). The genetic differentiation between ancestral and derived populations represents the evolution that occurred since colonization. The total phenotype divergence between ancestral and native populations each in their home environment represent combined effects of differentiation and plasticity. B) When the direction of phenotypic plasticity in the ancestral population matches the direction of genetic differentiation between the ancestral in derived populations in the derived environment, that plasticity is likely adaptive, and may have aided colonization of the derived environment. When plasticity and genetic differentiation are mismatched, that suggests maladaptive plasticity.

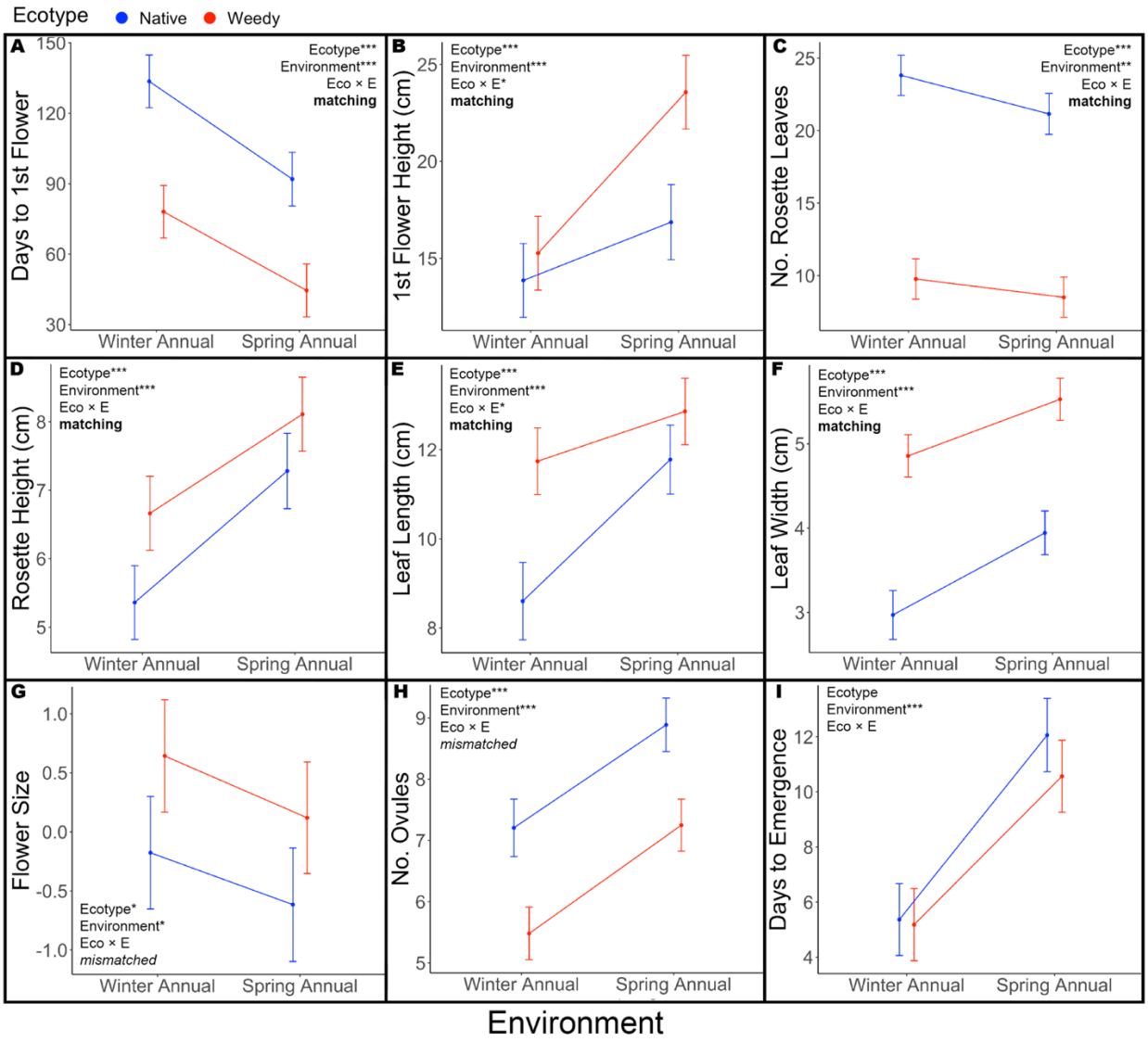

Figure 1-2. Least square means for the two ecotypes in the two environments for A) days to first flower, B) height of first flower, C) number of rosette leaves, D) rosette height from soil to highest leaf point, E) leaf length, F) leaf width, G) flower size, calculated as the first principal component of all floral measurements, H) number of ovules, and I) days to emergence. The labels "matching" and "mismatched" refer to the directions of phenotypic plasticity and genetic differentiation. Error bars are ±2SEM. *$P< 0.05$, **$P<0.001$, ***$P<0.0001$.

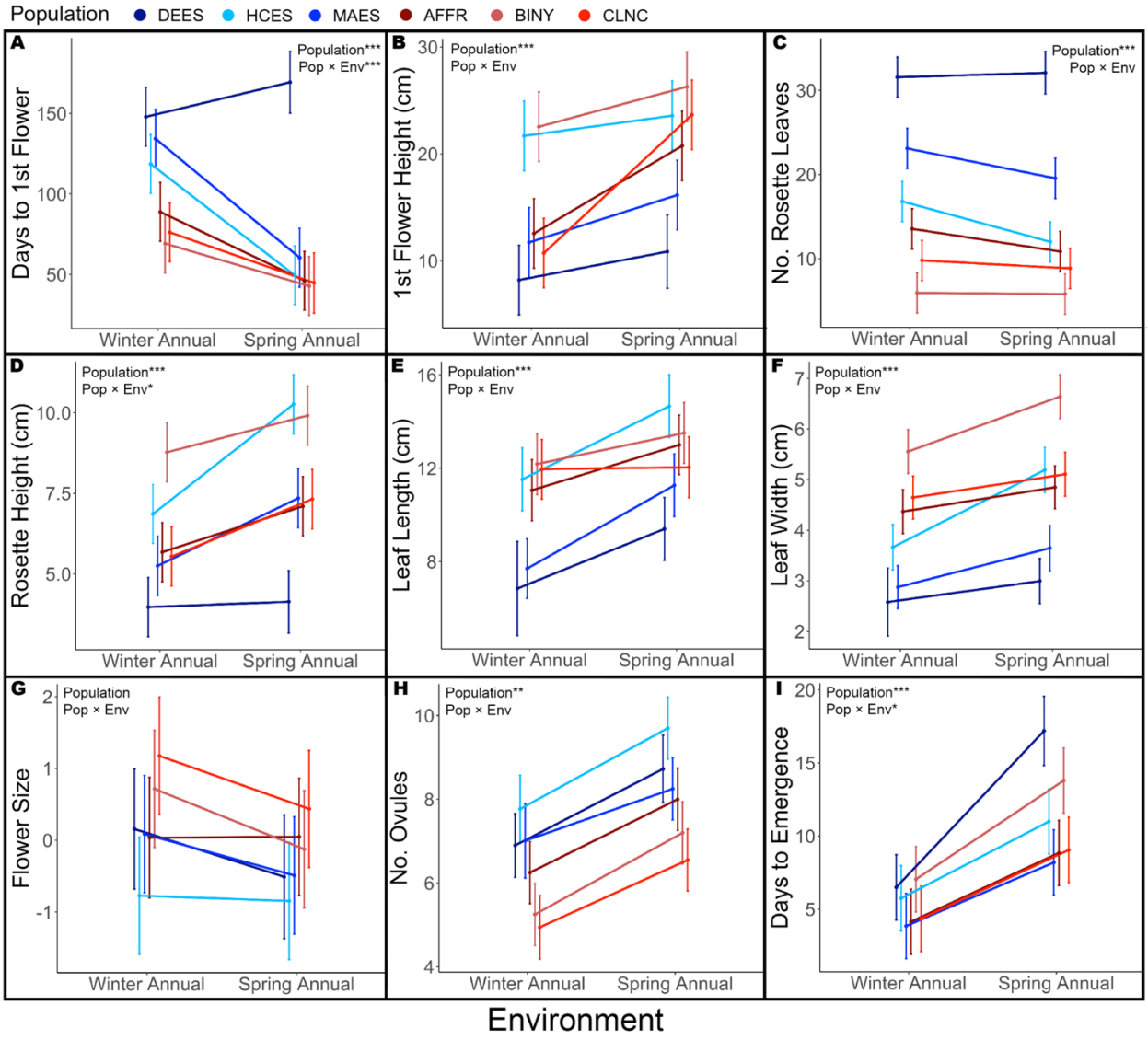

Figure 1-3. Population least square means for A) days to first flower, B) height of first flower, C) number of rosette leaves, D) rosette height from soil to highest leaf point, E) leaf length, F) leaf width, G) flower size, calculated as the first principal component of all floral measurements, H) number of ovules, and I) days to emergence. Native populations are on shades of blue while weedy populations are in shades of red. Error bars are ±2SEM. *$P< 0.05$, **$P<0.001$, ***$P<0.0001$.

Table S1-1. Correlation table of all response variables. Values are averages of 12 correlations, separated by population and chamber environment. Only one p-value average (bolded) was significant, the correlation between leaf length and width (p=0.024).

	Days to First Flower	First Folwer Ht.	No. of Rosette Leaves	Rosette Height	Leaf Length	Leaf Width	Flower Size	Ovule Count	Days to Emergence	Anther Separation
First Flower Height	0.001									
No. Rosette Leaves	0.452	-0.205								
Rosette Height	-0.064	0.346	-0.098							
Leaf Length	-0.188	0.044	0.000	0.277						
Leaf Width	-0.175	-0.003	-0.042	0.292	**0.716**					
Flower Size	0.128	-0.124	0.107	-0.010	-0.025	-0.072				
Ovule Count	-0.171	-0.035	-0.126	-0.028	0.118	0.133	-0.057			
Days to Emergence	0.121	0.048	0.042	0.148	0.049	0.012	-0.015	0.028		
Anther Separation	-0.064	0.028	-0.091	0.063	0.045	0.078	-0.281	0.087	0.027	
Anther Exsertion	-0.058	0.086	-0.064	-0.004	-0.084	0.014	-0.556	-0.094	-0.012	0.322

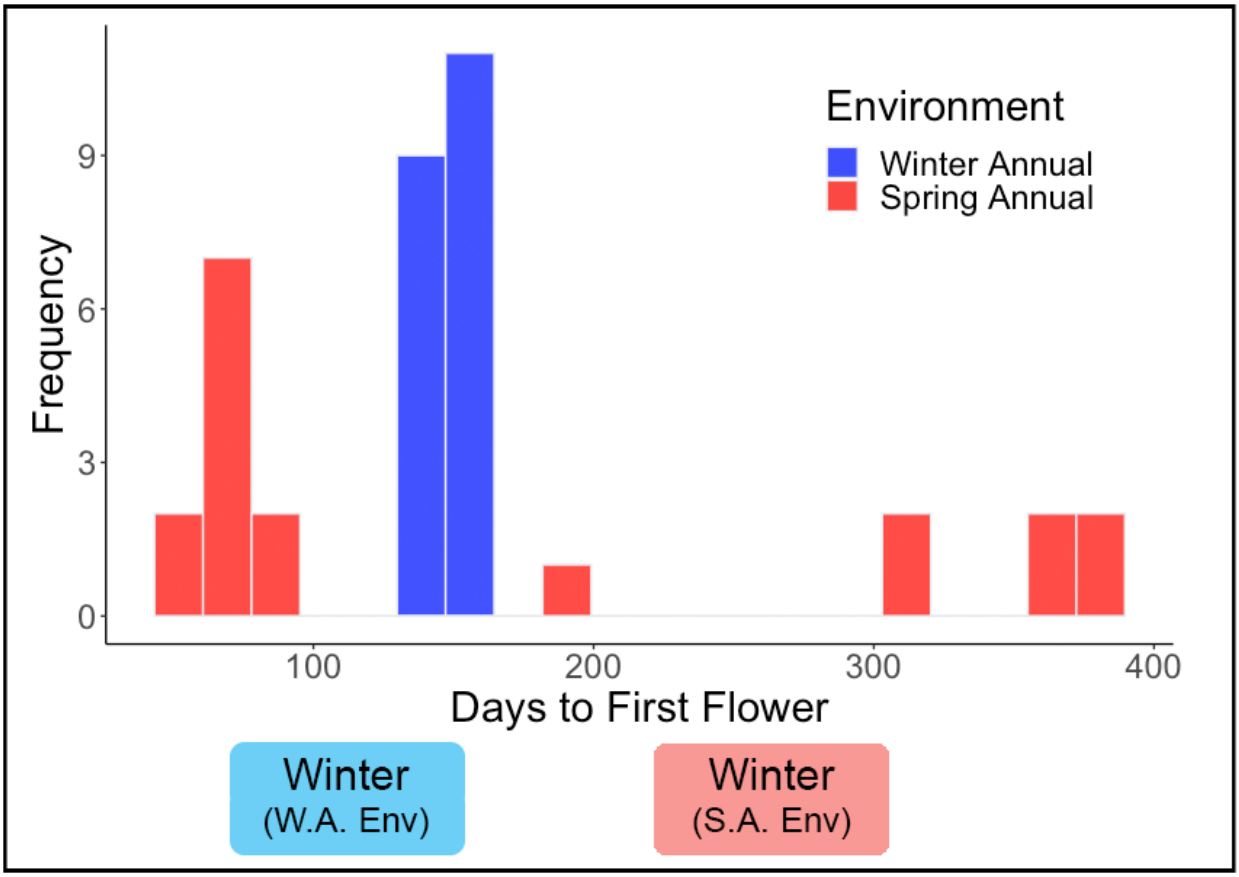

Figure S1-1. Flowering time distribution in native population DEES. Winter vernalization periods (Dec. 1 – Feb. 28) in the Winter Annual (left) and Spring Annual (right) environments are indicated by light blue and red boxes, respectively.

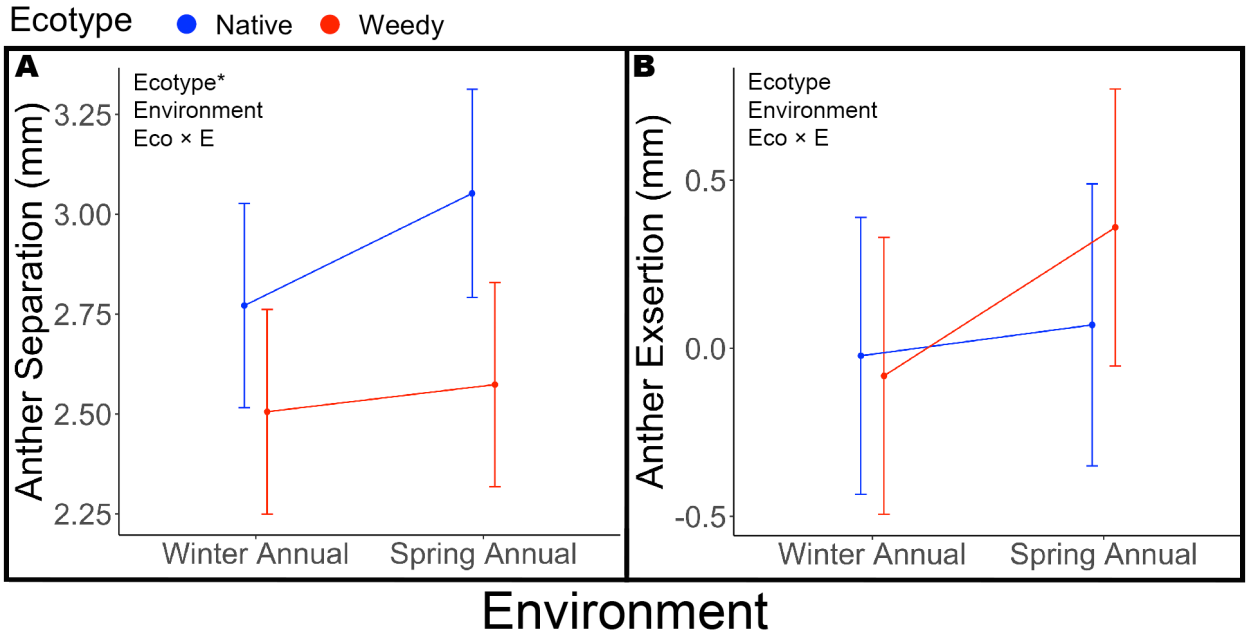

Figure S1-2. Least square means for the two ecotypes in the two environments for A) anther separation calculated as the difference in length of a short stamen filament and a long stamen filament, and B) anther exsertion, calculated as the length of a long stamen filament minus the length of the floral tube. Error bars are ±2SEM. **P< 0.05, **P<0.001, ***P<0.0001*.

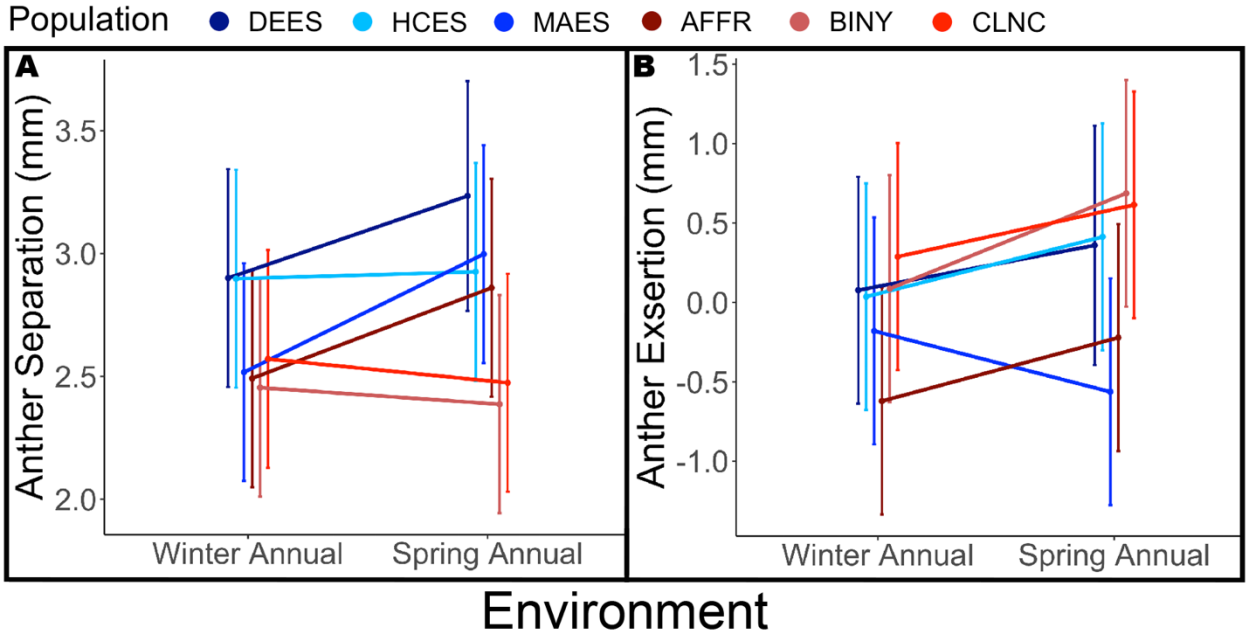

Figure S1-3. Population least square means for A) anther separation calculated as the difference in length of a short stamen filament and a long stamen filament, and B) anther exsertion, calculated as the length of a long stamen filament minus the length of the floral tube. Native populations are on shades of blue while weedy populations are in shades of red. Error bars are ±2SEM.

CHAPTER 2:
PLASTICITY AND ADAPTATION TO AGRICULTURAL ENVIRONMENTS MEDIATED BY GIBBERELLIC ACID IN A SERIOUS WEED

Introduction

When a population experiences a novel environment, either post-colonization or due to an environmental change, it faces both the need to establish in the short-term and to persist in the long-term. Phenotypic plasticity, or the ability of a single genotype to express multiple phenotypes in different environments (Bradshaw 1965; West-Eberhard 1989) can enable a population to survive in a novel environment long enough for adaptive evolution to occur (Baldwin 1896). This concept of phenotypic plasticity "buying time" for adaptive evolution has been described previously (Pfennig 2021; Diamond and Martin 2021 & refs within), but the examples are limited (citations in Diamond and Martin 2021; Garrison Chapter 1). Even less commonly explored are the mechanisms underlying both the phenotypic plasticity and subsequent adaptive evolution that occur in populations buying time in novel environments, and whether the same gene pathways are used in both plastic and genetic change.

Gene expression machinery, and more specifically the induced vs. constitutive expression of certain genes, determine when traits are plastically shifted by the environment vs. traits that are less responsive to environmental variance (López-Maury, Marguerat, and Bähler 2008). A common example of this distinction between induced and constitutive expression is in plant defense, where production of defensive compounds are constitutively higher in populations experiencing frequent herbivory than in plants that experience it less commonly (Heil et al. 2004; Rasmann et al. 2015). Generally, plastic or induced expression will be favored when in variable environments where the selective agent is weak or not consistently present (Via 1993; Scheiner and Callahan 1999), or if the phenotype or trait induction is costly (Lively 1986; Moran 1992). In

populations that are buying time in a novel environment, an inducible trait that confers fitness benefits in the new environment may evolve to be constitutively expressed as the population adapts. But whether this change in trait expression is controlled by a shift from induced to constitutive expression of the same gene networks, or if different gene networks are involved in constitutive vs plastic trait expression, has been less explored (but see Heil et al. 2004).

Agricultural fields are an important context for studying phenotypic plasticity buying time for adaptation. Agricultural fields are a widespread and novel environment, with row crops covering approximately 12% of the earth's land (FAO 2003) and with selective forces not found in nature, such as synthetic fertilizer, herbicides, intense competition from rapidly and uniformly growing crops, and regular, complete aboveground disturbance (e.g., tilling and harvesting). Agricultural weeds from a wild origin have succeeded several times at not only establishing under these extreme selective regimes, but persisting in competition with the crops that are grown there (Vigueira, Olsen, and Caicedo 2013). Although many agricultural weeds are known to have traits in common (e.g., rapid growth and flowering, high seed output, increased competitive ability; Baker 1965; 1974; Ellstrand et al. 2010), few studies address the underlying genetic mechanisms that lead to these common adaptations.

Gibberellic acid (GA) is an important plant hormone that plays a role in growth and development (Srikanth and Schmid 2011; Martins et al. 2018) and changes in its regulation of gene expression may play an important role in weed establishment and adaptation in agricultural fields. Although commonly known for its role in the plant-defense tradeoff (Yang et al. 2012; Smakowska et al. 2016; Li et al. 2022), more broadly it is important in the developmental shift in resource allocation from growth to reproduction (Fornara, de Montaigu, and Coupland 2010; Martins et al. 2018; Srikanth and Schmid 2011). GA has previously been found to speed up

flowering when applied exogenously, also reducing long-term growth structures like overwintering rosettes (Dill, Jung, and Sun 2001). Compared to other gene pathways that control flowering, the GA pathway is less constrained by environmental cues (Fornara, de Montaigu, and Coupland 2010) and could therefore be important for adaptation to agricultural fields where growth and reproduction must be consistently rapid.

We explored the potential role of GA in weed establishment and adaptation to a novel environment via buying time using *Raphanus raphanistrum*, a harmful agricultural weed that is found in crop fields across the world (Holm 1997). Weedy radish likely adapted to agricultural fields from a native ecotype of the same species and subspecies (hereafter "native radish"; see Methods), during which time the two ecotypes diverged (Sahli et al. 2008; Charbonneau et al. 2018). There is also evidence that phenotypic plasticity in some traits may have been crucial to buying time for adaptation to the agricultural environment. A previous growth chamber experiment reciprocally transplanted weedy and native ecotypes across a native radish winter annual growth cycle and a weedy radish spring annual growth cycle (Garrison Chapter 1). There, matching directions of phenotypic plasticity between the ancestral and derived environment and genetic differentiation between the native and weedy ecotype were used to identify traits that may have been adaptively plastic upon initial colonization of agricultural fields, buying time for those populations to adapt. Among the traits measured, early flowering, taller flowers, and fewer, larger, more upright rosette leaves were all found to be adaptively plastic in the agricultural environment (Garrison Chapter 1). The specific mechanisms underlying that plasticity and the ecotype differentiation are still unclear.

In this study, we used exogenous application of GA to weedy and native radish in three different environments to investigate its potential as a mechanism for phenotypic plasticity,

genetic differentiation, or both in weedy and native radish. GA was experimentally applied in both a greenhouse common garden and a growth chamber reciprocal transplant to answer the following questions: (1) do traits show plasticity in response to exogenous GA application? (2) do weedy and native radish differ in their response to GA application? and (3) do trait responses to GA match patterns of ecotype divergence, plasticity between environments, or both? If constitutive upregulation of the GA pathway was involved in the evolution of weedy radish, we expect to find a greater plastic response to GA application the native ecotype, and we expect that shift to be in the direction of weedier phenotypes (i.e., faster flowering, taller flowers, reduced investment in overwintering rosettes). Moreover, if GA is an important mechanism in trait plasticity, we expect trait response to GA application to match trait plasticity between environments.

Methods

Study system

Weedy wild radish (*Raphanus raphanistrum ssp. raphanistrum*) is a model system in plant ecology and evolution, especially in pollination and herbivory studies (Stanton, Snow, and Handel 1986; Conner and Via 1993; Strauss, Conner, and Rush 1996; Agrawal et al. 2002; Irwin and Strauss 2005). It is also is a serious weed in small grain crops, especially those in winter annual fields (Schroeder 1989; Hashem and Wilkins 2002), that can decrease crop yield in wheat by up to 55% (Eslami et al. 2006), and has evolved resistance to multiple herbicides (Walsh et al. 2004; Walsh, Owen, and Powles 2007). There is also evidence of a wild origin for weedy radish, and thus weedy radish could be a model for describing the poorly-understood wild-to-weedy route of agricultural pests; according to a cDNA phylogeny of the *Raphanus* genus (Shen et al. 2013), the closest relative to weedy radish is a plant in the same subspecies that is native to the

Mediterranean. Molecular marker analyses including large numbers of populations also suggest that native radish are most closely related to the weeds, with possible introgression from other members of the *Raphanus* genus (Charbonneau et al. 2018). Comparisons to the closest living relative of weedy radish will help identify weedy traits that are adaptations to agriculture.

Some key trait differences between native and weedy radish have been identified in previous work. Sahli et al. (2008) found using Fst/Qst comparisons that more rapid flowering, taller flowers, and fewer rosette leaves were likely adaptive traits in weedy radish. Faster flowering is beneficial in weedy radish, which must grow and reproduce in the time between tilling and harvesting of the crop fields they occupy, while taller flowers may enable them to be seen by pollinators among tall cereal crops. Native radish on the other hand overwinters in its native range and often require vernalization (cold temperatures) before flowering (Charbonneau et al. 2018). Additionally, growing in more sparsely vegetated areas (Arroyo and Gómez, pers. comm.) does not need to grow as tall. Similarly, native radish also produce an overwintering rosette comprised of many leaves that grow low to the ground, whereas weedy radish produce a more upright rosette with fewer leaves. This loss of rosette leaves may have resulted from greater resource allocation to reproduction, and more upright rosette leaves may increase light capture in competition with rapidly-growing crops.

Greenhouse Common Garden

We chose four populations of *R. raphanistrum ssp. raphanistrum* for our common garden: two of the native, winter annual ecotype collected in non-agricultural lands, and two of the weedy ecotype collected from active agricultural fields (Table 2-1). The weedy radish populations were chosen for their geographic diversity, with one occurring in a winter barley field in the native range (AFFR), and the other in a summer alfalfa field far outside of the native

range (BINY). Native populations were chosen for their diversity in habitat, with one growing in a montane park (DEES) and the other in a woodland (AZES). All populations were also included in previous experiments. Seeds were planted on June 1, 2017 in proportion to their previously calculated germination rates into 32 pots per population, with 128 pots total. Excess seedlings were transplanted to pots with no germinants or randomly thinned to one plant per pot. Plants were grown in the greenhouse with natural light as well as supplemental metal halide lights set to 16-hour days. After some early mortality, 106 plants survived to at least bolting to be used in the analyses.

Half the plants in each population were randomly assigned to either the GA treatment or control. Plants were then sorted into 16 flats in a stratified random design, with eight flats of each treatment type each containing two plants per population. Flats were arranged alternating between treatments (Figure S2-1), and rotated one position clockwise every week to reduce the effects of environmental variation within the greenhouse. Once the first two true leaves emerged, experimental plants were sprayed every other day with a single spray (~0.625mL) of 100μM gibberellic acid (GA), which covered the surface of the leaves. Control plants were similarly sprayed with one spray (~0.625mL) of DI water. To avoid contamination of the controls by GA, the experimental flats were moved aside before they were sprayed, and then left for approximately 10 minutes before returning them to the main bench.

Growth Chamber Reciprocal Transplant

The four *R. r. raphanistrum* populations we chose for the reciprocal transplant were the same two native populations AZES and DEES plus two new weedy populations AUFI and NAAU (Table 2-1). While the same weedy populations could not be used between the two experiments due to a lack of seeds, the AUFI and NAAU were similarly both geographically

diverse (Finland and Australia, respectively) and represent weeds from both spring and winter annual fields (Table 2-1). As in the greenhouse study, seeds were planted in proportion to their previously calculated germination rates into 80 pots per population, with 320 total pots, and excess seedlings were transplanted to pots with no germinants or randomly thinned to one plant per pot. All plants survived to at least bolting, for a total of 320 plants used in analyses.

Following the methods of Garrison (Chapter 1), growth chambers were used to mimic the temperature and daylength environments of Grenada, Spain (LEGR station) beginning October 1st, and Battle Creek, Michigan (KBTL station) beginning April 1st, the approximate dates that native plants in Spain and weeds in Michigan would germinate. These represent the winter annual native conditions and the spring annual northern temperate weedy conditions respectively. Temperature and day length information was collected from www.wunderground.com, using average maximum and minimum temperatures for the day and night, respectively.

Each environment was replicated across two chambers with 20 plants from each population grown in each chamber. The plants were randomly arranged in four flats per chamber (two per shelf), well-spaced to avoid competition for light. Two of those flats were assigned to the GA treatment and the other two were the control. The position of the flats was rotated every Monday, Wednesday, and Friday to reduce the effects of environmental differences within the chamber. Once the first two true leaves emerged, experimental plants were sprayed every other day with a single spray (~0.625mL) of 100μM gibberellic acid (GA), and control plants sprayed with one spray (~0.625mL) of DI water. To avoid contamination of the controls by GA, the experimental flats were moved out of the growth chambers before they were sprayed, and then left for approximately 10 minutes before returning them to the chambers.

Data Collection and Analyses

For each plant, we recorded the date of emergence and the date of first flower, with the number of days to first flower calculated from date of emergence. On the day of bolting (when flower buds were first seen), we counted the number of rosette leaves and the rosette height as the vertical distance from the soil to the highest point of the freestanding rosette. On the day the first flower opened, we recorded the first flower height as the vertical distance from the soil to where the flower's pedicel connects to the stem. We collected the third flower of every plant (or the most recently opened flower after the third) to take side-view and overhead photos of the flower. The number of ovules was also measured on the third flower by gently pressing the pistil between two glass slides and counting the visible ovules under a dissecting scope. Plants were discarded after the third flower was collected.

The floral photographs were used to measure eight floral traits using ImageJ (Schneider, Rasband, and Eliceiri 2012): petal width and length, corolla tube length, length of long and short filaments, length of long and short anthers, and length of pistil. These measurements were then used to calculate the composite trait flower size, which is the geometric mean of the eight floral traits. Additionally, we used these traits to calculate anther exsertion (the difference between long filament length and flower tube length) and anther separation (the difference between long filament length and short filament length).

For the greenhouse common garden, data were analyzed using an ANOVA, testing for differences in the traits listed above (response variables) with respect to plant Ecotype (native or weedy), GA Treatment (+GA or DI water control), and their interaction. The test also included population (nested within ecotype) and Population[Ecotype]*GA Treatment. In the growth chamber, a similar multi-way ANOVA was used with the additional main effect of chamber

environment, random effect of chamber nested within environment and new interaction terms Ecotype*Environment, Environment*GA Treatment, and Ecotype*Environment*GA Treatment.

Results

Trait differences by ecotype and environment

The weedy radish ecotype differed from the native ecotype in most traits measured in agreement with previous work (Sahli et al. 2008; Charbonneau et al. 2018). Averaged across treatments, weedy radish rosettes contain fewer leaves that in the greenhouse were larger and more upright than native radish rosettes (Table 2-2; Fig. 2-1). Weedy radish flower faster and grow taller than the native ecotype, and in the chamber also produce smaller flowers with fewer ovules (Table 2-2; Fig. 2-2).

In the growth chamber, many traits that differed by ecotype also were plastic between winter and summer annual environments, with the ecotype differentiation matching the plasticity in all but one trait (Table 2-2 & Table S2-2; Fig 2-1, Fig. 2-2, & Fig. S2-3). Weedy radish on average flower 37 days faster and produce 0.61mm smaller flowers that are 8.8cm taller than the native ecotype, as well as leaves that are 0.83cm wider. Similarly, in the weedy spring annual environment both ecotypes plastically produced smaller (0.88mm) taller flowers (4.2cm) more quickly (50 days) with leaves 0.63cm wider than in the environment that matches the native growth conditions (winter annual). Ovule count, which also differed by both ecotype and environment, did not fit the pattern as the weedy ecotype produces 1 fewer ovule on average, while plants in the weedy spring annual environment produce 1 more ovule on average.

Plasticity between growth chamber environments did differ between ecotypes for several traits, and in most of these cases plasticity was greater in the weedy ecotype (Ecotype*Environment interaction; Table 2-2 & Table S2-2). In the spring annual environment,

both ecotypes tended to make longer and wider leaves (Fig. 2-1), flowered faster (Fig. 2-2), and produced smaller flowers (Fig. S2-3) compared to the winter annual environment, but these differences were greater (and more often significant) in the native ecotype (Table S2-4). Rosette number and height also had a significant Ecotype*Environment interaction but these traits did not differ by environment overall (Table 2-2).

Trait response to Gibberellic Acid treatment

We investigated the effect of exogenous GA application on wild radish plants across two ecotypes and three environments. All traits significantly responded to GA application in the growth chamber, and all but three in the greenhouse, and in all cases this response was in the direction of the more weedy phenotype. We found an overall effect of the GA treatment on wild radish that resulted in fewer, more upright, and longer rosette leaves compared to control plants (i.e., a significant GA Treatment effect in greenhouse and growth chamber environments; Table 2-2; Fig. 2-1). In the greenhouse, rosettes treated with GA had 4.1 fewer leaves that were 2.5cm more upright and 1.3cm longer (Fig. 2-1, column 1). In the growth chamber environments, these differences were even larger, with GA plants producing 5.9 fewer rosette leaves that were 3.4cm more upright and 1.7cm longer (Fig. 2-1, columns 2&3). Additionally, GA treatment also had a significant effect on leaf width in the growth chambers, resulting in rosette leaves that were 0.6cm wider on average compared to control plants (Fig. 2-2K&L); greenhouse plants showed a marginally significant difference in the same direction.

We also found an overall treatment effect of GA on flowering time, with plants across all environments and ecotypes flowering faster on average under the GA treatment compared to control (Table 2-2). This difference was larger in the greenhouse than in the chamber (32.5 vs. 9.1 days faster; Fig. 2-2). We saw a significant overall GA treatment effect on flower height and

ovule count in the chamber only; plants treated with GA produced flowers that were 7.0cm taller (Fig. 2-2E&F) with 1.0 more ovules on average (Fig. 2-2H&I).

Native and weedy radish ecotypes differed significantly in their response to GA treatment (i.e., a significant Ecotype*GA Treatment interaction; Table 2-2; Table S2-1) for the majority of traits, with weedy radish less responsive to GA application in each of these cases. In each of these cases, the trait response of native radish to GA application was in the direction of the weedy radish trait mean. Only three traits, number of rosette leaves, rosette height, and flower height, showed a significant response to GA in the weeds as well as the natives, and in these cases the trait shift is generally greater in the native ecotype (Fig 2-1A-F; Fig. 2-2D-F).

The growth chamber environments also affected the response to GA in some traits (i.e., Environment*GA Treatment interactions). Leaf width and flowering time responded more strongly to GA in the winter annual environment (Table 2-2). Radish in the GA treatment flowered 12.1 days sooner on average in the winter annual environment, and only 6.1 days sooner in the spring annual environment (Table 2-2; Fig. 2-2B&C). Similarly, in the winter annual environment the average leaf width of GA plants was 2.0cm greater than control plants, and this difference shrinks to 1.5cm in the spring annual environment.

Finally, there was one trait, leaf length, that differed in its response to GA application by both environment and ecotype (i.e., significant Ecotype*Environment* GA Treatment interaction; Table 2-2). In the winter annual environment, GA application led to an increase in leaf length in the native phenotype only, to approximately the same width as the weedy radish in the same environment (Fig. 2-1H). However, in the spring annual environment, leaf length in native radish under the GA treatment actually exceeded the leaf length of weedy radish in that environment (Fig. 2-1I). This is the only example of GA application leading to a more extreme

trait value in the native ecotype compared to the weedy, but the trait change was still in the direction of the more weedy phenotype.

Discussion

By applying gibberellic acid (GA) exogenously to native and weedy radish in different environmental conditions, we have uncovered patterns that suggest GA may be responsible for both evolved differentiation and plastic shifts in phenotype in the evolution of a harmful agricultural weed. Namely, the fact that nearly all traits in weedy radish were less responsive to GA application than in their native counterpart (Fig. 2-1A-L; Fig. 2-2A-F) suggests an evolutionary change in the role of GA in trait expression. Additionally, an advance in flowering time in the native ecotype after the application of GA reflects both ecotype differences between native and weedy radish, as well as plastic responses between growth environments, further indicating a role of GA in the evolution of an important trait in weedy radish. These findings add to a large body of research in other systems that show similar plastic responses to GA application, but are some of the first to show evolutionary change in gene regulation by GA.

The particular mechanism by which the GA pathway has likely evolved in weedy radish is not clear, but there are two probable means. Weedy radish may have evolved to constitutively produce higher levels of GA than native radish, which is one way to explain the overall lower level of response to exogenous GA application in weedy radish. Alternatively, GA production levels may be similar in weedy and native radish, but many traits in weedy radish may require less GA for gene expression than in native radish. This mechanism is more likely given the trait response differences among weedy radish populations, as well as both diversity of proteins involved in the GA pathway (Blázquez, Nelson, and Weijers 2020) and of trait responses that GA regulates (Martins et al. 2018; Pavlista, Santra, and Baltensperger 2013). To test this, it will

be important in future studies to compare GA levels in weedy and native radish that express similar phenotypes in order to determine if the two ecotypes differ in the level of GA necessary to elicit the same response, as in (Heil et al. 2004).

In weedy radish, rapid flowering is an important adaptation for persistence in agricultural fields where there is regular, extreme disturbance at the times of tilling and harvesting. Further, in the evolution of weedy radish, plasticity for early flowering was likely very important for initial survival as it colonized agricultural fields (Garrison Chapter 1). In this study, we found the weedy ecotype flowered much more rapidly than the native ecotype, and that genetic differentiation matched the direction of plasticity between the weedy and native environments, with all plants flowering faster in the weedy spring annual environment compared to the winter annual. Additionally, the GA-induced shift toward earlier flowering in native but not weedy radish may reflect an evolutionary change in trait expression, with GA as a potential mechanism for both the initial plastic response and the subsequent adaptation to a colonized agricultural field (Fig. 2-2A-C). Native radish on average flowers more slowly than weedy radish, but can be readily induced to accelerate flowering with GA application, whereas weedy radish has constitutively increased its flowering speed, as is required in agricultural fields to produce seeds between planting and harvest. Additionally, native radish often requires vernalization in order to flower (Charbonneau et al. 2018), so a change in the regulation of GA pathway, which is less constrained by environmental cues like photoperiod and temperature (Fornara, de Montaigu, and Coupland 2010), may have enabled the loss of this requirement in weedy radish.

The significant interaction between chamber environment and GA treatment in flowering time also suggests that GA may play a role in plasticity for flowering time (Table 2-2). Specifically, the plastic response of slower flowering in the winter annual environment is

significantly reduced by the application of GA in the native ecotype, which brought the flowering time closer to the spring annual mean. However, if GA were a major mechanism of plasticity for flowering time between environments, we would expect to see greater response to GA in the winter annual vs. spring annual environment. While the native radish response in the winter annual environment was an 17.9-day advance, compared to a 11.4-day advance in the spring annual environment, this difference is not significant (i.e., no Ecotype*Environment* GA Treatment interaction). Additionally, the effect of the environment is not completely mitigated by GA application, which suggests other mechanisms besides the GA pathway are likely involved in the plastic response in flowering time.

Flowering time was only one of six important traits that has appeared to evolve a different response to GA in weedy vs. native radish (i.e., significant Ecotype*GA Treatment interaction), with the response being smaller or non-significant in weedy radish in each case. The majority of the other traits to respond are related to the rosette: number of rosette leaves, rosette height, leaf length, and leaf width. In other systems, GA application has been found to reduce the production of new leaves (Chen et al. 2003) and in winter annual species specifically, to reduce the overwintering rosette (Dill, Jung, and Sun 2001). In weedy radish, the low overwintering rosette (Fig. S2-2) has been lost after colonization of agricultural fields, where fewer, larger, more upright rosette leaves may be beneficial for competing for sunlight among dense crops, before quickly shifting to reproduction.

It is also striking to note the similarity between rosette number (Fig. 2-1A-C) and flowering time (Fig. 2-2A-C) responses. The pattern we see of faster flowering and fewer rosettes in the weedy ecotype and the GA treatment is due to a switch from vegetative to reproductive growth (Geber 1990), reflecting the more rapid lifecycle in weedy radish that can

be induced by GA application. Despite these similarities, the within-environment and within-population correlation between flowering time and rosette height was found in a previous study to be only 0.45 (Garrison Chapter 1). Additionally, these traits differ in their response to environment, where flowering time advances in the spring annual, but rosette number does not decrease. It is worth noting that this is in contrast to a previous experiment in this system, which found a reduction in both rosette leaf number and height in the spring annual environment compared to the winter annual (Garrison Chapter 1).

The last trait to respond differently to GA application by ecotype was flower height, which did respond significantly to GA in both ecotypes in the growth chamber environments, but the response of taller flowers was smaller in the weedy ecotype. As an obligate outcrosser growing among agricultural weeds, taller flowers may have been important in the evolution of weedy radish in order to attract pollinators (Sahli and Conner 2011), and we do indeed see that the weedy ecotype produces taller flowers than the native ecotype in all three environments (Fig. 2-2D-F). There is additional literature indicating that GA application may result in higher flowers (Sajid et al. 2016). The differing ecotype responses to GA in this trait and the five others mentioned above reflect the evolved differentiation in these traits between ecotypes, pointing to GA as a potential mechanism behind these differences.

Ovule number was the only trait to respond to GA that did not show a different response by ecotype (Table 2-2). This trait does not consistently differ by ecotype in radish (Sahli et al. 2008), nor was there a significant ecotype difference in the greenhouse. However, we did find that GA application in the spring annual chamber environment led to fewer rosettes, as well as fewer ovules in the weedy ecotype (Fig. 2-2H&I). GA application has been found to decrease seed number per fruit, but also increase overall fruit set (Ogilvie et al. 1991), so the response

found in this study may represent a GA-mediated shift to higher flower production, but total flower number or seed set were not recorded in this study.

Although the GA pathway seems to have been involved in weedy radish evolution given the trait shifts we saw, it is unlikely to be the only mechanism involved. Other likely candidate genes are Flowering Locus T (FT), which is an important flowering time gene that is strongly influenced by day length (although it also worth noting that this gene has been found to be influenced by GA levels; Hisamatsu and King 2008), as well as Flowering Locus C (FLC) and FRIGIDA (FRI), which both play a role in the vernalization requirement for flowering that has been lost in weedy radish (Srikanth and Schmid 2011).

The physiological effects of GA have been well-defined in a large number of plant species, but less attention has been given to the role of GA in evolution. Using the species *Raphanus raphanistrum*, we present evidence of GA as a potential mechanism in the evolution of a weedy ecotype, particularly the shift toward faster flowering and reduced investment in rosette leaves. Most strikingly, we found that in the majority of traits measured, native and weedy radish differ in their response to GA application, suggesting a change in how the GA pathway regulates these traits. Finally, the trait shifts found in this study match both plastic shifts between growth environments and genetic differentiation between radish genotypes, suggesting GA may have been involved in not only the initial plastic response in the colonization of agricultural fields, but also the evolved ecotype differences that resulted in the subsequent adaptation of weedy radish.

APPENDIX

APPENDIX

Tables and Figures

Table 2-1. The six populations of *R. raphanistrum* studied.

Population	Ecotype	Location	Location Description	Experiment Used
AZES	Native	Seville, Spain (37°15'26.31" N, 6° 13'15.47" W)	A wild pine-olive tree-Quercus woodland; winter annual	Greenhouse and chamber
DEES	Native	Despeñaperros Natural Park, Spain (38°23'25.18" N, 3°29'39.88" W)	Natural habitat in a montane park; winter annual	Greenhouse and chamber
AFFR	Weedy	Fontfroide Abbey, France (43°08'260" N, 2°53'547" E)	Barley field; winter annual	Greenhouse
BINY	Weedy	Binghamton, NY; USA (42°11'2.3994" N, 75°50'7.08" W)	Alfalfa field; spring annual	Greenhouse
AUFI	Weedy	Aura, Finland (60°38'54" N, 22°33'53" E)	Pea field; spring annual	Chamber
NAAU	Weedy	Naracoorte, Australia (140°73' E, 36°96' S)	Agricultural field; winter annual	Chamber

Table 2-2. Selected ANOVA results from both the greenhouse and growth chamber experiments. Only traits with a significant effect of GA treatment in at least one experiment were included (see supplemental tables for full ANOVA results). For both experiments the main effects of plant ecotype and treatment and their interaction are shown. From the growth chamber experiment spring vs. winter annual environment and all possible interactions between the three main effects are also included. Bold values indicate significance at a level of $p < 0.05$.

		Greenhouse Experiment		Growth Chamber Experiment	
Trait	Predictor	F Ratio	Prob > F	F Ratio	Prob > F
# Rosette Leaves	**Ecotype**	**107.55**	**<0.001**	**1120.61**	**<0.001**
	GA Treatment	**21.04**	**<0.001**	**325.59**	**<0.001**
	Ecotype*GA Treatment	**5.47**	**0.022**	**173.11**	**<0.001**
	Environment			0.09	0.762
	Ecotype*Environment			**4.25**	**0.040**
	Environment*GA Treatment			0.05	0.818
	Ecotype*Environment*Treatment			2.65	0.104
Rosette Height	**Ecotype**	**23.47**	**<0.001**	0.13	0.715
	GA Treatment	**29.29**	**<0.001**	**286.98**	**<0.001**
	Ecotype*GA Treatment	**9.56**	**0.003**	**64.26**	**<0.001**
	Environment			1.42	0.234
	Ecotype*Environment			**5.32**	**0.022**
	Environment*GA Treatment			1.08	0.300
	Ecotype*Environment*Treatment			2.14	0.144

Table 2-2 (cont'd)

		Greenhouse Experiment		Growth Chamber Experiment	
Trait	Predictor	F Ratio	Prob > F	F Ratio	Prob > F
Leaf Length	**Ecotype**	**23.34**	**<0.001**	0.04	0.837
	GA Treatment	**4.45**	**0.039**	**62.73**	**<0.001**
	Ecotype*GA Treatment	2.50	0.120	**29.68**	**<0.001**
	Environment			**38.59**	**<0.001**
	Ecotype*Environment			**38.69**	**<0.001**
	Environment*GA Treatment			1.35	0.247
	Ecotype*Environment*Treatment			**9.81**	**0.002**
Leaf Width	**Ecotype**	**91.44**	**<0.001**	**113.97**	**<0.001**
	GA Treatment	3.62	0.062	**55.20**	**<0.001**
	Ecotype*GA Treatment	3.31	0.074	**18.02**	**<0.001**
	Environment			**64.78**	**<0.001**
	Ecotype*Environment			**16.30**	**<0.001**
	Environment*GA Treatment			**5.45**	**0.020**
	Ecotype*Environment*Treatment			0.12	0.735
Days to First Flower	**Ecotype**	**148.08**	**<0.001**	**1403.88**	**<0.001**
	GA Treatment	**19.03**	**<0.001**	**83.65**	**<0.001**
	Ecotype*GA Treatment	**11.40**	**<0.001**	**30.95**	**<0.001**
	Environment			**2462.13**	**<0.001**
	Ecotype*Environment			**409.75**	**<0.001**
	Environment*GA Treatment			**9.00**	**0.003**
	Ecotype*Environment*Treatment			0.06	0.807
Flower Height	**Ecotype**	**22.07**	**<0.001**	**170.34**	**<0.001**
	GA Treatment	0.88	0.351	**107.37**	**<0.001**
	Ecotype*GA Treatment	1.89	0.1729	**9.80**	**0.002**
	Environment			**38.93**	**<0.001**
	Ecotype*Environment			0.13	0.718
	Environment*GA Treatment			0.56	0.455
	Ecotype*Environment*Treatment			2.53	0.113
Ovule Count	**Ecotype**	2.97	0.090	**27.86**	**<0.001**
	GA Treatment	1.69	0.197	**30.91**	**<0.001**
	Ecotype*GA Treatment	0.04	0.848	0.02	0.895
	Environment			**29.52**	**<0.001**
	Ecotype*Environment			0.07	0.794
	Environment*GA Treatment			1.39	0.240
	Ecotype*Environment*Treatment			1.08	0.299

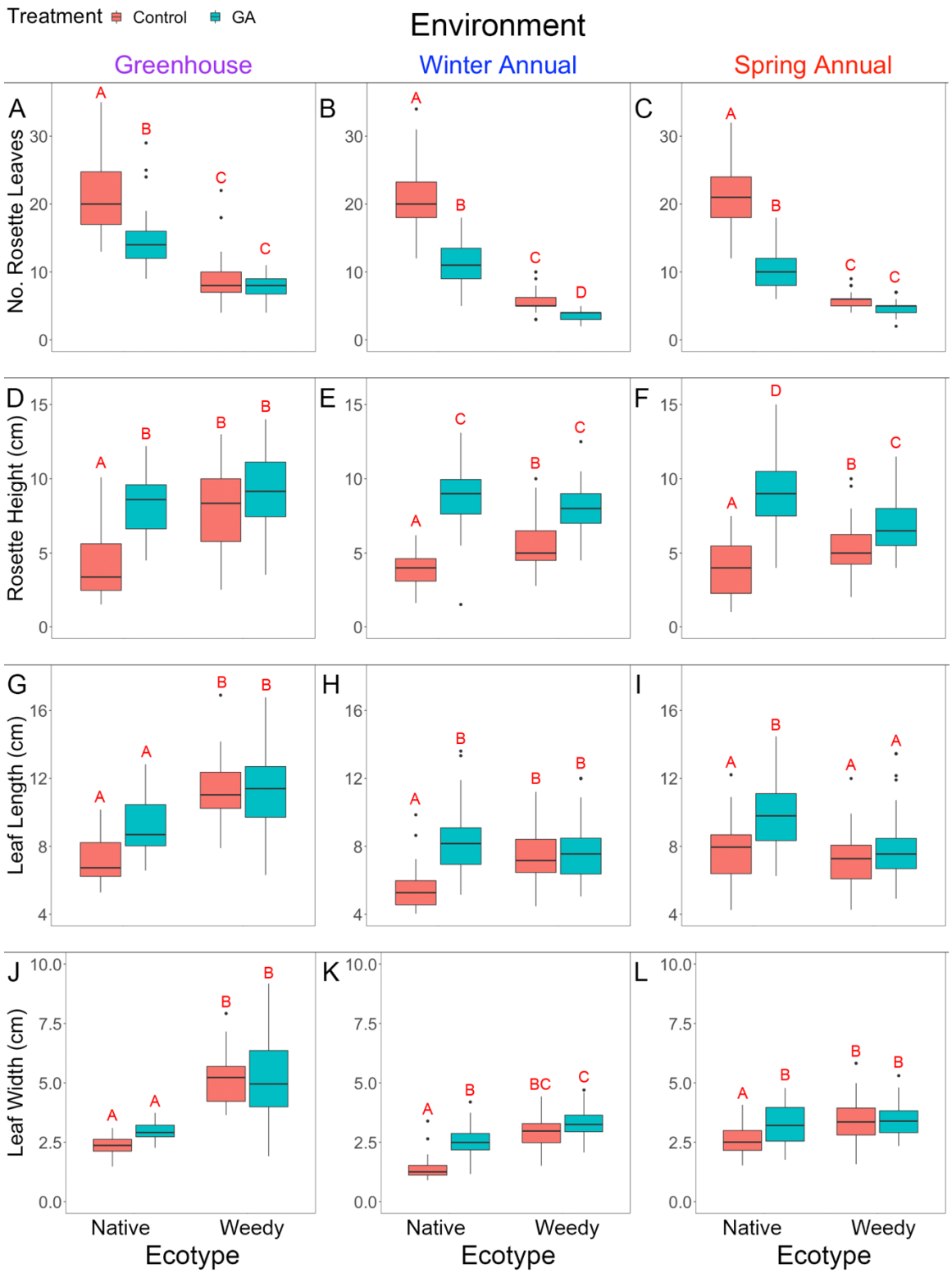

Figure 2-1. Ecotype-level effects of GA application on rosette leaf number (A-C), rosette height (D-F), leaf length (G-I), and leaf width (J-L) in three different environments. Winter Annual and Spring Annual environments were simulated in a growth chamber. Letters above boxplots represent different means by Tukey's HSD within each box.

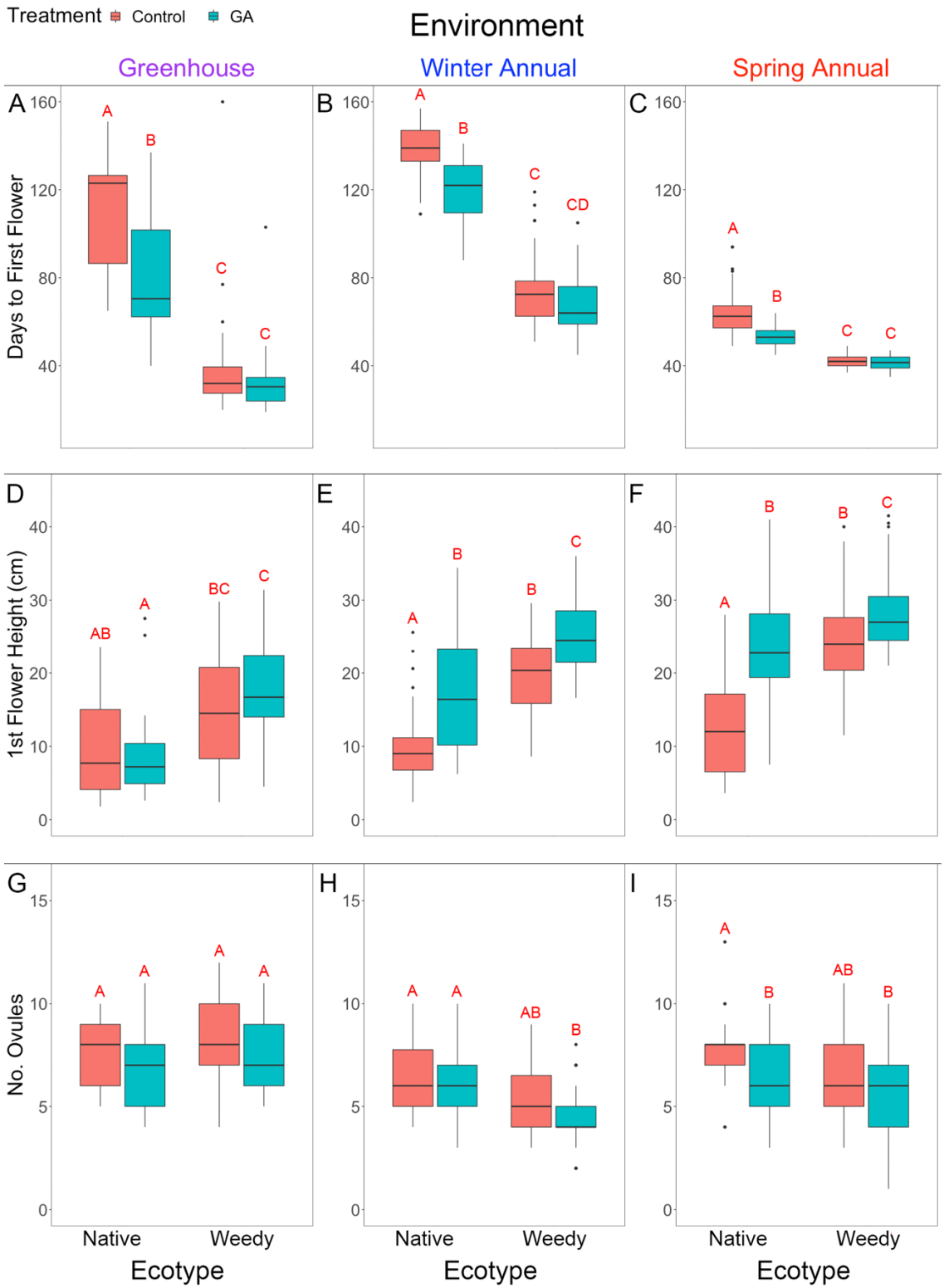

Figure 2-2. Ecotype-level effects of GA application on flowering time (A-C), flower height (D-F), and number of ovules (G-I). Winter Annual and Spring Annual environments were simulated in a growth chamber. Letters above boxplots represent different means by Tukey's HSD within each box.

Table S2-1. Greenhouse experiment ANOVAs for all plant traits as response variables with ecotype, treatment, population (nested within ecotype), and flat (nested within treatment) as main effects, and interaction terms for Ecotype × Treatment and Population × Treatment.

Trait	Predictor	DF	Sum of Squares	F Ratio	Prob > F
# Rosette Leaves	**Ecotype**	**1**	**2113.47**	**107.55**	**<0.001**
	GA Treatment	**1**	**413.50**	**21.04**	**<0.001**
	Ecotype*Treatment	**1**	**107.56**	**5.47**	**0.022**
	Population[Ecotype]	2	66.03	1.68	0.193
	Flat[Treatment]	14	284.57	1.03	0.429
Rosette Height	**Ecotype**	**1**	**124.55**	**23.47**	**<0.001**
	GA Treatment	**1**	**155.47**	**29.29**	**<0.001**
	Ecotype*Treatment	**1**	**50.73**	**9.56**	**0.003**
	Population[Ecotype]	**2**	**178.02**	**16.77**	**<0.001**
	Flat[Treatment]	14	77.26	1.04	0.424
Leaf Length	**Ecotype**	**1**	**137.45**	**23.34**	**<0.001**
	GA Treatment	**1**	**26.24**	**4.45**	**0.039**
	Ecotype*Treatment	1	14.71	2.50	0.120
	Population[Ecotype]	**2**	**45.37**	**3.85**	**0.027**
	Flat[Treatment]	14	34.50	0.42	0.963
Leaf Width	**Ecotype**	**1**	**89.23**	**91.44**	**<0.001**
	GA Treatment	1	3.53	3.62	0.062
	Ecotype*Treatment	1	3.23	3.31	0.074
	Population[Ecotype]	**2**	**29.36**	**15.04**	**<0.001**
	Flat[Treatment]	14	14.14	1.03	0.434
Days to First Flower	**Ecotype**	**1**	**180562.01**	**148.08**	**<0.001**
	GA Treatment	**1**	**23200.16**	**19.03**	**<0.001**
	Ecotype*Treatment	**1**	**13901.56**	**11.40**	**0.001**
	Population[Ecotype]	**2**	**25998.20**	**10.66**	**<0.001**
	Flat[Treatment]	14	10704.17	0.63	0.835
Flower Height	**Ecotype**	**1**	**867.33**	**22.07**	**<0.001**
	GA Treatment	1	34.68	0.88	0.351
	Ecotype*Treatment	1	74.42	1.89	0.1729
	Population[Ecotype]	**2**	**842.85**	**10.72**	**<0.001**
	Flat[Treatment]	14	418.19	0.76	0.708
Ovule #	Ecotype	1	9.67	2.97	0.090
	GA Treatment	1	5.52	1.69	0.197
	Ecotype*Treatment	1	0.12	0.04	0.848
	Population[Ecotype]	**2**	**31.65**	**4.85**	**0.011**
	Flat[Treatment]	14	31.16	0.68	0.783

Table S2-1 (cont'd)

Trait	Predictor	DF	Sum of Squares	F Ratio	Prob > F
Flower Size	Ecotype	1	1.23	2.53	0.116
	GA Treatment	1	1.90	3.88	0.053
	Ecotype*Treatment	**1**	**2.01**	**4.11**	**0.047**
	Population[Ecotype]	2	1.49	1.52	0.225
	Flat[Treatment]	14	5.56	0.81	0.653
Anther Exsertion	**Ecotype**	**1**	**4.94**	**11.52**	**0.001**
	GA Treatment	1	0.51	1.20	0.277
	Ecotype*Treatment	1	0.18	0.43	0.517
	Population[Ecotype]	2	1.06	1.23	0.298
	Flat[Treatment]	14	6.51	1.09	0.386
Anther Separation	Ecotype	1	0.38	0.84	0.362
	GA Treatment	1	1.16	2.60	0.112
	Ecotype*Treatment	1	0.50	1.11	0.296
	Population[Ecotype]	2	1.94	2.17	0.122
	Flat[Treatment]	**14**	**11.65**	**1.86**	**0.047**
Days to Emergence	**Ecotype**	**1**	**36.13**	**29.28**	**<0.001**
	Population[Ecotype]	**2**	**123.25**	**49.95**	**<0.001**
	Flat	14	18.97	1.10	0.368

Table S2-2. Growth chamber experiment ANOVAs for plant traits as response variables with ecotype, environment, treatment, population (nested within ecotype), and chamber (nested within environment) as main effects, and interaction terms for Ecotype × Environment, Ecotype × Treatment, Environment × Treatment, and Ecotype × Environment × Treatment.

Trait	Predictor	DF	Sum of Squares	F Ratio	Prob > F
Rosette Number	**Ecotype**	**1**	**9473.33**	**1120.61**	**<0.001**
	Environment	1	0.77	0.09	0.762
	GA Treatment	**1**	**2752.48**	**325.59**	**<0.001**
	Ecotype*Environment	**1**	**35.92**	**4.25**	**0.040**
	Ecotype*Treatment	**1**	**1463.41**	**173.11**	**<0.001**
	Environment*Treatment	1	0.45	0.05	0.818
	Ecotype*Environment*Treatment	1	22.43	2.65	0.104
	Population[Ecotype]	**2**	**169.13**	**10.00**	**<0.001**
	Chamber[Environment]	2	13.19	0.78	0.459
Rosette Height	Ecotype	1	0.47	0.14	0.706
	Environment	1	4.55	1.39	0.239
	GA Treatment	**1**	**936.84**	**286.72**	**<0.001**
	Ecotype*Environment	**1**	**17.18**	**5.26**	**0.023**
	Ecotype*Treatment	**1**	**210.02**	**64.27**	**<0.001**
	Environment*Treatment	1	3.50	1.07	0.301
	Ecotype*Environment*Treatment	1	6.96	2.13	0.145
	Population[Ecotype]	**2**	**123.75**	**18.94**	**<0.001**
	Chamber[Environment]	2	3.30	0.51	0.604
Leaf Length	Ecotype	1	0.16	0.04	0.8371
	Environment	**1**	**141.37**	**38.59**	**<0.001**
	GA Treatment	**1**	**229.79**	**62.73**	**<0.001**
	Ecotype*Environment	**1**	**141.72**	**38.69**	**<0.001**
	Ecotype*Treatment	**1**	**108.74**	**29.68**	**<0.001**
	Environment*Treatment	1	4.93	1.35	0.247
	Ecotype*Environment*Treatment	**1**	**35.92**	**9.81**	**0.002**
	Population[Ecotype]	**2**	**93.13**	**12.71**	**<0.001**
	Chamber[Environment]	2	10.06	1.37	0.255
Leaf Width	**Ecotype**	**1**	**52.86**	**113.97**	**<0.001**
	Environment	**1**	**30.04**	**64.78**	**<0.001**
	GA Treatment	**1**	**25.60**	**55.20**	**<0.001**
	Ecotype*Environment	**1**	**7.56**	**16.30**	**<0.001**
	Ecotype*Treatment	**1**	**8.36**	**18.02**	**<0.001**
	Environment*Treatment	**1**	**2.53**	**5.45**	**0.020**
	Ecotype*Environment*Treatment	1	0.05	0.12	0.735
	Population[Ecotype]	2	0.58	0.63	0.535
	Chamber[Environment]	2	2.72	2.93	0.055
Days to First Flower	**Ecotype**	**1**	**110268.62**	**1403.88**	**<0.001**
	Environment	**1**	**193390.03**	**2462.13**	**<0.001**
	GA Treatment	**1**	**6570.39**	**83.65**	**<0.001**
	Ecotype*Environment	**1**	**32184.27**	**409.75**	**<0.001**
	Ecotype*Treatment	**1**	**2431.30**	**30.95**	**<0.001**
	Environment*Treatment	**1**	**706.76**	**9.00**	**0.003**
	Ecotype*Environment*Treatment	1	4.68	0.06	0.807
	Population[Ecotype]	**2**	**7656.99**	**48.74**	**<0.001**
	Chamber[Environment]	**2**	**7078.21**	**45.06**	**<0.001**

Table S2-2 (cont'd)

Flower Height	**Ecotype**	**1**	**6102.47**	**170.34**	**<0.001**
	Environment	**1**	**1394.73**	**38.93**	**<0.001**
	GA Treatment	**1**	**3846.55**	**107.37**	**<0.001**
	Ecotype*Environment	1	4.70	0.13	0.718
	Ecotype*Treatment	**1**	**350.99**	**9.80**	**0.002**
	Environment*Treatment	1	20.09	0.56	0.455
	Ecotype*Environment*Treatment	1	90.48	2.53	0.113
	Population[Ecotype]	**2**	**659.64**	**9.21**	**<0.001**
	Chamber[Environment]	2	98.35	1.37	0.255
Ovule Count	**Ecotype**	**1**	**76.99**	**27.86**	**<0.001**
	Environment	**1**	**81.58**	**29.52**	**<0.001**
	GA Treatment	**1**	**85.42**	**30.91**	**<0.001**
	Ecotype*Environment	1	0.19	0.07	0.794
	Ecotype*Treatment	1	0.05	0.02	0.895
	Environment*Treatment	1	3.84	1.39	0.240
	Ecotype*Environment*Treatment	1	2.99	1.08	0.299
	Population[Ecotype]	**2**	**77.67**	**14.05**	**<0.001**
	Chamber[Environment]	2	14.53	2.63	0.074
Flower Size	**Ecotype**	**1**	**27.72**	**68.54**	**<0.001**
	Environment	**1**	**57.87**	**143.11**	**<0.001**
	GA Treatment	1	1.09	2.68	0.103
	Ecotype*Environment	**1**	**30.71**	**75.94**	**<0.001**
	Ecotype*Treatment	1	0.70	1.74	0.189
	Environment*Treatment	1	0.05	0.13	0.716
	Ecotype*Environment*Treatment	1	0.50	1.25	0.265
	Population[Ecotype]	2	1.99	2.46	0.088
	Chamber[Environment]	2	0.50	0.62	0.541
Anther Exsertion	Ecotype	1	0.45	0.75	0.386
	Environment	**1**	**3.59**	**5.94**	**0.015**
	GA Treatment	1	2.26	3.74	0.054
	Ecotype*Environment	1	0.14	0.23	0.635
	Ecotype*Treatment	1	0.64	1.05	0.306
	Environment*Treatment	1	0.51	0.84	0.359
	Ecotype*Environment*Treatment	1	0.03	0.05	0.819
	Population[Ecotype]	2	1.12	0.93	0.397
	Chamber[Environment]	2	1.44	1.19	0.305
Anther Separation	**Ecotype**	**1**	**4.95**	**11.19**	**<0.001**
	Environment	**1**	**3.54**	**8.00**	**0.005**
	GA Treatment	1	1.07	2.41	0.121
	Ecotype*Environment	1	0.86	1.95	0.164
	Ecotype*Treatment	1	0.55	1.24	0.266
	Environment*Treatment	1	0.47	1.05	0.305
	Ecotype*Environment*Treatment	1	0.09	0.20	0.654
	Population[Ecotype]	2	0.32	0.37	0.693
	Chamber[Environment]	2	0.34	0.38	0.684
Days to Emergence	Ecotype	1	13.20	3.20	0.075
	Environment	**1**	**1292.03**	**313.36**	**<0.001**
	Ecotype*Environment	1	12.40	3.01	0.084
	Population[Ecotype]	2	7.88	0.96	0.386
	Chamber[Environment]	2	1.91	0.23	0.794

Table S2-3. Greenhouse experiment means comparison by trait. Means comparisons for all pairs of ecotype and treatment combinations using Tukey-Kramer HSD.

	Ecotype & Treatment	Mean Grouping(s)			Mean
No. Rosette Leaves	Native Control	A			21.46
	Native GA		B		14.92
	Weedy Control			C	9.38
	Weedy GA			C	7.79
Rosette Height	Weedy GA	A			9.28
	Weedy Control	A			8.33
	Native GA	A			8.32
	Native Control		B		4.13
Days to First Flower	Native Control	A			151.42
	Native GA		B		94.44
	Weedy Control			C	40.91
	Weedy GA			C	32.69
First Flower Height	Weedy GA	A			17.47
	Weedy Control	A	B		14.90
	Native Control		B	C	9.75
	Native GA			C	8.74
Ovule Count	Weedy Control	A			8.23
	Native Control	A			7.61
	Weedy GA	A			7.35
	Native GA	A			6.96
Flower Size	Native Control	A			6.70
	Weedy GA	A	B		6.20
	Weedy Control	A	B		6.19
	Native GA		B		6.03
Leaf Length	Weedy GA	A			11.68
	Weedy Control	A			11.52
	Native GA		B		9.30
	Native Control		B		7.16
Leaf Width	Weedy GA	A			5.22
	Weedy Control	A			5.22
	Native GA		B		2.96
	Native Control		B		2.39
Anther Exsertion	Native GA	A			1.07
	Native Control	A			1.06
	Weedy GA	A			1.04
	Weedy Control	A			1.02
Anther Separation	Native GA	A			3.37
	Weedy GA	A			3.19
	Native Control	A			3.13
	Weedy Control	A			3.08

Table S2-4. Growth chamber experiment means comparison by trait. Means comparisons for all pairs of ecotype, environment, and treatment combinations using Tukey-Kramer HSD.

	Ecotype, Environment, & Treatment	Mean Grouping(s)						Mean
No. Rosette Leaves	Native, Winter Annual, Control	A						21.13
	Native, Spring Annual, Control	A						20.95
	Native, Winter Annual, GA		B					11.49
	Native, Spring Annual, GA		B					10.13
	Weedy, Spring Annual, Control			C				5.82
	Weedy, Winter Annual, Control			C				5.70
	Weedy, Spring Annual, GA			C	D			4.68
	Weedy, Winter Annual, GA				D			3.64
Rosette Height	Native, Spring Annual, GA	A						9.24
	Native, Winter Annual, GA	A						8.92
	Weedy, Winter Annual, GA	A	B					7.96
	Weedy, Spring Annual, GA		B	C				6.76
	Weedy, Winter Annual, Control			C	D			5.66
	Weedy, Spring Annual, Control				D			5.44
	Native, Spring Annual, Control					E		4.06
	Native, Winter Annual, Control					E		3.93
Days to First Flower	Native, Winter Annual, Control	A						137.85
	Native, Winter Annual, GA		B					119.62
	Weedy, Winter Annual, Control			C				74.50
	Weedy, Winter Annual, GA			C	D			67.92
	Native, Spring Annual, Control				D			64.97
	Native, Spring Annual, GA					E		53.50
	Weedy, Spring Annual, Control						F	42.45
	Weedy, Spring Annual, GA						F	41.63
First Flower Height	Weedy, Spring Annual, GA	A						28.66
	Weedy, Winter Annual, GA	A	B					25.23
	Weedy, Spring Annual, Control		B					24.35
	Native, Spring Annual, GA		B	C				23.29
	Weedy, Winter Annual, Control			C	D			19.82
	Native, Winter Annual, GA				D			17.32
	Native, Spring Annual, Control					E		12.64
	Native, Winter Annual, Control					E		9.73
Ovule Count	Native, Spring Annual, Control	A						7.76
	Weedy, Spring Annual, Control	A	B					6.65
	Native, Winter Annual, Control		B					6.39
	Native, Spring Annual, GA		B					6.33
	Native, Winter Annual, GA		B					5.81
	Weedy, Winter Annual, Control		B	C				5.55
	Weedy, Spring Annual, GA		B	C				5.55
	Weedy, Winter Annual, GA			C				4.51

Table S2-4 (cont'd)

	Ecotype, Environment, & Treatment	Mean Grouping(s)				Mean
Flower Size	Native, Winter Annual, Control	A				8.41
	Native, Winter Annual, GA	A				8.25
	Weedy, Winter Annual, Control		B			7.13
	Weedy, Winter Annual, GA		B	C		7.01
	Native, Spring Annual, Control		B	C		6.94
	Weedy, Spring Annual, GA		B	C		6.87
	Weedy, Spring Annual, Control		B	C		6.77
	Native, Spring Annual, GA			C		6.67
Leaf Length	Native, Spring Annual, GA	A				9.79
	Native, Winter Annual, GA		B			8.00
	Weedy, Spring Annual, GA		B			7.93
	Native, Spring Annual, Control		B			7.81
	Weedy, Winter Annual, GA		B			7.51
	Weedy, Winter Annual, Control		B			7.40
	Weedy, Spring Annual, Control		B			6.94
	Native, Winter Annual, Control			C		4.14
Leaf Width	Weedy, Spring Annual, GA	A				3.47
	Weedy, Spring Annual, Control	A	B			3.38
	Weedy, Winter Annual, GA	A	B			3.31
	Native, Spring Annual, GA	A	B			3.25
	Weedy, Winter Annual, Control		B	C		2.91
	Native, Spring Annual, Control			C		2.56
	Native, Winter Annual, GA			C		2.52
	Native, Winter Annual, Control				D	1.40
Anther Exsertion	Weedy, Spring Annual, GA	A				0.55
	Weedy, Winter Annual, GA	A				0.39
	Weedy, Spring Annual, Control	A				0.38
	Native, Spring Annual, GA	A				0.36
	Native, Spring Annual, Control	A				0.34
	Native, Winter Annual, GA	A				0.24
	Native, Winter Annual, Control	A				0.10
	Weedy, Winter Annual, Control	A				0.02
Anther Separation	Native, Winter Annual, Control	A				3.51
	Native, Winter Annual, GA	A	B			3.26
	Native, Spring Annual, Control	A	B			3.14
	Weedy, Winter Annual, Control	A	B			3.09
	Native, Spring Annual, GA		B			2.98
	Weedy, Spring Annual, GA		B			2.95
	Weedy, Winter Annual, GA		B			2.95
	Weedy, Spring Annual, Control		B			2.86

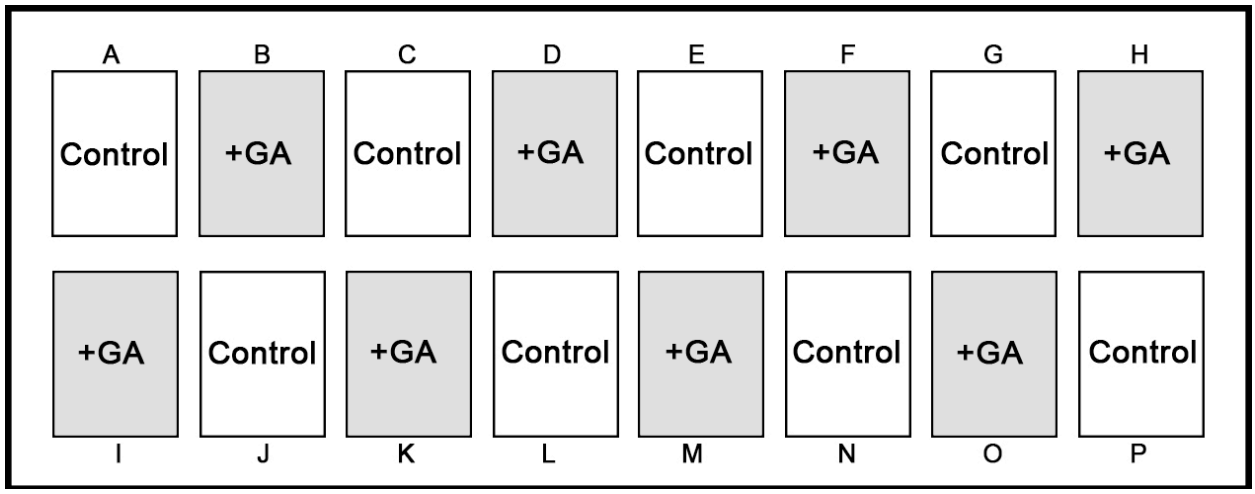

Figure S2-1. Layout of flats in the greenhouse. Each flat contained eight plants, two randomly selected from each population and treatment, and the flats were rotated clockwise every week (the starting layout is pictured).

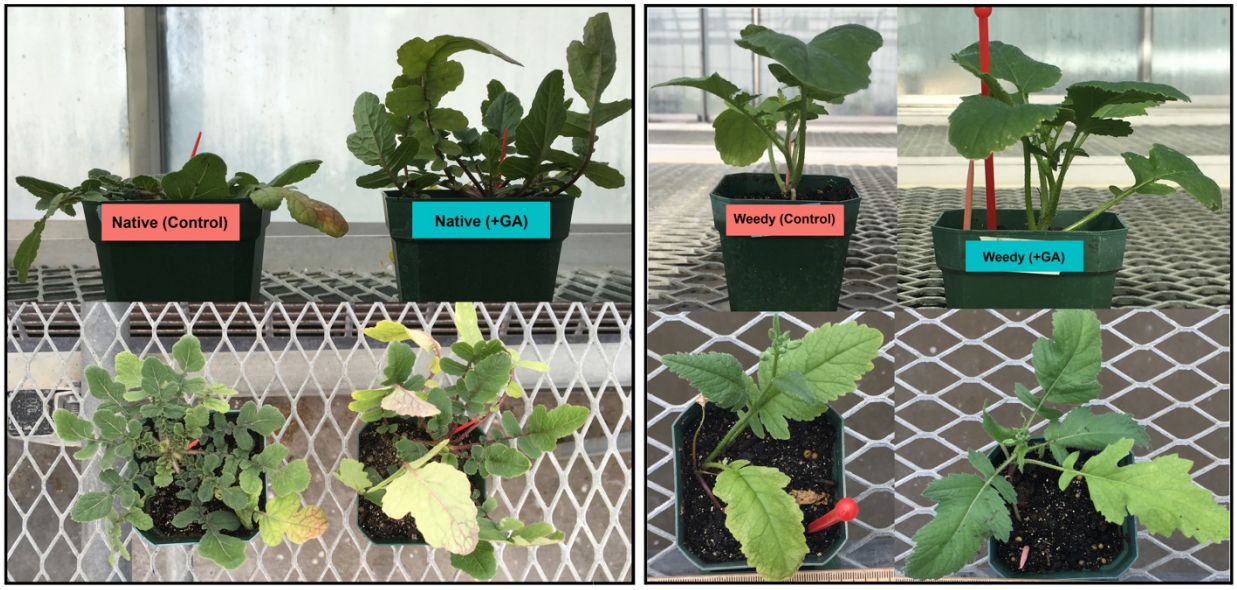

Figure S2-2. Side and top photos of native and weedy rosette under control and GA treatments.

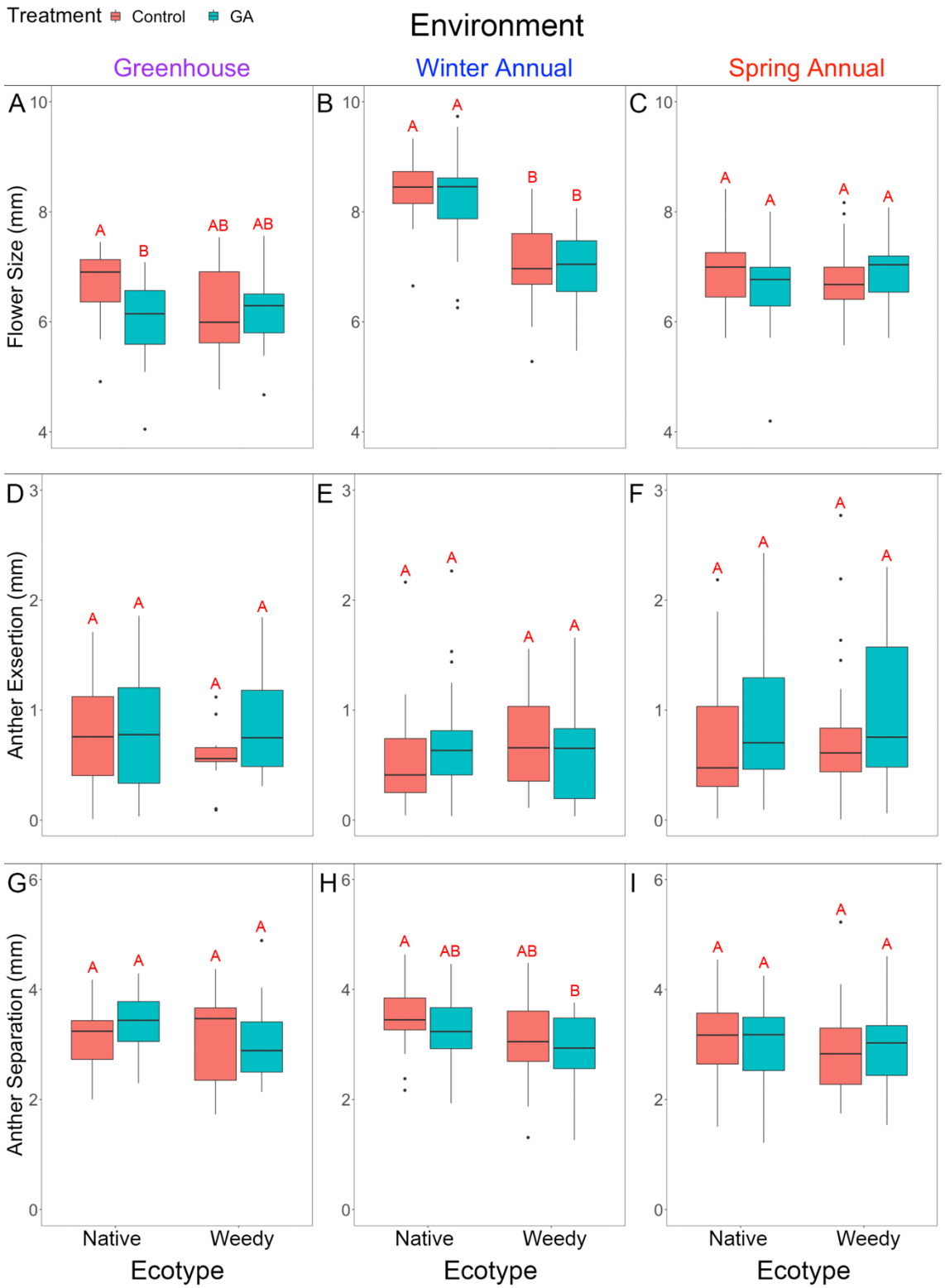

Figure S2-3. Ecotype-level effects of GA application on flower size (calculated as the geometric mean of eight floral traits), anther exsertion, and anther separation in three different environments. Winter Annual and Spring Annual environments were simulated in a growth chamber. Letters above boxplots represent different means by Tukey's HSD within each box.

CHAPTER 3:
ADAPTATION OF A WEED TO THE AGRICULTURAL ENVIRONMENT

Introduction

Agricultural weeds are a drain on resources globally and a major threat to food security. Weed management alone costs billions of dollars annually, with over $10 billion in global herbicide sales in 2004 (Oerke 2006). The cost of crop loss and damage is far greater, estimated to be $26.4 billion annually in the United States due alone (Pimentel et al. 2000). Further, agricultural weeds impact food security, with staple crops like rice facing up to 50% crop losses in some areas (Tshewang et al. 2016), and food price crises resulting from yield loss due to weeds (Yaduraju and Rao 2013). Once weeds have established in agricultural fields they are incredibly difficult to control due to their seemingly limitless ability to evade management (Clements and Jones 2021) but there is far less focus on how weeds establish in the first place. It is therefore imperative to investigate the origin of weed species, their colonization of agricultural fields, and how they successfully establish themselves as crop competitors.

A common way for weeds to establish in an agricultural field is from an existing crop plant, either through de-domestication or as the result of hybridization with wild relatives (De Wet and Harlan 1975). For weeds with crop origins, it is clear to see how they can quickly be successful as they already have traits that are adaptive in the agricultural environment (Vigueira, Olsen, and Caicedo 2013). However, the path to weediness is less clear in weeds with wild origins, as the wild ancestors of these weeds tend to be understudied (Vigueira, Olsen, and Caicedo 2013). Little is known about the role of adaptive evolution in the establishment of agricultural weeds from wild ancestors, and how often rapid evolution is a part of the path to weediness.

Adaptation in weeds has been more thoroughly explored in invasives, defined as introduced species impacting natural habitats, where recent evidence suggests that local adaptation to the invaded habitat may be more common than previously thought (Oduor, Leimu, and van Kleunen 2016). Due to the relatively short timescale of plant introductions (Colautti and Lau 2015), as well as the frequency of bottlenecks experienced by introduced species (Dlugosch and Parker 2008), it was thought instead that some wild plants were already suited to be weeds, either because of a plastic, general-purpose genotype (Baker 1974) or pre-adaptations (Gould and Vrba 1982). However, despite evidence for plasticity and pre-adaptation in invasive species (Davidson, Jennions, and Nicotra 2011; Schlaepfer et al. 2010), a meta-analysis (Oduor, Leimu, and van Kleunen 2016) found local adaptation was just as common in invasive species as in native species. In *Ambrosia artemisiifolia,* adaptations to local environments have been found in their introduced range of Europe, Australia, and China (Li et al. 2015; van Boheemen, Atwater, and Hodgins 2019). Even in species with evidence of pre-adaptation, local adaptation has been found to be important in the introduced range (Henery et al. 2010). Therefore, it is clear that adaptive evolution is important in plant invasions.

Local adaptation is less explored in agricultural weeds, where the "local" environment is actually a set of common characteristics in agricultural fields that exert selection on the plants that colonize (e.g., high competition with crops and frequent, complete aboveground disturbance when fields are tilled and harvested). While there are many examples of weed populations adapting to the management practices used against them (Barrett 1983; Heap 2014; Chavana et al. 2021), there have been few studies that show higher fitness of a weed compared to a native relative in agricultural fields (but see Weinig 2005). Further, selection on potentially adaptive traits in agricultural fields has rarely been estimated (but see Rausher and Simms 1989).

Therefore, the frequency with which agricultural weeds have had to adapt to the field conditions upon colonization is not known.

In this study, we provide evidence of weed adaptation to the agricultural field, including traits under selection, in wild radish (*Raphanus raphanistrum ssp. raphanistrum*). *R. r. raphanistrum* has two distinct ecotypes: the weedy ecotype, found growing in agricultural fields on every continent except for Antarctica (Holm 1997); and the native ecotype, which grows outside of agricultural fields in its native range of the Mediterranean. The weedy ecotype is an extremely damaging agricultural weed of small grain fields (Schroeder 1989; Hashem and Wilkins 2002) that has been found to decrease what yields up to 55% (Eslami et al. 2006). Additionally, weedy radish was likely to have evolved from a native ancestor according to cDNA phylogeny and molecular marker analyses (Shen et al. 2013; Charbonneau et al. 2018). Therefore, we can use the native ecotype as a proxy for the wild ancestor of weedy radish to look for evidence of weed adaptation to the agricultural field, as well as traits under selection in natives when introduced into this novel habitat.

The weedy ecotype flowers earlier, has taller flowering stalks, and fewer and taller rosette leaves compared to the native ecotype (Sahli et al. 2008; Charbonneau et al. 2018; Garrison Chapters 1 & 2), with evidence that differentiation in the first three of these traits is adaptive (Sahli et al. 2008). Faster flowering in the weedy ecotype is likely imperative in order for plants to grow and set seed in the time between tilling and harvesting in the field. This is in contrast to the native ecotype, where plants are winter annuals, germinating in the fall and flowering in the spring, often requiring a cold period (vernalization) before they can flower (Charbonneau et al. 2018). Taller flowers in the weeds may be useful to attract pollinators among tall crops as *R. r. raphanistrum* is an obligate outcrosser, while taller rosette leaves may be

beneficial for light competition with the growing crops. The native ecotype, on the other hand, typically grows in sparsely vegetated areas (Arroyo and Gómez, pers. comm.) and in those areas grow low overwintering rosettes and flowers that stay lower to the ground. However, the fitness of weedy radish has not been estimated in an agricultural field, so we do not have any direct evidence about either weedy radish adaptation or traits under selection in the agricultural field.

To address these knowledge gaps, we planted weedy and native populations of radish in an agricultural field to test for higher fitness in the weedy ecotype, which would be indicative of adaptation to the agricultural environment. Our second aim was to find traits under selection in the agricultural field, and to look for differences in selection on the weedy and native ecotype. If weedy radish is adapted to the agricultural field, then we would expect to see traits that are differentiated between weedy and native radish under selection in the agricultural field, with stronger selection on native populations in the direction of the weedy phenotype. We do not expect strong evidence of directional selection in weedy populations if weeds have adapted to agricultural fields, as their phenotype should have already shifted toward the trait optima.

Methods

Common Garden 1 - 2018

We grew eight populations from the weedy and native ecotypes in a common garden in an active oat field (Table 3-1). We used four populations of the weedy ecotype, collected growing in active agricultural fields, and four populations of the native ecotype growing outside of agricultural fields in the native range of Spain. Seeds from each population were kept in their fruit segments and planted in proportion to their germination rate with the goal of growing 60 plants per population. For half of our populations from each ecotype we planted seeds from

greenhouse crosses, but in the other half we used field-collected seeds (Table 3-1) as they were all we had available.

In May of 2018, the eight populations of *R. raphanistrum* were planted in an active agricultural field growing Saber oats (*Avena sativa*). The oats were planted as part of an oat variety trial, with pelletized poultry manure applied prior to planting, but no additional inputs after planting. The oat field was sown on April 13, 2018 and the radish seeds were planted 22 days later on May 5, 2018. Seeds were planted regularly between the rows of oats, 1-foot apart from each other in each row, with no plant less than a foot away from the margin of the oat field. Plants were randomly assigned their position in the field to minimize the effects of any environmental differences on each population. Plots with excess germinants were transplanted into empty plots of the same population, and excess germinants beyond that were randomly thinned down to one plant per plot.

After gemination, plants were allowed to grow with little intervention, but the plots were watered twice in July due to hot, dry conditions to prevent high mortality. Plants were removed from the agricultural field on July 25, 2018 when the oats were harvested. Thus, the experimental plants had a total of 81 days to germinate, grow, and set seed.

Common Garden 2 – 2019

In the second year of our study, only six populations of radish were used: three populations of the weedy radish ecotype, and three populations of the native radish ecotype (Table 3-1). The populations excluded were DAES and NAAU, due to low seed availability. As in the first year, seeds in their fruit segments were planted in proportion to their germination rate with the goal of growing 60 plants per population. The same seed sources were used in each year of the experiment.

In April of 2019, the six populations of *R. raphanistrum* were planted in an active agricultural field growing oats in similar variety trial as the previous year. The oat field was sown on April 4, 2019 and the radish seeds were planted no more than 4 days later, between April 6 and April 8, 2019. Planting was done by row, with populations randomized, so there was no overall difference in planting date by population or ecotype. Radish seeds were planted before oat germinants emerged, which differed from the previous year. Instead of pelletized poultry manure, dairy manure was applied before planting, and after planting no additional inputs, including water, were applied to the field. The remaining planting procedure was the same as the first common garden. Plants were removed from the agricultural field on July 30, 2019 when the oats were harvested, giving the experimental plants in this year between 111 and 113 days to germinate, grow, and set seed.

Trait Measurements and Data Analysis

A suite of traits was measured to look for evidence of traits under selection in the agricultural environment. The date of emergence from the soil was recorded for each plant, and the date of first flower opening was recorded, with flowering time calculated as the number of days from emergence to first flower. Rosette number (the number of basal rosettes on each plant) and rosette height (vertical distance from the soil to the tallest free-standing point of the basal rosette) were measured at the time of bolting, or on the day before plants were removed from the field in the case of plants that had not yet bolted by the end of the growing season. First flower height (the vertical distance from the soil to the point of attachment of the first flower's pedicel to the stalk) was measured on the day it opened. After the third flower opened, the most recently opened flower was photographed from the top and side and the number of ovules was counted under a dissecting scope. For the side photo, the sepal in front of a short stamen was removed, as

were the petal to the left and one long stamen to the left in order to get a clear view of the inner floral parts. These pictures were used to measure petal (limb) length and width, corolla tube (claw) length, pistil length, and the lengths of both the anther and filament of one short and one long stamen using ImageJ (Schneider, Rasband, and Eliceiri 2012). These measurements were then used to calculate the composite trait flower size (the geometric mean of the eight traits). All fruits produced by the plants were collected and seed number per fruit counted.

Data were analyzed using JMP (JMP, Version 15. SAS Institute Inc., Cary, NC, 1989-2021), with separate analyses for each year of the study. We first performed a chi-square test to find differences in the proportion of plants in each ecotype that successfully germinated. Because few plants set seed, we also performed a chi-squared test to determine if there was a difference by ecotype or population in the proportion of plants to set seed each year. We then performed an ANOVA to determine if fitness (seed number per plant) differed by ecotype and population nested within ecotype. For these analyses, all populations were included regardless of germination success.

For the remaining tests, we excluded any populations that failed to adequately germinate in a given year (fewer than 25% of the 60 desired plants). The populations from 2018 that we excluded were the native populations AZES (five plants germinated) and CNES (six plants germinated), as well as the weedy population CLNC (12 plants germinated). In 2019, we excluded same native populations with low germination in the previous year, AZES (five plants germinated) and CNES (seven plants germinated)

We used multiple regression to create selection gradients (β) to determine traits under direct selection (Lande and Arnold 1983). We performed two sets of multiple regressions, each performed separately for the two study years. In the first set, the response variable was fitness

(seed number) and the predictor variables were our suite of traits (fitness ~ [suite of traits]), and the regressions were performed within populations to look for direct selection on traits separately within each population. For these regressions, the only populations with an adequate number of trait measurements for the regression to run were AFFR (in both years), BINY and MAES (in 2019 only).

For our second set of multiple regressions we used ANCOVA, with ecotype and population nested within ecotype now also included as categorical predictors (fitness ~ [suite of traits] + Ecotype + Population[Ecotype]). This allowed us to look for both fitness differences by population and ecotype, as well as an overall look at direct selection (β) on each trait. To determine if strength of selection differed by ecotype, we also included the interaction between traits and ecotype for 2019 (the only year where both ecotypes set seed). Because we had a large number of plants in each year that did not produce any seeds, we used a generalized regression with a zero-inflated Poisson distribution for these ANCOVA analyses. We used the elastic net estimation method, which provides appropriate penalty for our large number of predictors and avoids overfitting (Crotty and Barker 2014).

Results

Adaptation to the agricultural field

In each year of the study, a greater proportion of plants germinated in the weedy ecotype (70.00% in 2018 and 70.56% in 2019) than in the native ecotype (54.17% in 2018 and 40.00% in 2019; 2018 $\chi^2 = 12.85$, $p < 0.001$; 2019 $\chi^2 = 34.57$, $p < 0.001$). Given the short duration of the crop growing season, few plants from either common garden were able to set seed: 38 plants out of the total 480 planted in 2018, and 59 plants out of the total 360 planted in 2019. However, in each of the two common gardens, there was a greater number of plants to set seed in the weedy

ecotype than in the native (2018 $\chi^2 = 55.95$, $p < 0.001$; 2019 $\chi^2 = 7.43$, $p = 0.006$; Fig. 3-1A&C). Within the weedy ecotype, the population AFFR had the greatest proportion of plants to set seed in both years (2018 $\chi^2 = 18.92$, $p < 0.001$; 2019 $\chi^2 = 17.66$, $p < 0.001$; Fig. 3-1B&D). There was no difference within the native ecotype in 2018, as no native plants were able to set seed, but in 2019 MAES had the greatest proportion of plants to set seed ($\chi^2 = 40.48$, $p < 0.001$)

Overall fitness (total seed set) differed significantly only in 2018, with the weedy ecotype producing more seeds than the native ecotype (Fig. 3-2A; Table S3-1). In that year, the weedy population AFFR produced the most seeds among all populations (Fig. 3-2B), while native populations produced no seeds at all. In 2019, there was a marginally significant trend of higher fitness in the weedy ecotype ($p = 0.1$; Fig. 3-2C). In that year, a weedy and a native population, AFFR & MAES, had the highest seed set among all populations (631 and 501 seeds total, respectively). Each weedy population was able to set seed in both years of the study, while only two native populations were able to set seed (MAES and AZES, which produced only 3 seeds), and only in 2019. Overall, the weedy ecotype had greater fitness in the agricultural field compared to the native.

Traits under selection in the agricultural field

We found evidence for four traits under direct selection (β) in the agricultural field: days to first flower, number of rosette leaves, first flower height and days to emergence (Table 3-2 & Table 3-3). As found previously (Sahli et al. 2008; Charbonneau et al. 2018; Garrison Chapters 1 & 2), the native ecotype flowered more slowly overall and produced fewer rosette leaves than the weedy ecotype in each common garden year (Fig. 3-3A&B; Table S3-1). Additionally, we found significant directional selection only in the native population MAES in 2019 (Table 3-2), for faster flowering and more rosette leaves (Fig. 3-4A&B). In both cases, selection in the native

population was toward the weedy ecotype mean. Two additional traits, first flower height and days to emergence were marginally significant predictors, with selection for taller flowers and faster emergence (Fig. 3-4C&D). Selection in first flower height was once again in the direction of the weedy radish mean (Fig. 3-3C), but not in emergence time, which was faster in the native ecotype each year (Fig. 3-3D). There was no significant selection detected in any of the weedy radish populations in either year (Table 3-2). However, direction of selection ($\pm\beta$) for days to emergence and flowering time was negative for all populations regardless of ecotype, but varied for other traits, with the weedy population BINY matching MAES more often than the weedy population AFFR.

Our ANCOVA with zero-inflated Poisson distribution did not reveal any additional traits under selection in the agricultural field, as only days to emergence and days to first flower were significant trait predictors (Table 3-3). However, selection on days to emergence was significant in 2018 as well as 2019 using this model, the only significant trait in 2018. The model also showed a significant effect of population within ecotype on fitness in 2018, in agreement with the ANOVA (Table 3-3; Table S3-1). However, this effect was not significant in 2019. Finally, while all other traits measured in this study differed by ecotype in at least one of the common garden years (Table S3-1), no traits besides the four already mentioned were found to be under selection in the agricultural field.

Discussion

We found clear evidence of adaptive evolution to crop fields in an agricultural weed, weedy radish, using two common garden experiments. Using its native counterpart as an ancestral proxy, we found higher fitness in the weedy ecotype compared to the native in both years, demonstrating that weedy radish has adapted to agricultural field conditions. Additionally,

we found evidence of directional selection on four traits that are differentiated between weedy and native ecotypes, with the direction of selection in three of these traits toward the weedy radish mean. Finally, directional selection within populations was only significant in native radish, further supporting our hypothesis that adaptive differentiation previously occurred in weedy radish.

Evidence of adaptation to the agricultural environment was strongest in 2018, where all populations of the weedy ecotype were able to set seed and none of the native radish populations were able to (Fig. 3-1A; Fig. 3-2A). It is important to note also that the 2018 common garden was a much shorter growing season for the radish plants, only 81 days, compared to 113 days in 2019. In the extended 2019 common garden, two native populations were able to set seed, with the population MAES producing nearly as many seeds as the most fecund weedy population, but AZES producing only 3 seeds total (Fig. 3-2D). It is possible that the high fitness of MAES in 2019 is due to potential past selection for earlier flowering in the greenhouse. The MAES seeds used in this study are from crosses in the greenhouse, and the average flowering time found here (53.9 days; Fig. 3-3A) is far faster than what has been previously reported for this population (Charbonneau et al. 2018). It may also be due to better seed quality from greenhouse-collected seeds, which could also account for higher proportion of plants to set seed in the weedy populations AFFR and NAAU compared to the field collected weedy populations BINY and CLNC. However, the last greenhouse-collected population was CNES, which failed to adequately germinate in either year.

The longer growing season in 2019 gave us the ability to estimate selection in both weedy and native populations. Most striking is that the only population with significant evidence of directional selection was the native MAES, and further that three of the traits under selection

were differentiated between ecotypes, with the direction of selection toward the weedy mean in each case (Fig. 3-3; Fig. 3-4). In short, the earlier flowering, taller flowers, and fewer rosette leaves that evolved in weedy radish appear to be adaptive in the agricultural field. Taller flowers are important for pollination, while flowering time and rosette number reflect an earlier resource allocation shift from growth to reproduction (Geber 1990; Adler et al. 2014) that is advantageous in the short growing season of the agricultural field. While we did not find that strength of selection on any of these traits differed by ecotype in our ANCOVA (i.e., no trait × ecotype interactions; Table 3-3), these findings overall support our hypothesis that weedy radish has adapted to agricultural field conditions since diverging from its native ancestor.

We also found faster emergence to be adaptive in the agricultural field, contributing to the rapid lifecycle favored in the field (Table 3-2; Table 3-3; Fig. 3-4D). However, the weedy ecotype was slower to emerge in each year (Fig. 3-3B) suggesting that the ecotype divergence found in this study may not be adaptive. Note that growth chamber studies did not find significant ecotypic differentiation for this trait (Garrison Chapters 1 & 2). Additionally, the native ecotype had lower germination success overall, and populations that failed to germinate in the span of this study were not included in these analyses. It is not known if these populations failed to germinate due to seed dormancy, or if the seeds were simply inviable. Differences in seed dormancy between the two ecotypes is a next step to explore in this system.

Our findings join a small number of studies that have found evidence of adaptive evolution in agricultural weeds beyond just adaptation to management practices, highlighting the importance of rapid evolution in weed establishment. The weed *Helianthus annuus* has been found to locally adapt to agriculture numerous times after diverging from nearby wild populations (Kane and Rieseberg 2008). Local adaptation was also found in *Abutilon*

theophrasti, where weedy populations had higher fitness in dense cornfields and non-weedy population were more fit in a no-competition control environment (Weinig 2005). Similarly, our study identified traits under selection by the agricultural environment itself, which adds to a growing understanding of how weeds initially adapt to agriculture (Garrison Chapters 1 & 2).

The success and ubiquity of agricultural weeds has largely been attributed to their high phenotypic plasticity and tolerance of a wide range of environmental conditions, but it is increasingly clear that the ability to rapidly adapt in agricultural fields is also an important characteristic of weeds. Weeds have clearly demonstrated the ability to adapt to management practices (Barrett 1983; Heap 2014; Chavana et al. 2021), which make them so difficult and costly to control. However, their ability to adapt to the field itself has also been demonstrated, highlighting the importance of further investigating the initial adaptations of agricultural weeds. More studies on initial weed adaptations should provide useful insights into weed management, as well as the tools to identify and control new weed populations before they become successful.

APPENDIX

APPENDIX

Tables and Figures

Table 3-1. The eight populations of *R. raphanistrum* studied. Asterisks signify years any population was excluded from multiple regression and generalized regression analyses due to low germination (<25% of target plant number).

Population	Ecotype	Location	Location Description	Years Planted
AZES	Native	Seville, Spain (37°15'26.31" N, 6° 13'15.47" W)	A wild pine-olive tree-Quercus woodland (field-collected)	2018* & 2019*
CNES	Native	Castillo de Calatrava de Nueva, Spain (38°39'59 "N 3°50'43" W)	Natural habitat among boulders	2018* & 2019*
DAES	Native	Seville, Spain (37°13'5.83"N 6°09'54.39"W)	Wild olive tree woodland (field-collected)	2018
MAES	Native	Near Madrid, Spain (40°39'37.5078"N, 3°46'14.1384"W)	Bordering a cereal field	2018 & 2019
AFFR	Weedy	Fontfroide Abbey, France (43° 08'260" N, 2° 53'547" E)	Barley field	2018 & 2019
BINY	Weedy	Near Binghamton, NY; USA (42°11'2.3994" N, 75°50'7.08" W)	Alfalfa field (field-collected)	2018 & 2019
CLNC	Weedy	Clayton, NC; USA (35°39'54"N, 78°30'31"W)	Winter wheat experimental plots (field-collected)	2018* & 2019
NAAU	Weedy	Naracoorte, Australia (140°73' E, 36°96' S)	Winter annual agricultural field	2018

Table 3-2. Selection gradients from multiple regression of radish traits on fitness (seed number) by population in each year of the study. Only populations with sufficient trait data could be included in the analyses. Bold values indicate significance at $p < 0.05$, while italicized values indicate significance at $p < 0.15$.

	2018			2019								
Population	AFFR (Weedy)			AFFR (Weedy)			BINY (Weedy)			MAES (Native)		
Trait	*β*	F Ratio	Prob > F	*β*	F Ratio	Prob > F	*β*	F Ratio	Prob > F	*β*	F Ratio	Prob > F
Days to Emergence	*-0.42*	*2.44*	*0.14*	-0.21	0.34	0.57	-1.33	1.40	0.36	*-0.37*	*2.63*	*0.12*
Days to First Flower	-0.25	0.75	0.40	-0.21	0.54	0.48	-1.18	1.44	0.35	**-0.44**	**4.40**	**0.05**
No. Rosette Leaves	0.15	0.17	0.69	0.21	0.38	0.55	-0.32	0.20	0.70	**-0.46**	**4.44**	**0.05**
Rosette Height	-0.31	0.36	0.56	-0.18	0.27	0.61	-0.25	0.06	0.83	0.24	0.86	0.37
First Flower Height	0.01	0.00	0.98	-0.06	0.03	0.87	0.58	0.33	0.62	*0.41*	*3.02*	*0.10*
No. Ovules	0.30	0.82	0.38	0.08	0.08	0.79	-0.05	0.01	0.95	-0.01	0.00	0.98
Flower Size	-0.17	0.27	0.61	0.02	0.00	0.96	-0.06	0.01	0.92	0.18	0.82	0.38

Table 3-3. Result of ANCOVA with fitness (seed number) as the response and with predictor variables ecotype, population nested within ecotype, all radish traits, and their interaction with ecotype in each year of the study. The analyses used a zero-inflated Poisson distribution and elastic net estimation method. Bolded values indicate significance at $P < 0.05$. Effect of ecotype and its interactions are not reported for 2018 because only plants from the weedy ecotype set seed. Bold values indicate significance at $p < 0.05$.

	2018			2019		
Trait	β	Wald $\chi 2$	P-Value	β	Wald $\chi 2$	P-Value
Days to Emergence	**-0.27**	**5.22**	**0.02**	**-0.17**	**7.29**	**0.01**
Days to First Flower	-0.03	1.02	0.31	**-0.05**	**4.31**	**0.04**
No. Rosette Leaves	-0.10	1.01	0.32	-0.03	0.04	0.85
Rosette Height	-0.03	1.14	0.29	0.00	0.00	0.95
First Flower Height	0.01	0.04	0.85	0.03	1.32	0.25
No. Ovules	-0.06	0.21	0.65	-0.02	0.19	0.66
Flower Size	0.45	1.35	0.24	0.03	0.10	0.75
Ecotype		-	-		0.01	0.92
Population		**13.51**	**<0.01**		0.54	0.46
Days to Emergence × Ecotype					0.04	0.84
Days to First Flower × Ecotype					0.36	0.55
No. Rosette Leaves × Ecotype					0.78	0.38
Rosette Height × Ecotype					0.14	0.71
First Flower Height × Ecotype					1.08	0.30
No. Ovules × Ecotype					0.68	0.41
Flower Size × Ecotype					1.97	0.16

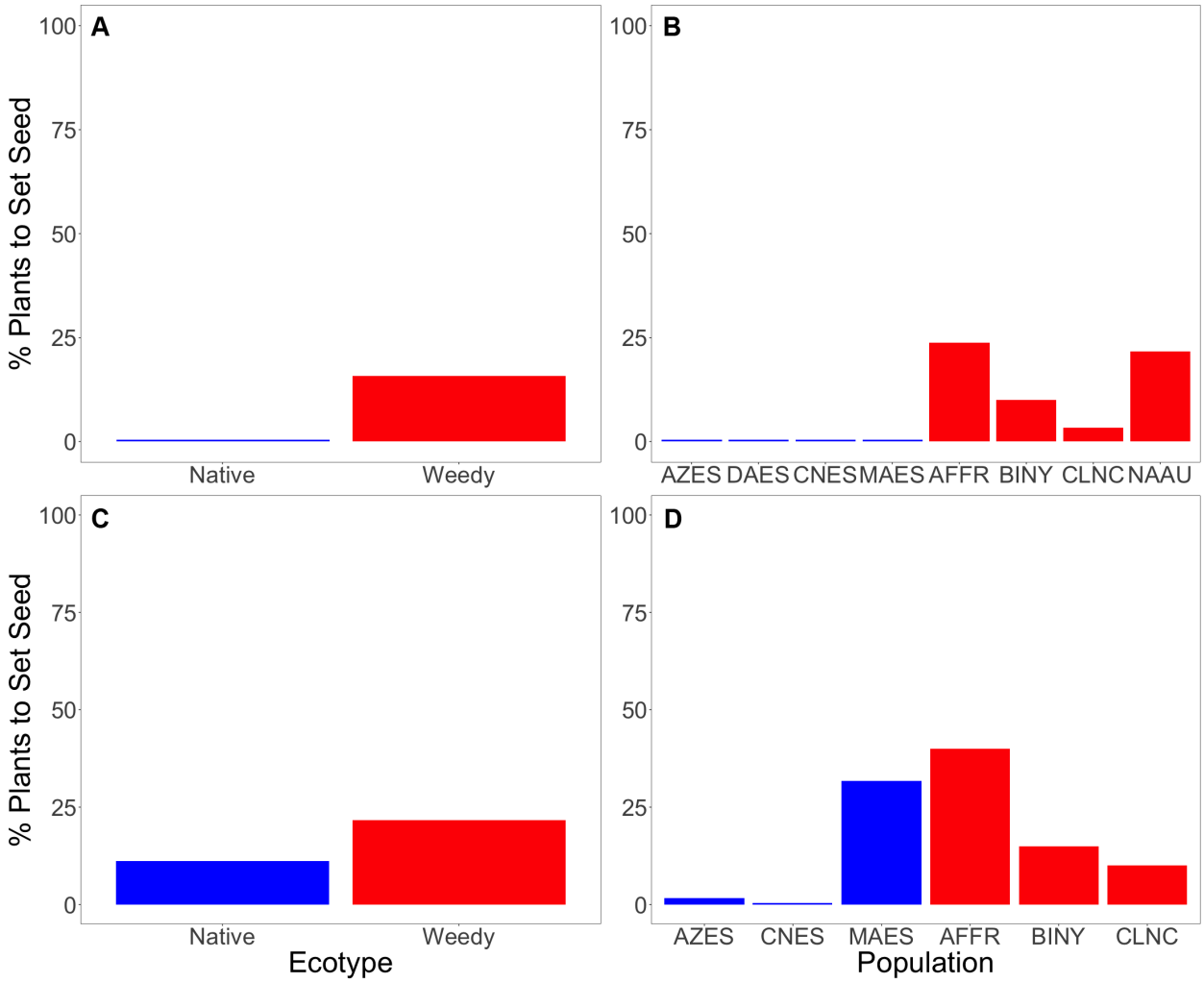

Figure 3-1. Percent of plants in each ecotype (left) and population (right) to set seed within the growing seasons of 2018 (A&B) and 2019 (C&D). The sample size of each percentage is 60 plants, the number of plantings for each population. Blue bars indicate native radish populations and red bars indicate weedy radish populations.

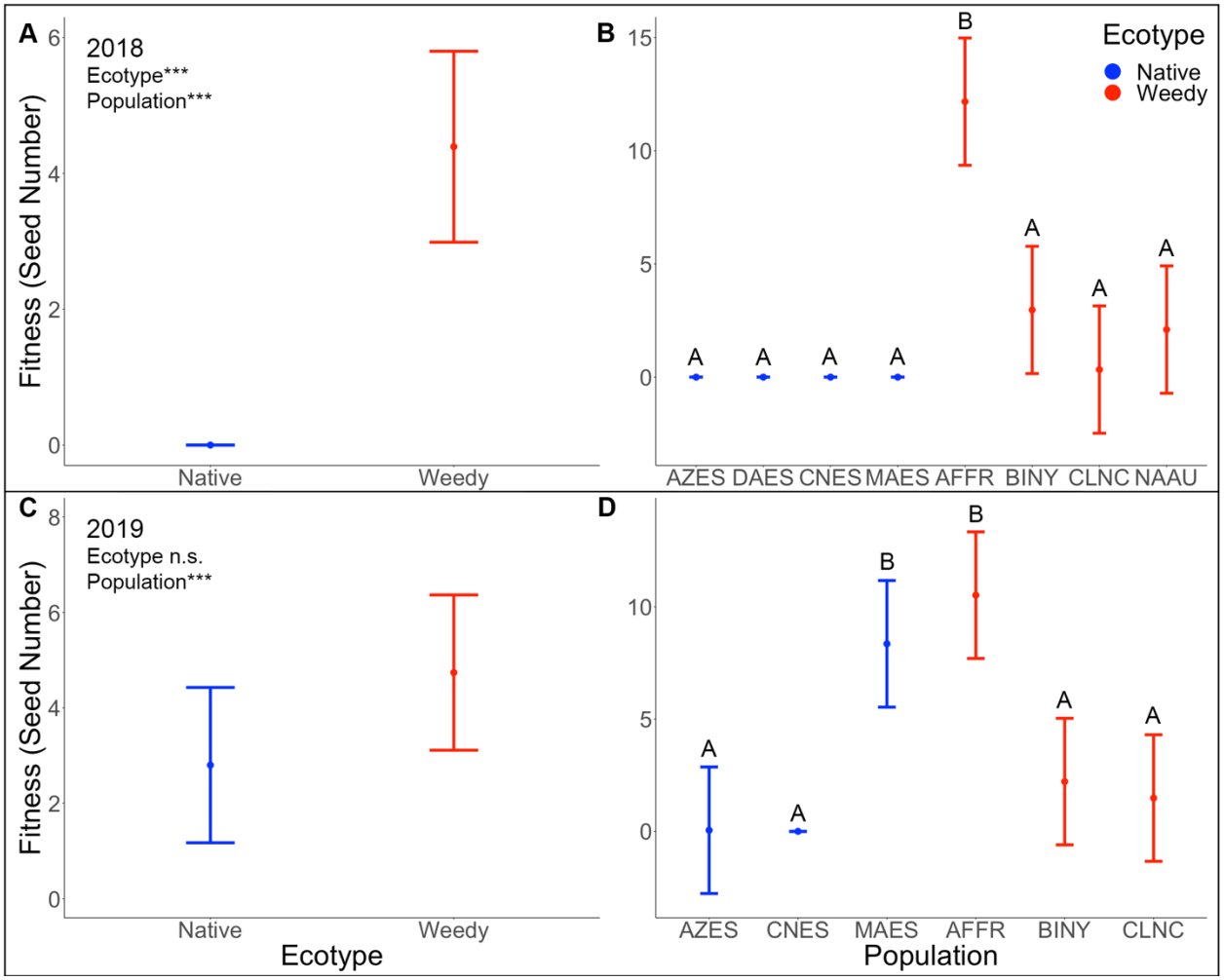

Figure 3-2. Fitness differences by ecotype (left) and population (right) in each common garden year. Least square means with 95% confidence intervals. Asterisks denote main effects that are significant at $P < 0.0001$ according to ANOVA, while letters denote means that are significantly different within each plot by Tukey's HSD.

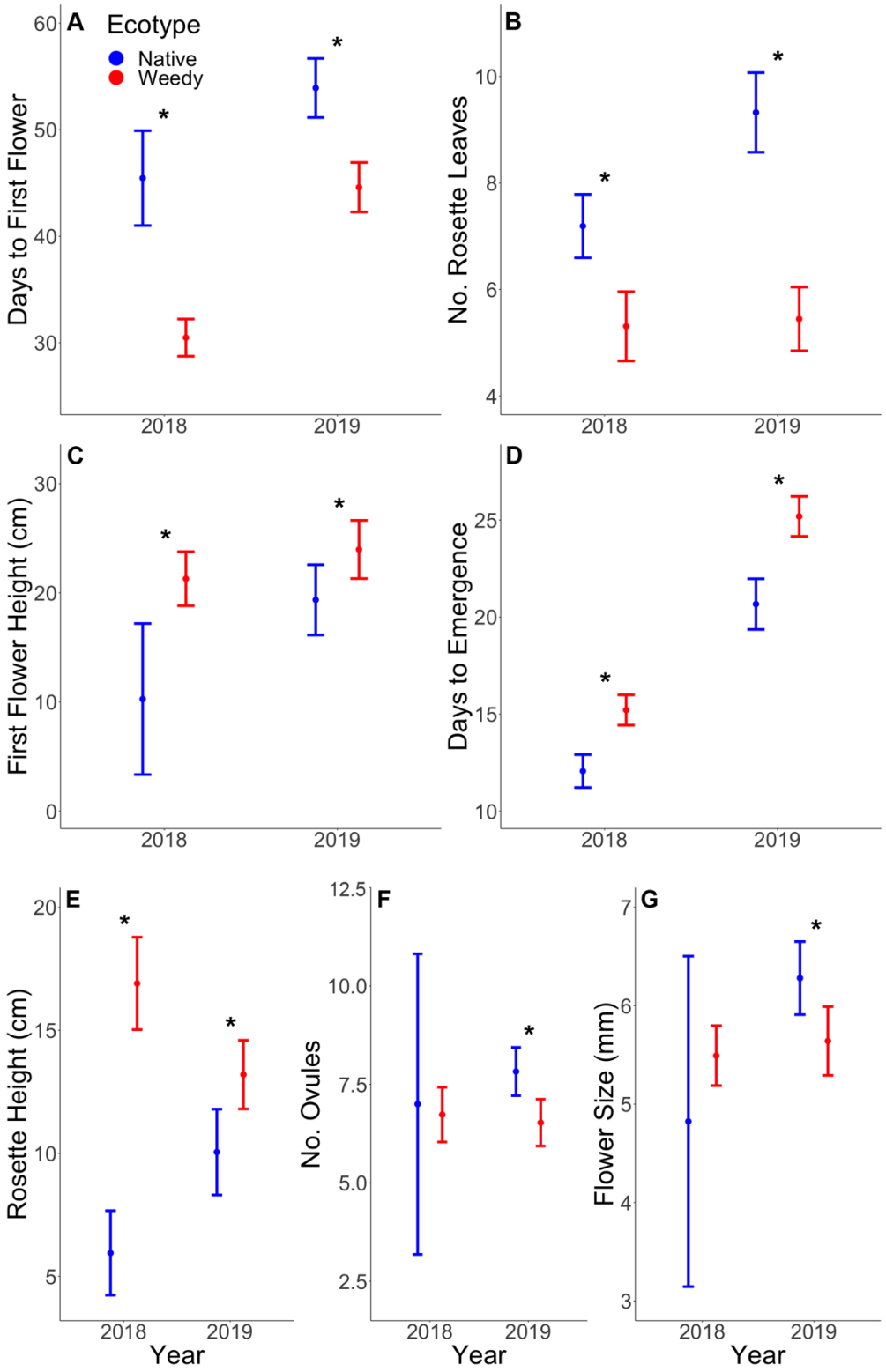

Figure 3-3. Trait means by ecotype. Least square means of days to first flower (A), number of rosette leaves (B), first flower height (C), days to emergence (D), rosette height (E), number of ovules (F), and flower size (G). Error bars are 95% confidence intervals, and asterisks over pairs indicate significant ecotype main effect at $P < 0.05$ according to ANOVA analysis.

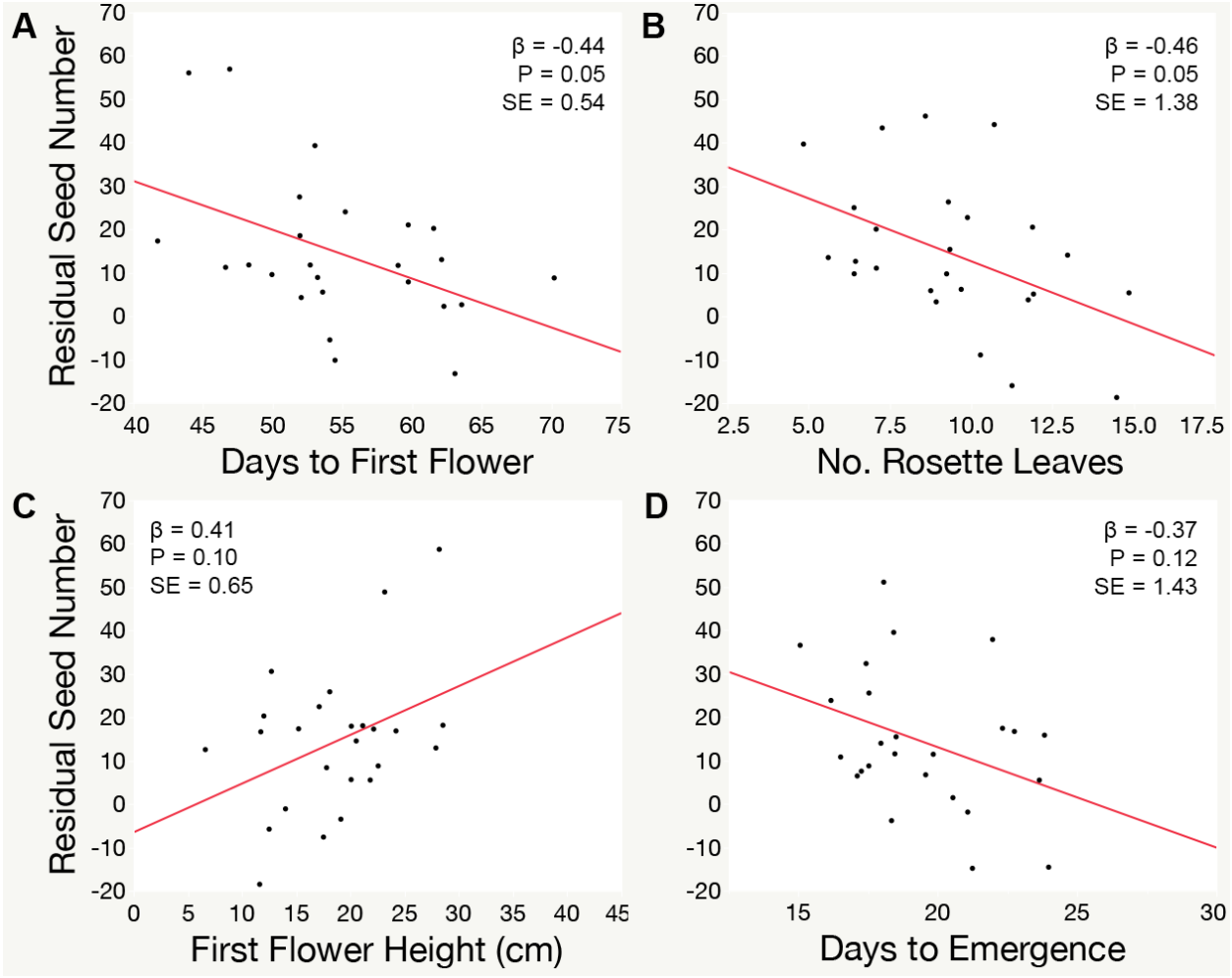

Figure 3-4. Traits under directional selection in the native population MAES: days to first flower (A), number of rosette leaves (B), first flower height (C) and days to emergence (D). Selection gradients (β), P-values, and standard errors from multiple regression analysis are reported.

Table S3-1. Result of ANOVA testing for mean trait differences by ecotype and by population nested within ecotype in each common garden. Bolded values denote significant differences at $p < 0.05$.

		2018		2019	
Trait	Predictor	F Ratio	Prob > F	F Ratio	Prob > F
Days to Emergence	Ecotype	**28.97**	**<0.0001**	**28.908**	**<0.0001**
	Population[Ecotype]	**4.27**	**0.006**	**8.170**	**0.000**
Days to First Flower	Ecotype	**38.97**	**<0.0001**	**26.055**	**<0.0001**
	Population[Ecotype]	2.58	0.060	0.262	0.770
No. Rosette Leaves	Ecotype	**17.73**	**<0.0001**	**64.350**	**<0.0001**
	Population[Ecotype]	2.26	0.083	**3.910**	**0.023**
Rosette Height	Ecotype	**72.20**	**<0.0001**	**7.792**	**0.006**
	Population[Ecotype]	2.29	0.081	2.440	0.092
First Flower Height	Ecotype	**8.93**	**0.004**	**4.790**	**0.031**
	Population[Ecotype]	0.27	0.847	0.914	0.404
No. Ovules	Ecotype	0.02	0.889	**9.266**	**0.003**
	Population[Ecotype]	1.15	0.330	**4.913**	**0.011**
Flower Size	Ecotype	0.63	0.432	**6.273**	**0.015**
	Population[Ecotype]	0.76	0.475	1.029	0.363
Seed Number	Ecotype	**18.85**	**<0.0001**	2.746	0.098
	Population[Ecotype]	**6.86**	**<0.0001**	**11.755**	**<0.0001**

CHAPTER 4:
ARTIFICIAL SELECTION FOR EARLY FLOWERING IN AN AGRICULTURAL WEED PREDECESSOR

Introduction

Populations colonizing a novel environment need to adapt rapidly in order to persist, and they can do so through either new mutations or standing genetic variation (Barrett and Schluter 2008). The relative frequency of populations adapting through either means is not known, but evolution via standing genetic variation is likely the faster route because beneficial alleles are already available and are at higher frequencies in the population than with a new mutation (Innan and Kim 2004). Additionally, standing genetic variation may be filtered by past selection in previous environments or ancestral populations, and thus may be more useful than novel mutations (Rieseberg et al. 2003). Therefore, information about a population's standing genetic variation, and more specifically the additive genetic variance in traits under directional selection in the new environment, can tell us about the potential of a population to adapt to that new environment (Lande and Shannon 1996).

Artificial selection experiments provide the strongest evidence for the presence of additive genetic variance in a trait (Conner 2003). By choosing which individuals reproduce based on a single trait value, one can learn not only how that trait responds to direct selection, but also correlated responses to that selection in other traits caused by genetic correlations (Conner 2003). Genetic correlations between traits may be helpful for adaptation to a new environment if correlated responses move traits toward their fitness optima, or may be a constraint if correlated responses move traits away from their fitness optima (Agrawal and Stinchcombe 2009). Therefore, information about additive genetic variance and genetic correlations in a population is crucial to understanding how it may adapt to a novel environment.

One important venue to study additive genetic variance and trait correlations is agricultural fields, because agricultural weeds are constantly adapting to management practices meant to eradicate them (Ashley et al. 2003; Neve et al. 2018), either through novel mutations standing genetic variation (Orr and Betancourt 2001). Although it is not clear how often weed adaptations result from standing genetic variation vs. novel mutations, there is evidence for high additive genetic variance in important weed traits. Resistance to herbicides has been found to evolve from standing genetic variation in populations that have never been exposed to the herbicide (Preston and Powles 2002; Neve and Powles 2005). Further, an overall lack of evidence for fitness costs in the evolution of herbicide resistance has been hypothesized to be due to evolution from standing variation involving fewer costs than novel mutations (Baucom 2019). Additionally, high additive genetic variation for several traits in *Alopecurus myosuroides* indicated the potential to adapt to non-chemical management practices like flower head mowing (Comont et al. 2022). In that same study, a lack of correlation between herbicide resistance and life history traits suggests that there was not a fitness cost associated with the evolution of herbicide resistance in that species (Comont et al. 2022).

For wild plants first colonizing agricultural fields, high additive genetic variance in key traits can enable rapid adaptation to the novel field environment. There is evidence that adaptation to agricultural fields will be fastest in populations that receive introgression from closely related crop species, which already carry some of the genetic variation needed to survive in the field (Campbell, Snow, and Sweeney 2009; Vigueira, Olsen, and Caicedo 2013). However, given adequate additive genetic variation in traits important for adaptation to the agricultural setting, wild populations should also be able to colonize agricultural fields without introgression.

This wild-to-weed route of evolution has been less explored, partly because the wild relatives of crop weeds are often poorly known (Vigueira, Olsen, and Caicedo 2013).

Weedy radish (*Raphanus raphanistrum ssp. raphanistrum*) is a costly and damaging agricultural weed. Weedy radish infests cereal fields on all continents but Antarctica (Holm 1997; Schroeder 1989; Alemseged, Jones, and Medd 2001; Hashem and Wilkins 2002), can decrease crop yield by up to 55% (Eslami et al. 2006), and has evolved resistance to multiple herbicides (Walsh et al. 2004; Walsh, Owen, and Powles 2007). In addition to this, weedy radish likely evolved from a wild ancestor as it is most closely related to a plant of the same species and subspecies native to the Mediterranean (hereafter "native radish"; Shen et al. 2013; Charbonneau et al. 2018). So despite belonging to the same genus as an agricultural crop (*R. sativus*), weedy radish may be a good model for studying the wild-to-weed route of evolution in particular (Vigueira, Olsen, and Caicedo 2013). However, it is unclear whether a wild ancestor of weedy radish would have been capable of adapting to the extreme environment of an agricultural field.

For agricultural weeds, one of the most important adaptations is a rapid lifecycle that allows the plant to grow and set seed in the short time between tilling the field and harvesting crops (Baker 1965; 1974). The weedy ecotype of *R. raphanistrum* has indeed evolved a rapid lifecycle, commonly flowering in as few as 30 days from emergence (Charbonneau et al. 2018), with evidence that advanced flowering is adaptive in agricultural fields (Garrison Chapter 3). Additionally, weedy radish has been shown to have high additive genetic variance for flowering time (Conner and Via 1993). Native radish, on the other hand, is a winter annual in its natural environment and has a much slower lifecycle, often requiring vernalization (cold temperatures) before it can flower (Charbonneau et al. 2018). In a 2018 common garden in an active summer oat field, no native radish plants were able to set seed in the 81 days between planting and

harvest (Garrison Chapter 3). Therefore it is important to determine whether a wild ancestor to weedy radish had enough additive genetic variance for flowering time to adapt to the accelerated life cycle required in an agricultural field.

Other traits have been found to differ consistently by ecotype, but it is unclear if these traits are the result of direct selection by the agricultural environment, or if they are the consequence of correlated responses to selection on flowering time. Using Fst/Qst comparisons it was found that taller flowers and fewer rosette leaves were potentially adaptive traits in weedy radish (Sahli et al. 2008), and more upright rosette leaves are also consistently found in the weedy ecotype (Garrison Chapters 1 & 2). Taller flowering stalks may have evolved in crop fields to increase visibility to pollinators among tall crops. A loss of rosette leaves in weedy radish may have resulted from greater resource allocation to reproduction, and more upright rosette leaves may increase light capture in competition with rapidly-growing crops. This is in contrast to native radish, which grows in natural to disturbed habitats with little surrounding vegetation (Arroyo and Gómez, pers. comm.) and produces overwintering rosettes with many leaves close to the ground. These rosette trait differences likely reflect a difference in life history strategy between the ecotypes (Adler et al. 2014), where native plants invest in vegetative growth and flower later, whereas weeds switch their investment to reproduction earlier to flower faster and produce fewer leaves overall. While the trait divergence between *R. raphanistrum* ecotypes is clear, the mechanisms by which these ecotypes diverged requires further study.

In this study, we asked the following questions: (1) could a wild ancestor of weedy radish have had enough additive genetic variation to rapidly evolve faster flowering in response to a shortened growing season, and (2) are any of the trait differences between the weedy and native ecotypes of *R. raphanistrum* the result of correlated responses to selection on flowering time? To

address these questions, we selected for early flowering time in two populations of native radish (a proxy for the wild ancestor of weedy radish) for two generations. A significant decrease in flowering time will provide evidence for additive genetic variation. Additionally, correlated trait responses to selection on flowering time that match ecotype differentiation (i.e., traits shift toward the weedy phenotype) suggests that some of the weedy radish traits differences may not be the result of direct selection. Finally, correlated trait responses in the opposite direction of the ecotype differentiation will provide evidence for genetic constraints that traits had to overcome in the evolution of weedy radish.

Methods

Study system

We selected for early flowering in two populations of the native *R. raphanistrum* ecotype: DEES and MAES. DEES is a population of plants growing in a natural, relatively undisturbed area in a montane park in Despeñaperros National Park in Jaén, Spain (Arroyo & Gómez, pers. comm.). MAES was also collected in Spain, in Colemnar Viejo just north of Madrid, but this population was found in a more disturbed area bordering a cereal field (Gómez-Campo, pers. comm.). Both populations both grow naturally as winter annuals and have similar flowering times in the greenhouse, although a higher proportion of the MAES population require vernalization to flower (Charbonneau et al. 2018).

Creation of selection lines

First generation of selection. In January 2015, 32 matrilines each of two populations of native radish (MAES and DEES) were started in a greenhouse, with 10 plants per matriline. Matrilines for MAES each consisted of full-siblings from a recent greenhouse-grown seed bulk-up, while matrilines for DEES were haphazardly assigned from bulked field-collected seeds.

Five plants from each matriline were randomly assigned to the Control line while the other 5 were assigned to the Selected line (earlier flowering). The full design was 2 populations X 32 matrilines X 2 selection lines X 5 plants per line = 640 plants total. However, due to limited germination in the DEES population, only 228 of the 320 pots planted produced plants.

Date of emergence and first flower opening was recorded for each plant. Following the methods of (Conner et al. 2011), the sibling with the fewest days from emergence to flowering was chosen to be crossed in the Selected line, while one of the five siblings in the Control line was randomly chosen. This design maximizes effective population size and minimizes genetic drift for crosses to produce the new generation (Hill 1980). The full design was 1 plant X 32 matrilines X 2 selection lines X 2 populations = 128 to be crossed total. Chosen plants were randomly mated within their selection line with each plant donating and receiving pollen from one other plant, with no reciprocal crosses.

Four plants from four different matrilines in the DEES population had not flowered by October (after crosses were completed), and were vernalized in growth chambers with eight-hour days at 6°C from October 5, 2015 until December 12, 2015. Two of the four flowered within two weeks of their return to the greenhouse, while the other two failed to flower and were thrown out of the study.

Second generation of selection. In 2018, the seeds collected from the first generation of the artificial selection experiment were grown in the greenhouse (i.e., Generation 1 plants). Not all matrilines from the parental generation produced seeds; the MAES Control line was missing two matrilines and DEES Selected was missing five. For the DEES selected line, five matrilines that produced many seeds were used twice. For the Control lines, bulk-up MAES seeds were used to restore the two matrilines. Five plants/matriline/population were grown for the Selected

lines, but only two plants/matriline/population were used for the Control lines to save space in the greenhouse. The full design was 7 plants X 32 matrilines X 2 populations = 448 Generation 1 plants total.

Date of emergence and flowering were once again recorded to measure flowering time, and crosses were assigned in the same manner as in the previous generation. 32 unique crosses were done within each population and selection line to produce Generation 2 seeds, but due to incompatibility and plant mortality a total of 20 plants were used as either a pollen source or recipient more than once in the 128 crosses.

Measurements of selection and correlated responses

To investigate trait changes after two generations of selection on early flowering, we performed a greenhouse common garden using seeds from our Selected and Control lines from the second generation of selection. Dates of emergence and first flower opening were recorded and flowering time was measured in the same manner as the first two generations. In addition, we looked for correlated responses to selection for early flowering in other traits that differ between native and weedy ecotypes. At the time of bolting (when flower buds first appear) we took top- and side-view rosette photographs, and counted the number of rosette leaves and the rosette height (the vertical distance from the soil to the highest point of the freestanding rosette). At the time of flowering we measured the first flower height as the vertical distance from the soil to where the flower's pedicel connects to the stem. We collected the third flower of every plant (or the most recently opened flower after the third) to take side-view and overhead photos of the flower. The rosette and floral photographs enabled us to measure the following additional traits using ImageJ (Schneider, Rasband, and Eliceiri 2012): leaf length and width, petal (limb) length and width, corolla tube (claw) length, pistil length, and the lengths of both the anther and

filament of one short and one long stamen. The floral measurements were then used to calculate three composite traits: anther exsertion (long filament length minus corolla tube length; Conner et al. 2011), anther separation (long filament length minus short filament length; Sahli and Conner 2011), and flower size (the geometric mean of the eight directly measured floral traits).

The data were analyzed using mixed effect ANOVA, with selection line as a fixed effect and matriline as a random effect nested within each selection line (JMP, Version 15. SAS Institute Inc., Cary, NC, 1989-2021). Separate analyses were done for each population. The plant traits measured in this study were each separate response variables, with selection line as the predictor variables and matriline included as a random effect.

Results

After only two generations of selection for early flowering, both populations used in this study flowered significantly faster compared to Control lines (Fig. 4-1; Table 4-1). The DEES Selected line flowered 50.5 days (33.9%) faster than the Control after two generations of selection, following a similarly large advance of 48.1 days (28.6%) after only one generation (Fig. 4-1A; Table 4-1). The MAES population was on average much faster to flower than DEES, but after two generations was still able to significantly advance its flowering in the Selected line by 15.8 days (22.3%) compared to Control (Fig. 4-1B; Table 4-1). There was also flowering time variation between years due to environmental variance in the greenhouse, with flowering time appearing slowest on average in the second year of the experiment, and fastest in the first (Fig. 4-1).

There was evidence of correlated responses to selection on five traits after two generations of selection for early flowering, with the direction matching the weedy phenotype in all but one trait (Fig. 4-2; Table 4-1). In both populations, plants in the Selected line produced

fewer rosette leaves on average, with the effect being slightly larger in DEES (8.1 fewer) than in MAES (6.0 fewer). While the Selected lines produced fewer rosette leaves, the leaves they produced were ~0.5cm wider in both populations, and 1.6cm longer in DEES. Flowers were ~0.3mm smaller on average in the Selected lines in both populations. Finally, flowers in DEES were 5.1cm shorter in the Selected line, in contrast to the weedy phenotype of taller flowers. All other traits measured did not significantly differ between selection lines after two generations of selection (Fig. S4-1).

Discussion

Artificial selection experiments are important tools for investigating the additive genetic variance in a population and its potential to adapt to new environments (Conner 2003). In this study we show that native radish, which likely shares a recent ancestor with the damaging agricultural weed of the same species and subspecies (weedy radish), can rapidly evolve its flowering time toward the weedy phenotype in just two generations of artificial selection. Artificial selection can also reveal trait correlations by measuring responses in traits not under direct selection, and these genetic correlations can either help or hinder adaptive evolution in the correlated traits. In this study we also found evidence for genetic correlations in several traits that are known to have been important in the evolution of weedy radish.

Native radish has previously been used as a proxy for the wild ancestor of weedy radish (Garrison Chapters 1 & 2), but little is known about its potential to adapt to an agricultural environment through standing genetic variation. Weedy radish has evolved to flower rapidly, taking between just 25 to 35 days on average (Charbonneau et al. 2018). In our study, two generations of artificial selection for earlier flowering shifted the average flowering time of one native population (DEES) 50 days closer to this weedy phenotype compared to the Control line.

In the population MAES, the overall difference between Control and Selected lines was smaller (15.1 days), but the Selected average flowering time was reduced to just 55 days, almost twice as fast as Selected DEES. This rapid response in both populations provides evidence that a native ancestor of weedy radish may have been able to adapt to earlier flowering using existing additive genetic variation after first colonizing an agricultural field.

This study adds to a broader body of literature that uses artificial selection to investigate additive genetic variance for flowering time. Compared to other systems, native radish showed a rapid response, with Selected lines flowering 33.9% and 22.3% faster than the Controls in DEES and MAES, respectively. For instance, three generations of selection for early flowering in *Campanulastrum americanum* led to a 13.3 day (14.7%) advance in flowering (Burgess, Etterson, and Galloway 2007), while the greatest selection differential among populations of *Mimulus cardinalis* after two generations of selection was 17.64 day (21.6%) advance (Sheth and Angert 2015). In agricultural relatives of native radish, however, there have been substantial shifts in flowering time that suggest similarly high levels of additive genetic variance. *Raphanus sativus*, the species of the radish crop, was made to flower in as few as 19 days after an unknown number of generations of selection for early flowering (Williams and Hill 1986), while *R. sativus* is known to take anywhere from 37 to 172 days to flower on average in the field (Charbonneau et al. 2018). In the weedy ecotype of *R. raphanistrum*, plants were able to advance their flowering time by 30 days after five generations of selection for early flowering, but were able to delay their flowering by 55 days after just three generations of selection for later flowering (Ashworth et al. 2016). However, it is worth noting that the weedy radish population was an escaped weed collected outside of the agricultural field in Australia (Walsh et al. 2004) and was much slower to flower than populations collected from agricultural fields (59 days on average).

In addition to additive variance for early flowering, we also found evidence for correlated responses to selection in several traits that differ between weedy and native radish. In four of the measured traits, the correlated response to selection matched the overall ecotype differences between native and weedy radish found in previous studies (Garrison Chapters 1-3; Sahli et al. 2008). Plants in the Selected lines produced fewer, larger rosette leaves compared to the Control, while the weedy ecotype produces fewer, larger rosette leaves on average compared to the native ecotype. Additionally, Selected plants made smaller flowers compared to the Control, while the weedy ecotype has also been found in previous studies to produce smaller flowers on average (Garrison Chapters 1 & 2; Sahli et al. 2008). These correlated responses to selection that match existing ecotype differences could suggest that these trait differences are not adaptive in agricultural fields, but rather just due to selection for early flowering. However, given previous evidence that both earlier flowering and fewer rosette leaves are adaptive in agricultural fields (Garrison Chapter 3; Sahli et al. 2008), this particular genetic correlation may have facilitated the adaptation of these traits in weedy radish.

Not all correlated responses matched the differences between the weedy radish ecotypes. Across two generations of selection, DEES plants in the Selected line produced shorter plants compared to the Control. Similarly, in a continuation of the previously mentioned study where the weedy radish ecotype was selected for early flowering (Ashworth et al. 2016), a reduction in flower height in the selected compared to control was also found (Sun et al. 2021). However, the weedy ecotype consistently produces taller first flowers compared to the native ecotype (Garrison Chapters 1 & 2), a trait that has been shown to be adaptive in the agricultural field (Garrison Chapter 3). This suggests that in the evolution of weedy radish, direct selection on flower height may have had to overcome negative correlated responses to selection between

flowering time and flower height in some radish populations. As an obligate outcrosser that is primarily bee pollinated (Sahli and Conner 2011), selection on taller plants is likely important for radish plants in agricultural fields, where flowers will need to grow higher to be seen by pollinators among the tall cereal grains that weedy radish is known to infest. However, it is worth noting also that plants with flowers and subsequently fruits that grow lower on the flowering stalk are more likely to have an advantage in a harvest weed seed control system, where fruits that are below the 15cm practical harvest cutting height of wheat may be able to avoid interception and enter the soil seed bank (Walsh and Powles 2014; Sun et al. 2021), so this correlated response to selection may be adaptive in fields with this weed control practice.

In addition to the patterns described above, we also saw notable differences in trait response by both population and generation. As noted previously, MAES had a much smaller response to selection for early flowering time, likely due to its faster flowering than DEES in the parental generation (Fig. 4-1). Surprisingly, MAES was not found to flower any faster than DEES in previous experiments (Charbonneau et al. 2018) despite growing in a more disturbed area than DEES. This may be due to our use of field-collected seeds for our initial generation of DEES and greenhouse-grown seeds for MAES. It is possible that in performing crosses to create these seeds, MAES was unintentionally selected for earlier flowering. Flowering time variation by generation is less easy to account for. The three greenhouse experiments in this study took place over three different years (2015, 2018, and 2021) and started in three different months (January, February, and June, respectively), with similar greenhouse temperature and supplemental lighting settings used in each year. Despite similar greenhouse conditions, the parental generation of DEES plants flowered much faster than the subsequent generation on average (Fig. 4-1A). The source of that generational variation, particularly in DEES, is not clear.

In this study, we used artificial selection to show that the native ancestor of weedy radish likely would have been able to adapt to the agricultural environment by evolving faster flowering time through additive genetic variation to fit a more accelerated growth cycle. Additionally, we found evidence that some traits in weedy radish like fewer, larger rosette leaves and smaller flowers may have evolved due to genetic correlations with faster flowering. On the other hand, a genetic correlation between faster flowering and shorter flowers revealed that taller flowers evolved in weedy radish despite that genetic constraint. As well as elucidating more on the evolution of a harmful agricultural weed, this study adds to a body of literature that uses artificial selection to learn more about the potential of populations to adapt to new environments.

APPENDIX

APPENDIX

Tables and Figures

Table 4-1. Tests for differences between Selected and Control trait means after two generations of selection for early flowering, and least square means with standard errors around the means (SEM). Bold values denote test p-values < 0.05, while underlined p-values denote a significant random effect of matriline at p < 0.05.

	DEES				MAES			
Trait	Control Mean (±SEM)	Selected Mean (±SEM)	F Ratio	Prob > F	Control Mean (±SEM)	Selected Mean (±SEM)	F Ratio	Prob > F
Days to First Flower	**149.00 (7.31)**	**98.56 (7.35)**	**29.50**	**<0.01**	**70.71 (2.91)**	**54.91 (2.79)**	**15.34**	**0.0002**
No. Rosette Leaves	**48.59 (2.10)**	**40.50 (2.12)**	**9.63**	**<u>0.002</u>**	**32.70 (1.84)**	**26.73 (1.77)**	**6.04**	**<u>0.02</u>**
First Flower Height	**14.97 (1.37)**	**9.90 (1.37)**	**7.99**	**0.01**	8.27 (0.69)	8.35 (0.65)	0.01	0.93
Flower Size	**7.13 (0.09)**	**6.84 (0.09)**	**4.70**	**0.03**	**6.69 (0.10)**	**6.40 (0.09)**	**4.40**	**0.04**
Leaf Width	**2.99 (0.13)**	**3.50 (0.13)**	**7.84**	**0.01**	**3.42 (0.17)**	**3.88 (0.15)**	**4.07**	**0.05**
Leaf Length	**11.09 (0.38)**	**12.76 (0.40)**	**8.99**	**0.003**	12.57 (0.55)	13.44 (0.47)	1.43	0.24
Rosette Height	7.70 (0.42)	7.56 (0.42)	0.06	0.81	6.58 (0.26)	5.99 (0.25)	2.77	0.10
Emergence	3.43 (0.31)	3.88 (0.32)	1.04	0.31	3.97 (0.34)	3.93 (0.32)	0.01	0.94
No. Ovules	9.57 (0.30)	10.08 (0.31)	1.60	0.21	7.68 (0.33)	8.34 (0.32)	2.21	0.14
Anther Exsertion	0.10 (0.11)	0.23 (0.12)	0.78	0.38	0.14 (0.10)	0.17 (0.10)	0.04	0.84
Anther Separation	3.21 (0.10)	3.19 (0.11)	0.02	0.88	3.47 (0.15)	3.27 (0.15)	0.86	0.36
	—One Generation of Selection—							
Days to First Flower	**168.48 (12.46)**	**120.37 (8.19)**	**12.40**	**0.0006**	84.60 (7.73)	75.12 (4.05)	1.27	0.26

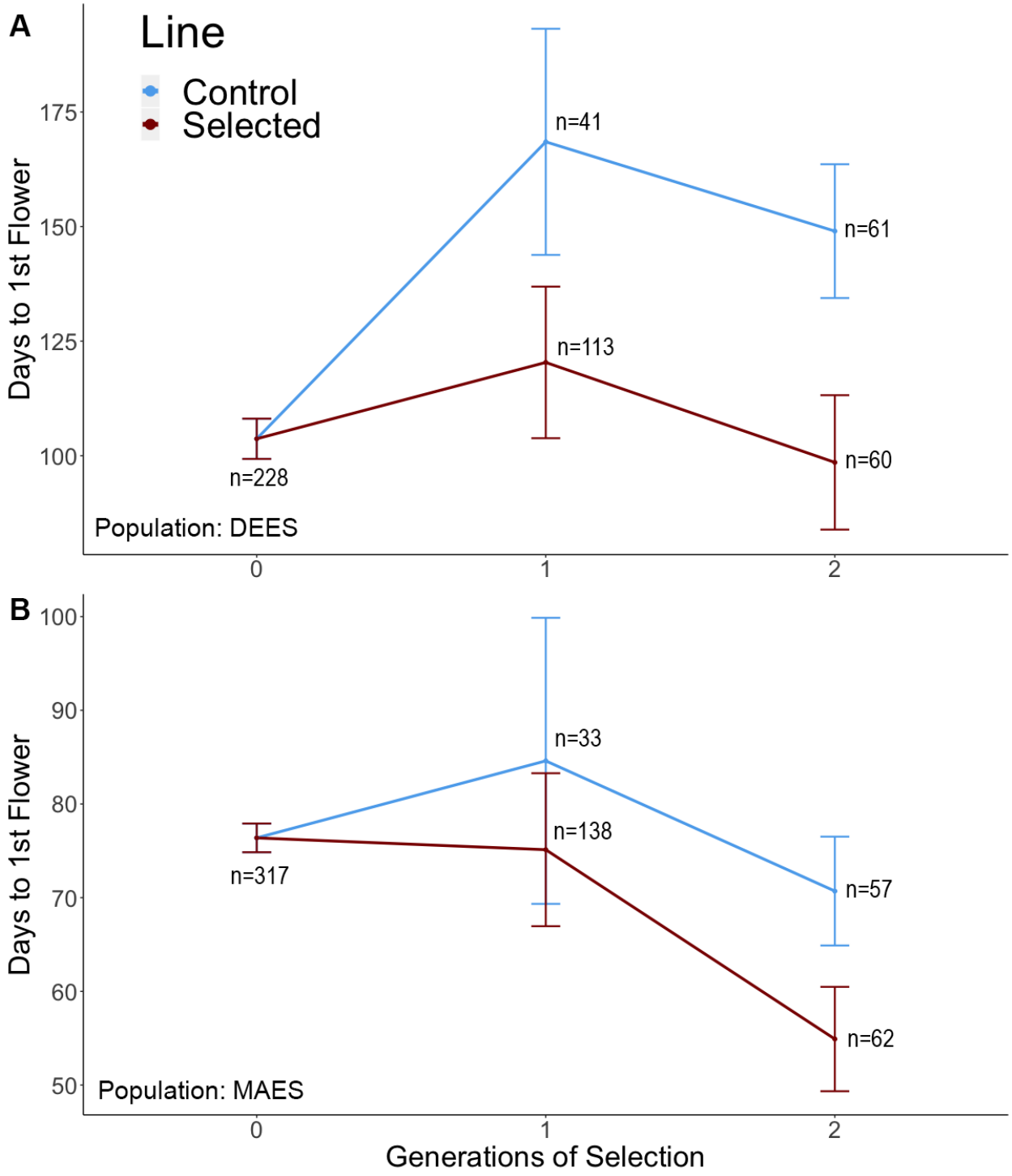

Figure 4-1. Flowering time after two generations of selection for early flowering in two populations of native radish, A) DEES and B) MAES. Error bars are 95% confidence intervals.

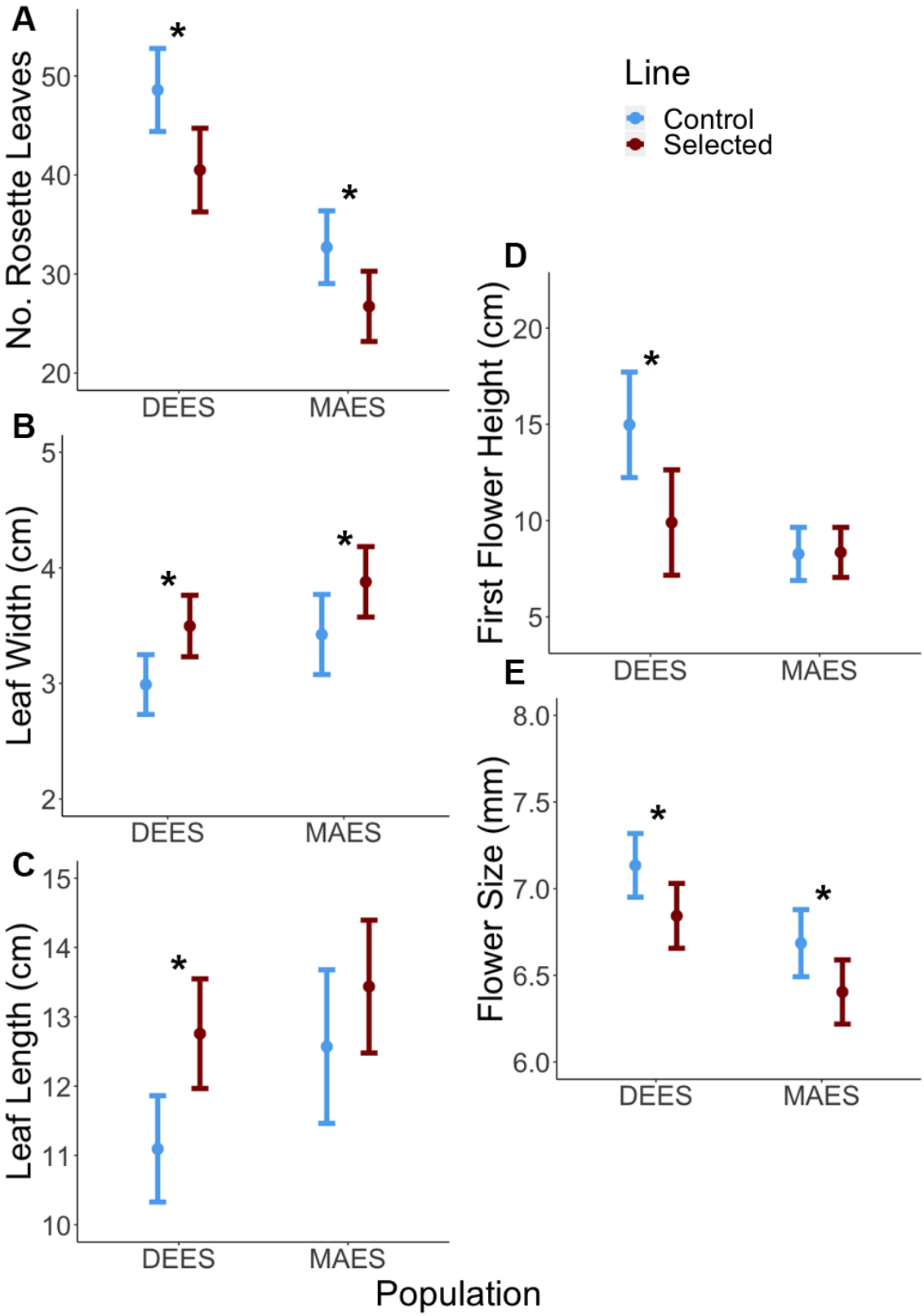

Figure 4-2. Indirect response to selection for early flowering in A) number of rosette leaves, B) leaf width, C) leaf length D) first flower height, and E) flower size. Asterisks above pairs denote significantly different means by t-test. Error bars are 95% confidence intervals.

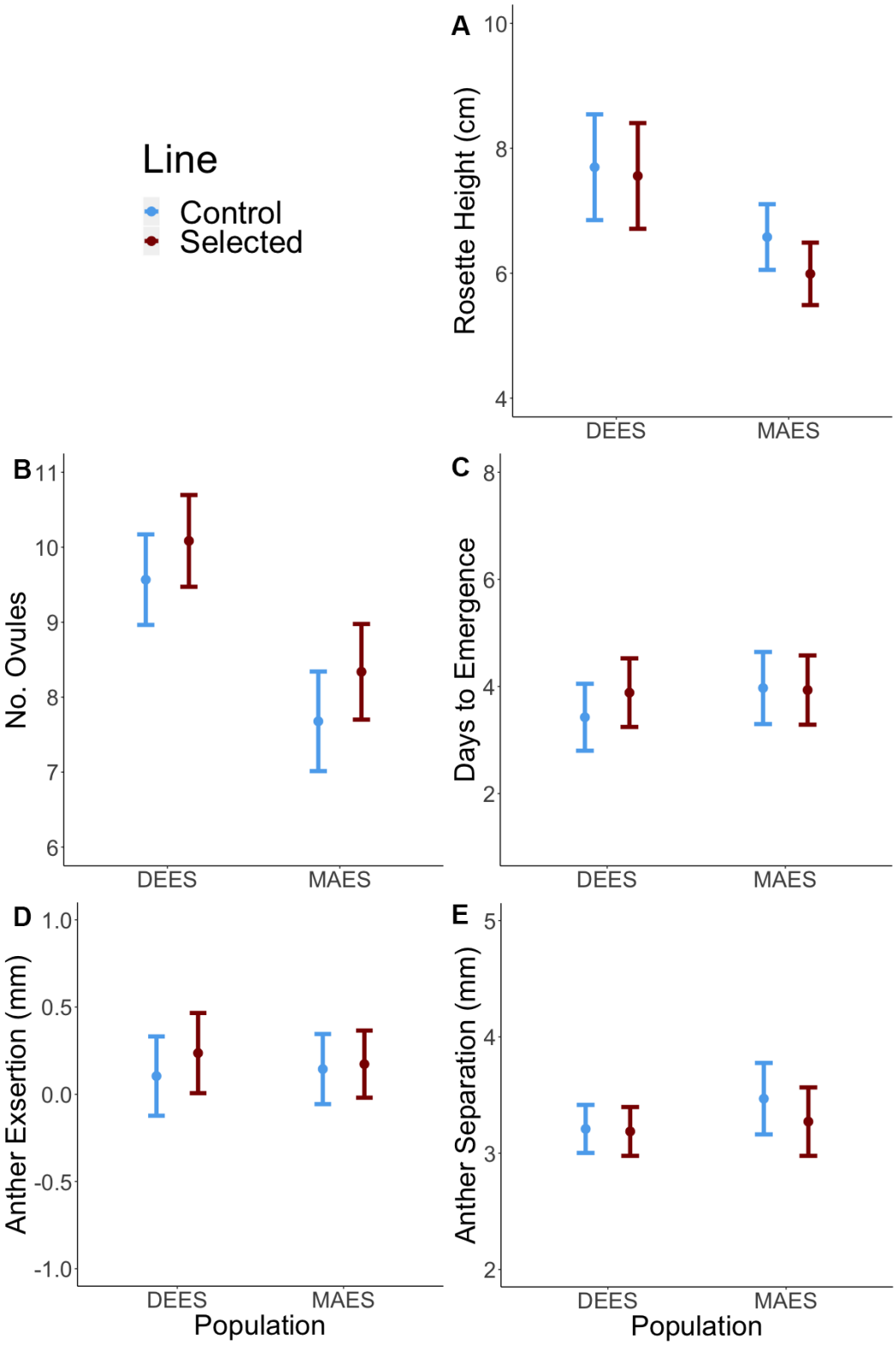

Figure S4-1. Line means after two generations of selection for early flowering in A) rosette height, B) number of ovules, C) days to emergence, D) anther exsertion, and E) anther separation. Error bars are 95% confidence intervals.

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