

Ecological contexts of balancing selection in nature

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the University Program in Ecology
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ABSTRACT

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Abstract

How genetic variation is maintained in the face of persistent natural selection is a central question in evolutionary biology. Here, I leverage a focal polymorphism, leaf chemical profile in a perennial wildflower (*Boechera stricta*, Brassicaceae) to investigate the ecological and genetic mechanisms that may influence the maintenance of variation in this trait.

In the first chapter, I present data from a suite of common garden and greenhouse experiments showing that the alleles underlying variation in chemical profile have contrasting fitness effects across environments. I identify two putative selective drivers on chemical profiles, and utilize phenotype-environment associations and molecular genetic analyses to test for additional evidence of past selection by these drivers. Together, these data are consistent with balancing selection on chemical profile, likely caused by pleiotropic effects of genes that influence secondary chemical biosynthesis on herbivore defense and drought response.

In the second chapter, I utilize a multi-year, manipulative field experiment to test for the effects of variation in selective drivers on genotypes conferring contrasting chemical profiles. I integrate variable effects of the environment on fitness components across life history and across environments to assess patterns of lifetime fitness. These

data suggest that environmental conditions in which contrasting genotypes can both persist may be widespread.

Together, these chapters provide complementary perspectives on the question of persistent natural variation, suggesting that variation in secondary metabolic profiles in *B. stricta* may persist at present due to balancing selection, and may continue to persist in the future under a range of varying environmental scenarios.

Dedication

For all the plants I've yet to see,
for a world that can grow them, and
for the process of coming to know them
tomorrow.

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Introduction

General premise and approach

How genetic variation is maintained in the face of persistent natural selection is a central question in evolutionary biology. Neutral models of mutation-selection balance are possible, but do not explain intermediate-frequency polymorphisms that have been observed in many populations and species. An alternative explanation is balancing selection, in which natural selection actively maintains polymorphisms at higher than neutral levels over space and/or time. This phenomenon is frequently invoked, but rigorous tests demonstrating balancing selection operating in nature are scarce, particularly on complex traits, which frequently display high levels of variation.

Moreover, balancing selection is an evolutionary process that may be generated by a variety of mechanisms, including overdominance, spatial and temporal variation in selection, frequency-dependent selection, and antagonistic pleiotropy, which may result from diffuse and/or multivariate ecological interactions. Although elucidating the functional mechanisms influencing balancing selection is critical to understanding how variation is expected to persist in future environments, concrete links between the genetic underpinnings of complex traits, ecological variation within and across environments, and subsequent selection on variation are limited.

Conversely, ecological approaches, which often aim to identify environmental mechanisms driving organismal performance and fitness, rarely employ strategies to

tease apart the effects of genotype and environment. This, however, is essential to understanding the capacity of populations to adapt to changing conditions.

In this dissertation, I leverage strengths of both of these approaches. By utilizing tractable genetic tools in field and greenhouse experiments, I aim to dissect the molecular underpinnings of ecological interactions and fitness in nature. Conversely, by utilizing population modeling, I aim to understand the role of ecological variation and covariance in determining lifetime fitness. Together, these approaches offer an integrated perspective on the forces contributing to the maintenance of natural variation.

Collaborative contributions

Here, I present a synthesis of collaborative work spanning field experiments, greenhouse experiments, molecular genetic analyses, and demographic modeling. All co-authors contributing to each project are listed at the beginning of the research chapters. As is required by The Graduate School at Duke University, I outline the original and collaborative contributions to the two research chapters below:

For the first chapter, data were generated by myself and others, each of whom is named as a co-author. I analyzed all of the presented data, with two exceptions: (1) assembly of the *Boechera stricta* version_2 reference genome, and subsequent analyses of *BCMA* copy number variation and the evolutionary history of gene duplications; (2) SNP calling and calculation of population genetic summary statistics such as F_{ST} , π , and

r^2 . Analyses in the former section were led by Cheng-Ruei Lee; those in the latter were led by Baosheng Wang and Tom Mitchell-Olds. I synthesized, interpreted, and described all results, with input from collaborators when necessary, especially regarding methods in components that I did not generate data for myself.

For the second chapter, I carried out the experimental design, data collection, and analyses independently, with input from the named co-authors and logistical assistance from students and research assistants. I synthesized, interpreted, and described all results independently, with feedback from co-authors.

1. Ecological factors influence balancing selection on a complex trait locus

Lauren N. Carley, Julius P. Mojica, Baosheng Wang, Chia-Yu Chen, Ya-Ping Lin, Kasavajhala V. S. K. Prasad, Emily Chan, Che-Wei Hsu, Rose Keith, Chase L. Nuñez, Catherine Rushworth, Maggie R. Wagner, Jing Wang, Pei-Min Yeh, Kathy Ghattas, Cheng-Ruei Lee, and Tom Mitchell-Olds

1.1 Introduction

In the face of natural selection, why do diverse ranges of phenotypes persist in the wild, rather than solely combinations of optimal traits? Population genetics theory demonstrates that both directional and stabilizing selection should result in the reduction of genetic variation within populations over time (Falconer & Mackay 1996). Following the development of methods for quantifying phenotypic selection (Lande 1979, Lande & Arnold 1983), hundreds of studies have generated thousands of measurements of selection in nature. Meta-analyses reveal that directional selection is common in nature and can be strong (Kingsolver et al. 2012). Despite this, high levels of additive genetic variance are frequently documented within and among natural populations (Mitchell-Olds & Rutledge 1986, Houle 1992, Barrett & Schluter 2007). This incongruity between empirical evidence – persistent and widespread genetic diversity – and theoretical predictions – depletion of genetic variation in response to selection – is perplexing.

In addition to posing a theoretical puzzle, the persistence of genetic variation also has implications for the ability of populations to persist. Genetic variation is the “raw material” by which populations adapt to environmental changes (Barrett & Schluter 2007); thus, the ability of a population to respond to selection is influenced by the amount of genetic variation existing in that population for the trait(s) underlying adaptation. Because anthropogenic activity has dramatically increased the rate of environmental change in the past century (Steffen et al. 2007), the question of whether and how populations can adapt to changing selective pressures is of critical importance (Lande & Shannon 1996, Etterson & Shaw 2001, Anderson et al. 2012).

The neutral explanation for persistent genetic diversity within populations is mutation-selection balance (Zhang & Hill 2005). In this model, recurrent mutations introduce new alleles into loci. Some mutations may persist due to genetic drift if they are neutral, but selection acts to remove those that are deleterious. Because of this, the expected outcome of mutation-selection balance is a relatively high number of polymorphisms occurring at relatively low frequencies, because deleterious mutations are removed by selection before they become common, and because neutral mutations only drift to intermediate frequencies over very long periods of time (Johnson & Barton 2005). While a variety of empirical studies have supported the mutation-selection balance hypothesis, including several regarding human genetic variation (Kryukov et al. 2007, Keinan & Clark 2012), many natural populations display levels of polymorphism

that are higher than expected from neutral models (Caballero & Keightley 1994, Charlesworth & Hughes 1996, Zhang & Hill 2002).

Considering these shortcomings, an alternative explanation for high levels of genetic diversity is balancing selection, in which natural selection actively maintains genetic diversity within or among populations (Mitchell-Olds et al. 2007). While balancing selection is clearly theoretically plausible and has been documented empirically for some traits in nature (*e.g.* flower color [Subramaniam & Rausher 2000] and self-incompatibility [Charlesworth 2006] in some plants and immune traits in humans [Hedrick & Thompson 1983]), our understanding of it is still incomplete. First, the majority of studies of balancing selection have focused on genetically simple traits controlled by major genes; support demonstrating its role in the evolution of complex traits in nature is lacking (but see Mojica & Kelly 2010, Mojica et al. 2012, Ågren et al. 2013, Troth et al. 2018). Second, balancing selection is a pattern that can be generated by a variety of mechanisms, including overdominance, spatial and temporal variation in selection, frequency-dependent selection, and antagonistic pleiotropy (Hedrick et al. 1976, Delph & Kelly 2014). However, the relative importance of these various mechanisms remains understudied. Inference about the contributions of different mechanisms of balancing selection to observed patterns of natural variation in complex traits is limited by experimental and genetic tractability of study systems.

The ecology surrounding these mechanisms is also often nebulous; indeed, as in most studies of natural selection (Anderson et al. 2014), it remains a challenge to concretely identify the environmental drivers of balancing selection and how they vary across space and time. Understanding the ecological contexts of natural selection is especially important for determining how environmental changes might influence evolutionary trajectories (Lawrence et al. 2012, Merilä & Hendry 2013, Alberti 2015). Making predictions about evolutionary outcomes under changing environments requires a mechanistic understanding of how ecological species-environment relationships influence fitness (Anderson & Wadgymar 2020) and, ultimately, the maintenance or depletion of genetic diversity.

Here, we leverage a genetically and experimentally tractable system to explicitly test multiple ecological and genetic mechanisms that may contribute to balancing selection on a complex trait locus. We focus on anti-herbivore defense, a complex plant trait comprising many constituent phenotypes – *e.g.* physical characteristics such as leaf toughness and hairiness (Agrawal & Fishbein 2006), life-history traits, structural features such as size and branching architecture (Carmona et al. 2011), and chemical properties such as primary (DeLucia et al. 2012) and secondary metabolite content (Mithöfer & Boland 2012), among others – all of which may have their own complex genetic architectures. In *Boechera stricta* (Brassicaceae), a wild relative of *Arabidopsis*, secondary metabolic profile contributes substantially to insect resistance, and, subsequently,

fitness; furthermore, chemical profile is controlled by a known gene of major effect (Prasad et al. 2012). Thus, secondary metabolite profile in *B. stricta* is an ideal intermediary phenotype trait linking complex trait variation to a tractable genetic basis. In this study, we used a combination of field experiments, greenhouse experiments, and molecular genetic analyses to test for balancing selection on a biochemical polymorphism, explicitly testing multiple contrasting ecological and genetic mechanisms that may contribute to balancing selection.

1.2 Methods

1.2.1 Study system and focal trait

Boechera stricta (Brassicaceae) is a short-lived perennial and emerging model organism for evolutionary ecology and genetics. Its short life span, small size, and self-compatibility make it amenable to experimental manipulation and crossing, and its close relationship to *Arabidopsis* affords a wealth of genetic and genomic resources (Rushworth et al. 2011). *B. stricta* is native to the Rocky Mountains in western North America and occupies a broad array of habitats spanning elevational and latitudinal gradients, making it an ideal system for studying the genetic bases of adaptive traits in nature.

Boechera, like all members of the Brassicaceae, produces amino-acid derived secondary chemicals called glucosinolates (GS), which mediate ecological interactions with herbivores and pathogens (Hopkins et al. 2009). A polymorphism present in *B.*

stricta causes variation in enzyme function in the first step of GS biosynthesis, allowing some GS to be synthesized from branched-chain amino acid precursors (valine and isoleucine; BC-GS) rather than the typical methionine (Met-GS) (Prasad et al. 2012). The gene controlling this flux is called branched-chain/methionine allocation (*BCMA*); it influences the proportion of total GS that are derived from branched-chain amino acids (BC-ratio). This chemical phenotype has previously been demonstrated to influence the complex traits of insect resistance and reproductive fitness in the lab and in some field environments, and shows high broad-sense heritability (Prasad et al. 2012).

After the *BCMA* QTL was identified (Prasad et al. 2012), representative genotypes showing high and low BC-ratio phenotypes (called “LTM” and “SAD” from MT and CO, respectively) were used to generate genetic tools for this and other studies. Specifically, SAD and LTM were used as parents in a biparental crossing design to generate near-isogenic lines (NILs) with a homogenous genetic background except for a small segregating region containing the *BCMA* locus (Prasad et al. 2012). These NILs are homozygous for either the SAD or LTM parental *BCMA* allele, conferring methionine-derived GS (hereafter referred to as the MM homozygote, from CO) or branched-chain amino acid-derived GS (the BB homozygote, from the MT-ID border), respectively. Additional detail on methodologies for developing genetic tools is provided in Appendix A.

Although transgenic approaches can assay the fitness effects of alleles at a finer genetic resolution than natural crosses, which are inherently limited in resolution by crossing-over frequency and linkage disequilibrium, we focus on NILs derived from crosses between naturally occurring *B. stricta* genotypes for several reasons. First, T-DNA can mutants display a range of intra- and interchromosomal rearrangements, as well as altered chromatin structure and function (Jupe et al. 2019), so multiple independent inserts are required to infer the functional effects of a transgene. In addition, our non-transgenic approach allowed us to avoid strict regulatory restrictions associated with the use of transgenic organisms, especially outdoors. By using NILs, we could allow plants to reproduce without concern for transgene escape, thus allowing us to measure consequences of allelic variation on reproductive fitness directly in a variety of natural habitats.

A note on genetic terminology

Previous research revealed that the *B. stricta* genome contains multiple copies of the *BCMA* gene (Prasad et al. 2012). Specifically, there is one copy on chromosome 2, *BCMA2*, which synthesizes GS using methionine, and has similar function in both LTM (MT) and SAD (CO) parental genotypes. *BCMA2* does not predict BC-ratio. In a long-read assembly of the LTM reference (see 1.2.6), there are two additional copies on chromosome 7, *BCMA1* and *BCMA3*, both of which fall within the *BCMA* haplotype. Mutations changing amino acid residues at positions 134 and 536 in the *BCMA* enzymes

differentiate both *BCMA1* and *BCMA3* from *BCMA2*. Cloning of the LTM and SAD parental alleles at each of these loci and expression in *Arabidopsis* revealed that *BCMA3* explains observed variation in the BC-ratio phenotype; mutations changing amino acid residues at positions 145 and 265 differentiate the LTM and SAD parental alleles, conferring the enzyme's activity targeting branched-chain amino acids vs. methionine during GS biosynthesis. The *BCMA1* gene copy is present in LTM but absent in the SAD parental genotype (Prasad et al. 2012).

Here, due to our primary focus on NIL crosses, we refer to “*BCMA* genotype” as the chromosome 7 haplotype, incorporating allelic variation at the casual locus explaining BC-ratio variation (*BCMA3*), the presence or absence of *BCMA1*, and possibly other loci in tight linkage with the *BCMA3* gene. Thus, we emphasize functional allelic variation between LTM and SAD parental genotypes at the *BCMA3* locus, acknowledging that other loci also occur in this region (see 1.2.6, 1.3.4, Appendix F, and Appendix I). Specifically, “*BB*” and “*MM*” genotypes identify NILs that are homozygous for the parental alleles conferring branched-chain GS and met-GS, respectively, as well as other loci in tight linkage. In this study, we assemble a new reference genome for *B. stricta* and use long-read sequence data to further characterize variation within the QTL (see 1.2.6).

1.2.2 Characterization of biochemical polymorphism

To assess the geographic extent and patterning of variation in BC-ratio, we characterized the chemical profiles of 337 genotypes collected from across the species range (Wang et al. 2019). Each genotype was collected from a different source location and self-pollinated for at least one generation under consistent greenhouse conditions to minimize maternal environmental effects on phenotypes. Specifically, we germinated seedlings of each genotype in petri dishes, and then transplanted germinants into potting soil (80% Fafard 4P Mix, Conrad Fafard, Agawam, MA; 20% Sunshine MVP, SunGro Horticulture, Vancouver, BC) in 4" cone-tainers (Stuewe & Sons Inc., Tangent, OR). We grew 20 replicates per genotype in a randomized, complete-block design under controlled greenhouse conditions (18-21° C day / 13-16° C night; 16 h day length; watering to saturation daily; fertilization at 300 ppm N weekly) in Durham, NC. When transplants reached 4 weeks in age, we harvested a single fresh leaf (~30 mg) per individual, flash-froze the tissue in liquid N₂, and then freeze-dried the samples. We weighed each freeze-dried leaf and ground the tissue with two 2.38 mm steel ball bearings (V and P Scientific, San Diego, CA) in each 1.4 mL tube (COMO RACK™, Micronic, Aston, PA) using a Geno/Grinder® automated tissue homogenizer and cell lyser (Spex® Sample Prep, Metuchen, NJ, USA). We extracted desulfoglucosinolates from the ground, freeze-dried tissue according to Burow et al. (2006), with modifications for high-throughput analyses, and separated and quantified distinct compounds from

total leaf GS extracts via high-performance liquid chromatography (HPLC) (Appendix H). Thus, we determined the identity and quantity of the different GS in each sample; from the full GS profiles for each individual, we calculated BC-ratio as follows:

$$BC\text{-}ratio = \frac{BC\text{-}GS}{Total\ GS} = \frac{1ME + 2OH1ME + 1MP}{1ME + 2OH1ME + 1MP + 6MSOH + 5MSOP}$$

We estimated the least-squares mean value and standard error of BC-ratio per genotype using a restricted maximum likelihood (REML) mixed effects model in JMP Pro v. 13, regressing BC-ratio onto the random effects of genotype ID and position within the greenhouse (complete statistical block). We visualized variation in these genetic mean trait values in response to the location of origin for each accession using the ‘ggmap’ package v. 3.0.0 in R (Kahle and Wickham 2013).

1.2.3 Characterization of genetic polymorphism

To explore the extent of genetic variation underlying the BC-ratio polymorphism, we performed Sanger sequencing on a subset of 92 genotypes from the full panel of 337 accessions described above (Mojica et al., *unpub.*).

Sequence alignment revealed multiple genetic variants in the *BCMA* locus; in addition to the previously described gain-of-function changes altering activity of the BCMA1 and BCMA3 enzymes (Prasad et al. 2012; 1.2.1), we identified an 8 bp deletion (causing a frame shift) and a nonsense mutation (causing a premature stop codon), each of which was present in multiple genotypes in this panel (Appendix B: Table B1). To

assess the effects of different allelic variants on BC-ratio, we used linear models ('lm' function in the 'stats' package v. 3.6.0 in R v. 3.6.0) fitting BC-ratio in response to the presence or absence of the 8bp deletion, the presence or absence of the premature stop codon, and genetic group (Wang et al. 2019). We plotted the geographic distribution of allelic variation using the 'ggmaps' package (Kahle and Wickham 2013) in 'ggplot2' v. 3.1.1 in R v. 3.6.0. To characterize the known geographic range of each allele, we used the 'geodist' package (Padgham & Sumner 2019) to calculate the maximum pairwise geographic distance between accessions containing each variant.

1.2.4 Field experiments

Prior studies have demonstrated that variation in BC-ratio in *B. stricta* influences herbivore defense (Schranz et al. 2009, Manzaneda et al. 2010, Prasad et al. 2012). However, whether herbivory acts as a driver of balancing selection for glucosinolate diversity is unknown. To test for effects of *BCMA* on herbivore resistance and fitness in nature, we conducted a suite of greenhouse and field experiments testing the effects of *BCMA* across environments. In this context and in all statistical models, we consider "environment" to be the combination of site + year, since the biotic and abiotic environments (*e.g.* herbivore abundance and composition, edaphic factors) may vary across sites within years, and because interannual variation in climate can also yield different selective environments across years within sites.

Common gardens

We transplanted 6,860 *BCMA* NILs into three experimental gardens in central Idaho and two experimental gardens in southwest Colorado, near the source populations for the parental genotypes used to generate the NILs (Figure 1, inset boxes). We transplanted separate cohorts containing 360-1,350 individuals each during independent transplanting events between 2013 and 2015 (Table 1). Replicate individuals within each genotype spanned multiple genetic families to control for possible effects of unlinked loci outside of the *BCMA* region (Appendix C: Table C1).

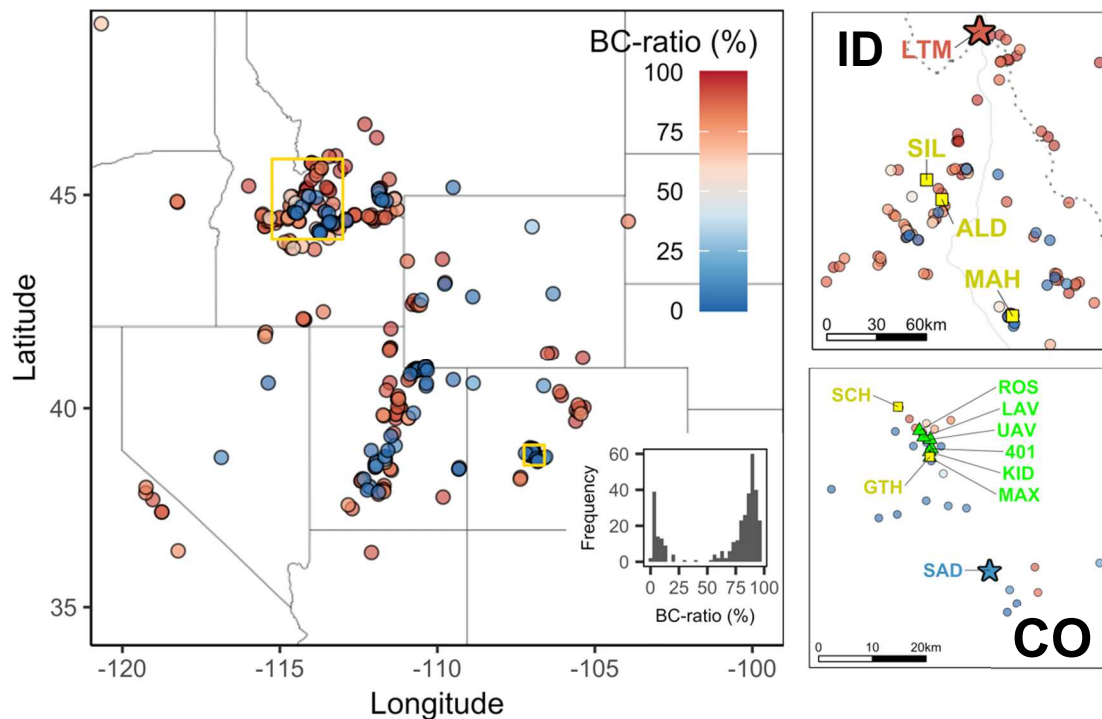


Figure 1. Glucosinolate variation in *B. stricta* is highly polymorphic and widespread. The proportion of glucosinolates derived from branched-chain vs. unbranched amino acids (“BC-ratio”) is a quantitative trait that is bimodally distributed (inset) and intermixed geographically across the species range. Boxes: Close-range view of northern (ID) and southern (CO) focal regions, showing the locations of the common gardens (yellow squares), temporary arrays (green triangles), and parental genotypes for crosses (red and blue stars) described in this study.

Prior to transplanting, replicate individuals of each *BCMA* genotype (full sibs derived from self-pollination) were germinated at Duke University and assigned to random positions within complete statistical blocks. We grew germinants for 1-2 months under controlled greenhouse conditions (as described in 1.2.2), and then shipped established rosettes overnight to field locations. In the field, we transplanted individuals

in a randomized complete block design at 10 cm intervals into the matrix of native vegetation in common gardens. Gardens were fenced to exclude megaherbivores, but insects and small vertebrates could freely browse experimental plants. Within each cohort, a minimum of four families were included per *BCMA* genotype to account for possible effects of unlinked loci outside of the *BCMA* region (see Appendix A).

Table 1. Summary of *BCMA* NIL transplants across years and sites.

State	Garden Site	Shorthand	Census Year	Transplant cohort	N planted	N obs. herbivory
ID	Alder	ALD	2013	Spring 2013	720	384
			2014	Fall 2013	360	302
			2015	Fall 2014	855	334
	Mahogany	MAH	2014	Fall 2013	540	252
			2015	Fall 2014	570	348
	Silver	SIL	2014	Fall 2013	450	265
			2015	Fall 2014	665	406
CO	Gothic	GTH	2016	Fall 2015	1350	293
	Schofield	SCH	2016	Fall 2015	1,350	1,090
Total:					6,860	3,674

Temporary arrays

In the common gardens, we planted *BCMA* NIL homozygotes with equal genotype frequencies, testing for spatial and temporal variation in herbivore defense and fitness. However, negative frequency-dependent selection can also maintain polymorphisms by conferring fitness advantages to genotypes or phenotypes that are rare (Bergelson et al. 2001, Hodgkins & Barrett 2006, Trotter & Spencer 2009). Thus, in

addition to transplants in common gardens, we implemented temporary arrays in six additional environments in Colorado in 2016 (Figure 1, green triangles) to test for frequency-dependent effects of *BCMA*. For this experiment, we germinated 5,880 *BCMA* NILs under greenhouse conditions (as described in 1.2.2) and transplanted germinants into 98-cell “cone-tainer” racks containing ~100 mL of potting soil per 3.8 cm diameter cone (Steuwe & Sons, Inc., Tangent, OR). In the greenhouse, we assigned racks to three treatments with different starting frequencies of the *MM* genotype: high = 66% *MM*, mid = 50% *MM*, and low = 34% *MM*. Individuals were assigned to random positions within racks. We shipped racks overnight to the Rocky Mountain Biological Laboratory and arranged 10 racks (3 high, 4 mid, and 3 low *f(MM)*) in a random configuration in each of the six array sites. Replicate individuals of each NIL genotype spanned multiple independent family lines to control for possible effects of unlinked loci outside of the *BCMA* region (Appendix C: Table C2).

To facilitate insect access to arrays, we sunk each rack flush with the soil and native neighbor vegetation by digging a separate rectangular hole for each rack, and excluded megaherbivores with wire cages (Appendix C: Figure C1). Because plants in racks were confined to a relatively small volume of soil, we watered all arrays twice per week for the first two weeks of the experiment. Despite this, plants began to show signs of drought stress (wilting, browning of leaves; Appendix C: Figure C2); thus, we watered all arrays every other day for the duration of the 2016 growing season. After 8

weeks, we removed the arrays from the field, scored them for survival and herbivore damage (described below), and terminated the experiment.

Field measurements

Because mortality in the field is high, we followed most transplants through one summer following the planting of each cohort, during which sample sizes were largest. For all experimental plants (transplants and arrays), we censused each plant in mid to late growing season to score survival (0 or 1) and estimate total percent tissue lost to herbivores as follows:

$$\text{Herbivore damage (\%)} = \frac{\text{damaged leaves}}{\text{total leaves}} \times \text{avg. \% damage per damaged leaf}$$

B. stricta requires cold-cueing (vernalization) in order to flower; as such, we could only measure reproductive output in cohorts of transplants which were planted in the autumn prior to the census year. This included all common garden environments except for ALD-2013 in Idaho (Table 1). We scored all individuals for reproduction (0/1) and counted the number of fruits per plant at the end of the growing season as estimates of reproductive fitness. In one environment where survival and reproduction was high (SCH-2016), we also measured fruit length, and estimated total reproductive output by multiplying fruit number by mean fruit length, since total fruit length is a reliable proxy for total seed number (Wadgymar et al. 2017).

Natural selection for herbivore resistance.

For experimental individuals in environments that allowed reproduction ($N = 3,094$; see above), we tested for natural selection on herbivore defense by regressing reproductive success (0/1) on herbivore damage using a generalized linear mixed effects regression with a binomial distribution and logit link function ('glmer' function in 'lme4' package v. 1.1-21 in R v. 3.6.0). Among individuals that did reproduce, we modeled the number of fruits per individual in response to herbivore damage using a negative binomial GLMM to accommodate overdispersion in fruit number. Finally, among reproductive individuals in the SCH-2016, we modeled log-transformed total fruit length in response to herbivore damage using a linear mixed effects regression. All three models of reproductive fitness components included a random effect of garden block (*i.e.*, complete statistical blocks)

We also tested for the effects of herbivore damage on survival in a subset of transplants in one environment (SCH-2016 in Colorado; $N = 1,219$) which we monitored for multiple years. Specifically, we regressed survival in 2017 upon herbivore damage in 2016 using a generalized linear mixed effects model ('glmer' function in the 'lme4' package v 1.1-21 in R v. 3.6.0) with a binomial distribution, accounting for position within the garden (*i.e.*, complete statistical block) as a random effect.

Variation in herbivore resistance across environments

Spatial and temporal variation in selection can maintain polymorphisms (Turelli & Barton 2004). To assess the role of heterogeneous selection on the evolution of GS diversity in *B. stricta*, we tested for variation in the effects of *BCMA* alleles on herbivore resistance, a component of reproductive fitness (Figure 3A; Prasad et al. 2012).

Specifically, we used restricted maximum likelihood (REML) mixed-effects models in JMP Pro v. 14.3.0 to test for *BCMA* × environment effects on herbivore damage. Because ecological conditions and experimental designs varied between permanent transplants and temporary arrays, we fit separate models for each experiment.

The model for common garden transplants included random effects of statistical block nested within environment and family line nested within *BCMA* genotype.

Because we manipulated starting genotype frequencies in the array experiment, that statistical model included fixed effects of genotype frequency category and the *BCMA* × frequency interaction, in addition to random effects of statistical block nested within environment and family line nested within genotype. Because herbivore damage could only be measured on plants that survived to the census date, our models retained 3,674 and 5,193 observations for garden transplants and temporary arrays, respectively.

We extracted least squares mean and standard error values of herbivore damage for each genotype in each environment as estimated in the REML models after accounting for other model effects, and plotted this variation using ‘ggplot2’ v. 3.1.1 in R

v. 3.6.0. If our statistical models identified a significant *BCMA* × environment interaction, we used pairwise contrasts to compare damage levels received by the two *BCMA* homozygotes within each environment.

Variation in survival across environments

Viability selection can have significant impacts on total fitness (Schluter et al. 1991, Crone 2001, Hadfield 2008, Mojica & Kelly 2010, Wadgymar et al. 2017). Therefore, spatial and temporal variation in survival may also influence the maintenance of polymorphisms. We tested for variation in the effects of *BCMA* on survival across environments using a parallel approach to that described above for variation in herbivore resistance. We fit separate generalized linear mixed effects models for the two experiments ('glmer' function in the 'lme4' package v. 1.1-21 in R v. 3.6.0). For transplants, we modeled survival as a function of *BCMA*, environment, and the *BCMA* × environment interaction, including random effects of garden block and family line. For temporary arrays, we included all of the model effects in the transplant model, plus additional fixed effects of genotype frequency and the *BCMA* × frequency interaction. We tested the significance of model effects and interactions using type III ANOVA ('Anova' function in the 'car' package; Fox and Weisberg 2019).

We extracted least squares mean and standard error values of the probability of survival for each genotype in each environment as estimated in the models described above ('emmeans' function using the package 'emmeans' v. 1.3.4), and plotted this

variation using 'ggplot2' v. 3.1.1 in R v. 3.6.0. If our statistical models identified a significant *BCMA* × environment interaction, we used pairwise contrasts to compare survival of the two *BCMA* homozygotes within each environment.

Frequency-dependent effects

We tested for frequency-dependent effects of *BCMA* in the temporary array experiment, in which we manipulated the starting frequencies of the NIL genotypes. To determine frequency-dependent effects of *BCMA* on herbivore resistance, we assessed the significance of the frequency and *BCMA* × frequency model effects in our array models for herbivore resistance and survival (described above).

Response to selection

In addition to individual-level fitness, we assessed population-level trends in genotype frequency changes as a test for response to drought-mediated selection in our array experiment, in which plants showed signs of drought stress (Appendix C: Figure C2). To do so, we calculated the final frequency of the *MM* genotype within each array as follows:

$$Final\ f(MM) = \frac{final\ MM}{(final\ MM + final\ BB)}$$

and the proportion mortality per array as follows:

$$Mortality = 1 - \frac{(final\ MM + final\ BB)}{(initial\ MM + initial\ BB)}$$

for each of the 60 arrays. We regressed the final frequency of the *MM* genotype upon the population mortality proportion, accounting also for fixed effects of environment and the starting *MM* genotype frequency, using a linear model (`'lm'` function in the package `'stats'` v. 3.6.0 in R v. 3.6.0). We visualized the relationship between the strength of viability selection and final $f(MM)$ using `'ggplot2'` v. 3.1.1.

1.2.5 Greenhouse experiments

We complemented our field experiments with several greenhouse experiments to assess genetic variation in herbivore resistance and drought response under controlled conditions.

Herbivore resistance

We grew juvenile *BCMA* NILs in a randomized complete block design under controlled greenhouse conditions (described in 1.2.2) for 6 weeks before challenging them with a model herbivore to determine insect resistance. In this experiment, five complete blocks each contained 45 individuals spanning five replicates per family (4 *BB* families and 5 *MM* families; Appendix C: Table C3) yielding a total sample size of 225 individuals. We confined trays containing full statistical blocks to plexiglass chambers with mesh panels for ventilation. In these chambers, we applied a single second-instar cabbage looper larva (*Trichoplusia ni*, Lepidoptera: Noctuidae; a generalist caterpillar commonly used in laboratory herbivory experiments with plants in the Brassicaceae [e.g.

Lambrix et al. 2001, Traw & Dawson 2002, Cipollini et al. 2003, Schranz et al. 2009, Manzaneda et al. 2010, Müller et al. 2010]) to each individual plant. We allowed larvae to roam freely within the chambers, letting insects feed on different individual plants within a block according to their preference. After five days of feeding, we ceased the herbivory treatment and scored each plant for insect damage as described in 1.2.4. When phenotyping was complete, we autoclaved the contents of all flats to prevent insect escape.

We used a restricted maximum likelihood (REML) mixed-effects model in JMP Pro v. 14.3.0 to test for the effect of *BCMA* on resistance to *T. ni*, accounting for random effects of position within the greenhouse (complete statistical block), and family line nested within genotype. We extracted least-squared mean and standard error values of herbivore damage on each genotype as estimated in the REML model, and plotted this variation using 'ggplot2' v. 3.1.1 in R.

Drought response

We validated the effects of *BCMA* on drought stress in several controlled experiments, since drought occurred unintentionally, under un-manipulated circumstances in the field.

<i>Controlled dry-down: BCMA NILs</i>

We implemented a progressive dry-down experiment (Mojica et al. 2016) to gradually induce drought stress in *BCMA* NILs. We germinated 1,000 NIL seedlings in petri dishes and transplanted them into 2" × 2" square pots. The 1,000 individuals

included 100 replicate individuals for each of five family lines per genotype, half of which were randomly assigned to a drought treatment. We assigned each pot to a position in one of 32 greenhouse racks using a completely randomized design (Appendix E: Table E1), such that each rack contained replicated individuals of multiple family lines per genotype, some of which were assigned to each experimental treatment.

We transplanted germinants into pots filled with known masses of fritted clay potting medium (Profile® Greens Grade™ Porous Ceramic; mean clay mass = 34.35 ± 0.16 g per pot). Fritted clay absorbs and releases water more evenly than potting soil, and the relationship between volumetric water content (VWC) and water potential (Ψ_w) is well-characterized in this substrate (van Bavel et al. 1978, Mojica et al. 2016). We grew germinants under well-watered conditions for six weeks until juvenile plants were established. Because fritted clay is an inorganic substrate that is chemically inert, we saturated pots with 0.5x strength Hoagland's solution prior to transplanting germinants, and then again twice weekly until beginning the drought treatment. Growth conditions in the Duke University greenhouse included a 16h day with minimum light levels $600 \mu\text{mol}/\text{cm}^2$, and temperatures ranging $18.3\text{-}21.1^\circ \text{C}$ daily / $15.0\text{-}17.7^\circ \text{C}$ nightly.

For individuals assigned to the drought treatment, after the 6-week growth period, we began withholding water to induce drought stress. Specifically, we weighed each pot in the dry treatment daily and divided the current weight by the saturated

weight (measured for each pot prior to transplanting) to calculate the current percent saturation for each individual i as follows:

$$\% \text{ sat}_i = \frac{\text{current mass}_i}{\text{saturated mass}_i}$$

After weighing all pots in the drought treatment, we identified the individual in the experiment with the highest percent saturation ($\max(\% \text{ sat})$), and watered every other pot i with the volume of water required to achieve an equal saturation to the wettest pot, as follows:

$$\text{volume to add}_i = (\max(\% \text{ sat}) * \text{sat mass}_i) - (\% \text{ sat}_i * \text{sat mass}_i)$$

In this way, we gradually reduced the substrate VWC (and thus water potential) over the span of several days, drying down the plants gradually and allowing them to respond morphologically and physiologically to drought conditions. Throughout the span of the drought treatment, plants in the wet treatment were watered to saturation daily. We ceased the dry-down after 12 days, when the plants in the dry treatment had begun to wilt, and measured a variety of morphological phenotypes on all individuals. In addition, we estimated several physiological traits, including leaf water content (LWC):

$$\text{LWC} = \frac{\text{rosette fresh weight} - \text{rosette dry weight}}{\text{rosette fresh weight}}$$

leaf mass per area (LMA):

$$\text{LMA} = \frac{\text{longest leaf dry mass}}{\text{longest leaf length} * \text{longest leaf width}}$$

and daily water use for each day:

$$water\ use_t = final\ mass_{t-1} - initial\ mass_t$$

where the final mass is the mass following watering, and the initial mass is the mass prior to watering. For the latter, we then calculated the least squares mean values of daily water use per individual by regressing the mass of water used per day onto day number, including a random effect of individual plant ID to account for repeated measures on the same individuals over time. For tissue measurements, we obtained fresh weights immediately following the completion of the experiment, and dry weights from samples dried at room temperature for a minimum of 6 months.

For plants in the drought treatment, we analyzed variation in water use with linear mixed effects models ('lmer' function in the 'lme4' package v. 1.1-21) to fit daily water use in response to the fixed effect of a single morphological variable, *BCMA*, and the *BCMA* × morphology interaction, as well as random effects of greenhouse position (block) and family line nested within genotype. For morphological variables which were significant in these individual models, we fit a multivariate model assessing variation in water use in response to *BCMA* and all morphological predictors, as well as random effects of block and family line.

We used data from all plants (wet and dry treatments) to test for genetic variation in drought response. Specifically, we used linear mixed-effects models ('lmer' function in the 'lme4' package) to fit each morphological variable measured in response

to *BCMA*, drought treatment, and the *BCMA* × drought interaction, as well as starting height as a fixed covariate and random effects of block and family line nested within genotype. We extracted least square mean and standard error values for each genotype in each treatment as estimated in these models and plotted variation in response to drought using ‘ggplot2’ v. 3.1.1. If our models detected a significant genotype × treatment effect, we assessed pairwise differences across all categories using a Tukey HSD post-hoc test.

<i>Effects of BCMA on drought response in a genetically diverse sample</i>
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To test for pleiotropic effects of *BCMA* on drought response in variable genetic backgrounds, we performed an additional controlled greenhouse dry-down experiment as described above for the *BCMA* NIL genotypes. We grew 6 biological replicates of each of 350 genotypes collected from across the species range in a randomized complete block design in the greenhouse. These accessions largely overlap with the panel of genotypes used to determine range-wide variation in glucosinolate profile (see section 1.2.2) and its underlying genetic variation (see section 1.2.3). We used the same methods described above except that we grew plants for 8 weeks (vs. 6 weeks for NILs) prior to initiating the drought treatment.

Following the dry-down, 1,317 individuals representing 325 genotypes survived. We harvested a leaf from each plant to measure its fresh weight (immediately) and dry weight. We used these measurements to calculate leaf water content (LWC) per individual plant as described above for *BCMA* NILs. We used a generalized linear

mixed model fitted using restricted maximum likelihood estimates (REML) to calculate the least square mean LWC per genotype, treating genotype, block, and the genotype $\times$ drought treatment interaction as random effects, and the main effect of treatment as a fixed effect. We calculated genotypic LS means in JMP 12 (SAS Institute, Cary, NC, USA).

We assigned 225 of the 325 accessions into genotype categories near *BCMA3* locus using SNP data from whole genomic sequencing (Wang et al. 2019). Specifically, we grouped accessions into categories based on genotype at the SNP position with the strongest statistical association with BC-ratio in the BCMA region, as determined in a GWA study on glucosinolate profile (Mojica et al., *unpub.*). The *B. stricta* LTM reference genome, which was constructed from the LTM genotype from Idaho (Figure 1), produces branched-chain GS and is homozygous for CC at this locus (SNP position 10,419,878 on chromosome 7). The majority of sampled accessions (203) were also homozygous CC. The remainder were homozygous for the alternative allele, GG.

We analyzed genetic mean LWC under drought across this panel of accessions, in two ways. First, we regressed LWC under drought upon the genetic mean BC-ratio for each accession in a linear model also accounting for a fixed effect of genetic group (Wang et al. 2019). We used a second linear model to we test whether LWC under drought was significantly predicted by SNP genotype in *BCMA3*. We tested the significance of main effects in these models using type III ANOVA as described above.

For the second model, we calculated least squares and standard error values using means for each *BCMA3* SNP genotype using the ‘emmeans’ package (Lenth 2019), and plotted variation across genotypes using ‘ggplot2’ (Wickham 2016).

1.2.6 Molecular signatures of selection

Nucleotide diversity

Balancing selection leaves detectable genomic signatures, including levels of nucleotide diversity in target genes that exceed genome-wide averages (Mitchell-Olds et al. 2007). To determine whether *BCMA3*, the *BCMA* copy that confers functional variation in glucosinolate phenotype (Prasad et al. 2012; sections 1.2.3 and 1.3.1), showed molecular signatures of balancing selection, we compared the observed level of nucleotide diversity (π) in *BCMA3* to the distribution of π in a subset of comparable genes in the *B. stricta* genome. Beginning with Sanger sequence data on the *BCMA3* locus in 91 genotypes across the species range (described in 1.2.3), we focused on a subset of 55 genotypes belonging to the COL and UTA genetic groups (Wang et al. 2019). We excluded sequence data for genotypes from other genetic groups because of residual population structure within WES and low variation in BC-ratio within NOR.

From this subset of genotypes, we calculated the observed π in *BCMA3* as described in Wang et al. (2019). We also calculated the number of silent sites in this gene, which was 364. We then calculated π in other comparable genes in the same 55

genotypes. Beginning with all genes in the genome, we filtered based on two criteria: (A) $\pi > 0$, and (B) number of silent sites > 360 . We implemented criterion A to exclude genes for which sequence data was missing or of low quality, resulting in no estimation of π . We implemented criterion B to account for the effect of gene length on π (Appendix D).

Our filtering retained 1,775 genes. We compared the observed value of π_{BCMA} to the genome-wide distribution of π for those loci among our 55 focal genotypes. We determined whether π_{BCMA} was a statistical outlier by comparing its value to the cutoff of the 5% upper tail of π values in the subset of comparable genes.

Phenotype-environment correlations

Natural selection by abiotic factors may result in phenotype-environment correlations, in which the trait responding to selection is correlated with the selective driver across the landscape (Lee & Mitchell-Olds 2011; The 1,001 Genomes Consortium 2016; Anderson & Wadgymar 2020). To test for past selection on *BCMA* in *B. stricta*, we assessed phenotype-environment correlations between BC-ratio and climatic variables from the locations of origins in a panel of 267 genotypes across the species range. This subset retained genotypes for which we had estimated genetic mean BC-ratio and which were assigned to one of four primary genetic groups, excluding admixed genotypes (Wang et al. 2019). First, we used the latitude and longitude of origin for each genotype to extract 19 bioclimatic variables from the WorldClim database (<http://www.worldclim.org/bioclim>). Because many climate variables are correlated, we

reduced axes of climate variation by conducting a principal components analysis in JMP Pro v. 14.3.0 and extracting multivariate climatic coordinates for each genotype in PCA space. We obtained PCA coordinate values up to the 5th PCA axis, at which point >90% of climate variation was explained (Appendix H).

To test for climatic predictors of BC-ratio, we used a standard least squares multiple regression in JMP Pro v. 14.3.0 to fit BC-ratio in response to genetic group, latitude, longitude, and elevation of origin, and the first 5 principal components axes of climate variation (all fixed effects). We identified significant climatic predictors of BC-ratio, and then used axis loading (Appendix H) to determine which raw climate variables contributed to the most significant axes. We then fit a suite of reduced univariate models assessing variation in BC-ratio in response to each of these raw climate variables.

Disentangling pleiotropy and linkage

Using NIL genotypes derived from crosses of natural accessions confers several advantages over transgenics, particularly regarding legal and permitting issues for field experiments. However, NIL crosses are limited in the level of precision with which phenotypic effects can be causally attributed to specific genes, as the variable region of the genome may contain other tightly linked loci in addition to the gene of interest. In this case, the target region is small, but other loci within the *BCMA* haplotype might contribute to some of the phenotypic effects we observe in this study. To clarify this, we

used genomic sequencing to characterize other genetic variation between *BCMA* NIL genotypes.

<i>Assembly of B. stricta version_2 reference genome.</i>

Initial characterization of possible linked loci in the *BCMA* region using the version_1 *B. stricta* reference genome (Lee et al. 2017) was unsatisfactory. The version_1 reference genome was constructed from the LTM genotype, which contains two previously known copies of *BCMA*, as well as other repetitive elements (see 1.2.1); alignment of new reads to this region yielded poor consensus sequences. To resolve ambiguity about the identity of nearby loci in the *BCMA* region, we assembled a version_2 *B. stricta* reference genome using long-read sequencing technology using the Colorado (SAD12) genotype. Full details explaining the genome assembly, resolution of repetitive elements, and characterization of the *BCMA* haplotype is described in Appendix F.

<i>Genotyping-by-sequencing</i>

With a new high-quality genome to use as a reference, we characterized genetic variation within the *BCMA* region using genotyping-by-sequencing. Specifically, we grew 216 *BCMA* NILs (112 BB homozygotes and 104 SS homozygotes spanning 23 independent family lines) in potting soil under standard greenhouse conditions (see 1.2.2) for approximately 2 months, until rosettes were well established. We harvested ~100 mg leaf tissue from a subset of 67 individuals (34 BB homozygotes and 33 MM homozygotes; Appendix A) after keeping plants in the dark for 2 days to promote

carbohydrate breakdown, and stored tissue at -80° C until processing. We also harvested additional leaf and root material from all 216 plants into 70% MeOH for validation of GLS phenotypes using HPLC (see section 1.2.2 and Appendix G for additional detail). We ground frozen leaf tissue at high speed using a Geno/Grinder® automated tissue homogenizer and cell lyser (Spex® Sample Prep, Metuchen, NJ, USA) and extracted genomic DNA using a DNeasy® Plant Mini Kit (Qiagen® N.V., Hilden, Germany) according to the manufacturer's instructions. We used extracted DNA to prepare genomic libraries for sequencing according to Andolfatto et al. (2011), with some modifications (Perera 2019), as follows.

We digested 10µL of 2ng/µL DNA with 0.6 µL of MSE1 restriction enzyme (stock concentration; New England Biolabs) in 2 µL of NEB Buffer 4 (New England Biolabs) with 0.2 µL of bovine serum albumen (stock concentration; New England Biolabs) and 7.2 µL ddH₂O, totaling 10 µL per reaction per sample. We incubated the digestion for 3h at 37° C and then 20 min at 65° C. We added unique adaptor sequences (Appendix A) to each sample by adding 10µL of digested genomic DNA to 8µL of 5µM adapter solution along with 0.2µL T4 DNA Ligase (400U), 5µL T4 DNA Ligase buffer, and 22.8µL ddH₂O, totaling 46µL per sample. We incubated these mixtures at 16° C for 3h to ligate adapters to the sheared genomic fragments. We pooled uniquely identified samples, concentrated, cleaned, and resuspended DNA according to Andolfatto et al. 2011. We size-selected genomic fragments ranging from ~250-300 bp in length using a 2% agarose

gel with 1x TAE buffer and a GeneRuler 50bp DNA ladder (ThermoFisher Scientific).

We purified excised agarose bands using a QiaQuick Gel Extraction Kit (Qiagen® N.V., Hilden, Germany) according to the manufacturer's instructions.

Finally, we added GBS indexed primers and amplified genomic fragments using PCR. Specifically, we mixed 2µL of purified, size-selected library DNA with 12.5µL Phusion® High Fidelity PCR Mastermix (New England Biolabs) 1.25µL 10µM indexed FC1 primer, 1.25µL 10µM indexed FC2 primer, and 8µL of ddH₂O (see Appendix A for index sequences). We used the following PCR protocol: hot start for 30s at 98° C; 15 cycles of 10s at 98° C, 15s at 60° C, and 15s at 72° C; and final extension for 7 min at 72° C. We purified the PCR products using Ampure XP PCR purification beads (Beckman Coulter Life Sciences) according to the manufacturer's instructions, and in the final step eluted DNA into 30µL of TE buffer. We measured the final concentration of each library using a Qubit™ dsDNA HS Assay Kit according to the manufacturer's instructions, and adjusted the concentrations of the libraries to 10 nM as necessary using ddH₂O.

We sequenced the GBS libraries using the Illumina HiSeq 4000 platform (150 bp paired-end reads) at the Duke University Sequencing and Genomic Technologies Shared Resource center. We demultiplexed sequence reads using fastq-multx with default settings (<https://github.com/brwnj/fastq-multx>) and aligned them to the *Boechera stricta* version_2 reference genome (Appendix F) using the Burrows-Wheeler Aligner (BWA; Li 2013). We called SNPs using GATK as described in Wang et al. (2019), assigning

genotype values of -1, 0, 1, and 2 for missing, homozygous for the reference allele, heterozygous, and homozygous for the alternative allele, respectively. The GBS sequence reads were aligned to the version_2 reference, which was constructed from the SAD12 (CO) genotype, so reference alleles reflect those derived from the SAD (Met-GS) NIL parent and alternate alleles reflect those derived from the LTM (BC-GS) NIL parent (see 1.2.1 and Appendix A for additional information).

We filtered aligned SNP data to focus on chromosome 7, where the *BCMA* polymorphism is located. After filtering to remove indels, sites with mean read depth >20 (to exclude repetitive regions), sites with missing data at a frequency > 0.9, and sites with >2 alleles, we retained 3,646 SNPs among the 65 sampled NIL genotypes. To determine the physical size of the variable interval in the NILs, we calculated F_{ST} according to Wang et al. (2019) between the sequenced *MM* and *BB* homozygotes in 20 kb non-overlapping windows along chromosome 7.

We used BLAST to locate the position of the *BCMA3* gene within this region. Using gene annotations from the *B. stricta* version_1 LTM reference genome, we identified nine other genes within the variable region in the NILs and identified their putative functions from Arabidopsis orthologs, when available (The Arabidopsis Information Resource [TAIR] 2020). We used this list to determine whether or not genes other than *BCMA3* are likely to contribute to any phenotypes characterized in this study.

1.3 Results

1.3.1 Widespread variation in BC-ratio and *BCMA3*

Multiple genetic polymorphisms contribute to variation in BC-ratio

Our phenotypic survey of 337 genotypes revealed high levels of glucosinolate polymorphism across the species range of *B. stricta* (Figure 1), including at small spatial scales within our focal regions. While a genetic basis of variation in BC-ratio was previously elucidated (Prasad et al. 2012), new sequence data in a subset of our phenotyped accessions also revealed further, previously unknown genetic variation associated with BC-ratio (Figure 2).

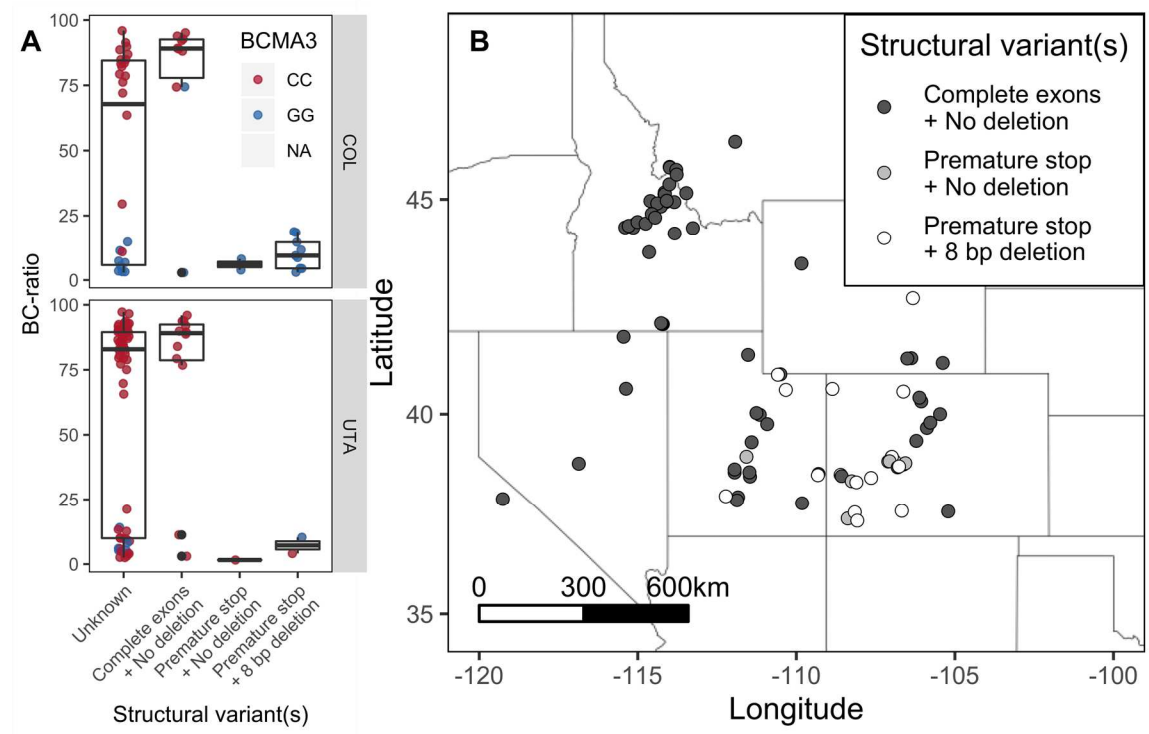


Figure 2. Several old, widely distributed alleles are correlated with BC-ratio. A: At the *BCMA* locus, at least two sequence variants predict the BC-ratio phenotype: SNP variation (colored data points) and a premature stop codon (boxplots). Both of these genetic variants are geographically widespread (B).

One allelic variant, a nonsense mutation causing a premature stop codon in a *BCMA* exon, disrupts function of the BC-AA active *BCMA* allele, yielding low BC-ratio phenotypes carrying loss-of-function alleles (Figure 2A). Another allelic variant, an 8 bp deletion causing a frameshift in the *BCMA* gene, is found within a subset of the premature-stop alleles (Appendix B: Table B1). This deletion mutation, which appears to have arisen after the nonsense mutation, is also correlated with BC-ratio (Figure 2A), but does not explain additional phenotypic variation beyond the effect of the nonsense mutation with which it co-occurs (Table 2). The alleles conferring variation in BC-ratio are geographically widespread (Figure 2B); of the accessions we assayed for allelic variation, we found complete exon variants, stop codon only variants, and stop codon + deletion variants spanning 1,217 km, 438 km, and 729 km of the species range, respectively. These widespread variants in largely undisturbed habitats suggest that the functional genetic bases of variation in chemical phenotypes are not recent in origin.

Table 2. Effects of *BCMA3* structural variation and SNP variation on BC-ratio.

Model effect	Sum sq.	df	F	P	
Genetic group	1195.1	1	2.2769	0.141444	
BC-ratio SNP	2410.1	1	4.5919	0.040084	*
Premature stop	6831.0	1	13.0146	0.001073	**
8 bp deletion	253.4	1	0.4828	0.492348	

Copy number variation in the *BCMA* locus

We used long-read sequence data to assemble a new reference genome for *B. stricta* ("version_2"), reducing the amount of missing data and the number of scaffolds substantially from the previous version (Appendix F: Table F1). New long-read assemblies of both NIL parents, LTM (MT) and SAD12 (CO), revealed substantial variation in the number of *BCMA* copies present in the NIL haplotype (Figure 3A). While the CO parent has a single copy of *BCMA* (*BCMA3*) on chromosome 7, the *BCMA* region in the MT parent is rich in transposons and tandem repeats, containing two functional copies of *BCMA* (*BCMA1* and *BCMA3*) as well as ten non-functional duplicated pseudogenes.

Using phylogenetic reconstruction, we estimated the evolutionary history of these gene copy events (Figure 3B). We found that the first duplication event of *BCMA2* on chromosome 2 (orthologous to *CYP79F1/2* in *Arabidopsis*) producing *BCMA1/3* on chromosome 7 happened following the divergence of the genera *Boechera* and *Arabidopsis*. Further, a sister species within the genus, *B. retrofracta*, has only a single copy of *BCMA1/3*, and is a sequence outgroup to all *B. stricta* paralogs on chromosome 7. This suggests that the duplication of *BCMA1/3* occurred within *B. stricta*, following divergence from *B. retrofracta*. Finally, the reconstruction demonstrates that duplication of the *BCMA1* and *BCMA3* functionally divergent copies (Prasad et al. 2012) occurred prior to differentiation between the two *B. stricta* subspecies, represented by the MT and

CO parents, respectively (Lee et al. 2017, Wang et al. 2019), indicating that there was a secondary loss of *BCMA1* from the CO parental genotype. A graphical model of this evolutionary history is provided in Appendix F (Figure F2).

To characterize the extent of *BCMA* copy variation in *B. stricta*, we estimated read coverage depth using previously published sequence data (Wang et al. 2019) in a panel of 484 genotypes. We found that most genotypes in the COL, UTA, and NOR groups have 2 or 3 *BCMA* copies, consistent with a single copy (*BCMA2*) on chromosome 2 and 1-2 copies on chromosome 7. Genotypes in the WES group, however, have elevated copy number and increased variation in copy number, with some genotypes possessing 10 or more *BCMA* copies (Figure 3C). Copy number is correlated with the BC-ratio, but this is unlikely to be causal. Rather, high *BCMA* copy number is found in genotypes which are homozygous for the SNP variant near *BCMA3* that is correlated with BC-ratio (Figure F3).

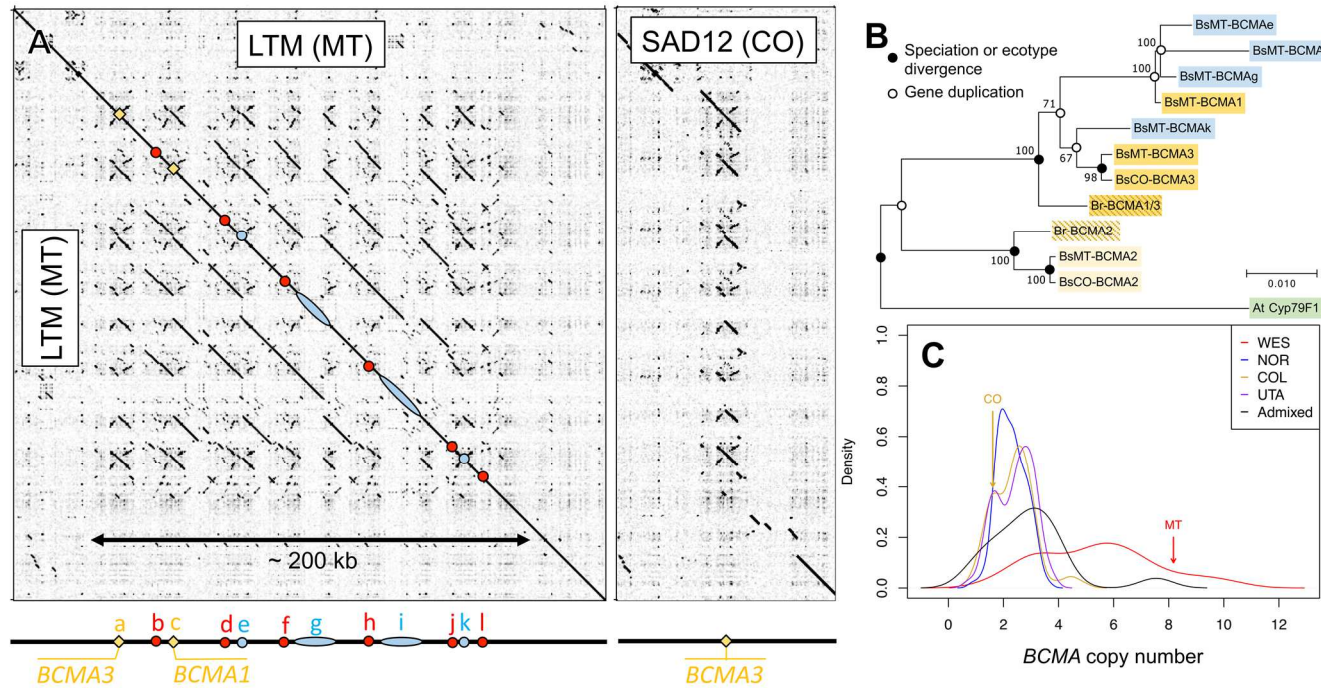


Figure 3. *BCMA* copy number variation across *B. stricta* genotypes. A: Long-read assemblies of the LTM and SAD12 chromosome 7 NIL genotypes reveal substantial variation in tandem repeats and *BCMA* copy number in a 200 kb region on chromosome 7. Functional *BCMA* gene copies are indicated in yellow; red circles show severely truncated, non-functional *BCMA* copies; blue ellipses indicate close-to-full-length copies of *BCMA* containing deletions or transposon insertions. B: Phylogenetic reconstruction of *BCMA* copy sequences (excluding severely truncated copies) elucidates the evolutionary history of *BCMA* duplications in *Boechera*. Colored boxes behind (pseudo)gene names match features in part A; blue boxes contain nonfunctional *BCMA* pseudogenes on chromosome 7, light yellow boxes contain functional copies of *BCMA2* on chromosome 2, and dark yellow boxes contain functional copies of *BCMA3* on chromosome 7. Shaded boxes indicate paralogs in *B. retrofracta*, and green box indicates the *A. thaliana* ortholog (*CYP79F1*). Scale bar shows genetic distance in nucleotide differences per base pair. C: Gene duplication and loss events yield variable *BCMA* copy number across genotypes. Colors indicate genetic groups as characterized in Wang et al. (2019).

1.3.2 Herbivory influences fitness in nature

To determine whether herbivore-mediated selection may contribute to balancing selection on glucosinolate profile, we tested the fitness consequences of herbivore resistance, a complex trait that is influenced in part by BC-ratio in *B. stricta* (Schrantz et al. 2009, Manzaneda et al. 2010, Prasad et al. 2012). We found that herbivore damage reduces fitness, primarily by decreasing reproductive output (Figure 4).

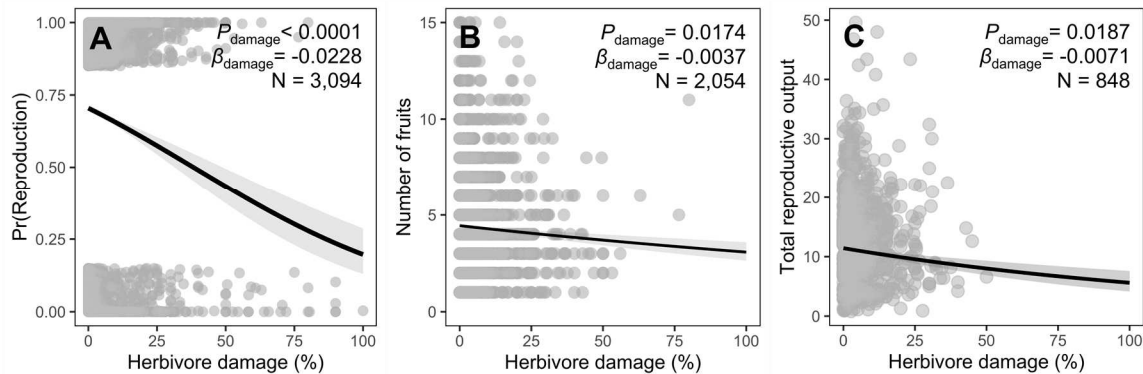


Figure 4. Herbivore damage decreases fitness. A: Herbivore damage greatly reduced the probability of reproducing. Among individuals that reproduced, herbivore damage also decreased total reproductive output, causing slight reductions in the number of fruits produced (B) and the total size of all fruits (C). In A and B, fitted line and statistics show the main effect of damage across 8 environments. In C, fitted line and statistics shows the main effect of damage in a single environment (SCH-2016). Black line indicates model prediction and shaded ribbon indicates ± 1 SE of that prediction.

Specifically, herbivore damage significantly decreased the probability of reproduction ($N = 3,094$, $\chi^2 = 21.11$, $P = 4.33 \times 10^{-6}$; Figure 3A). Among individuals that did reproduce, herbivore damage also had significant but less pronounced negative effects on the number of fruits produced ($N = 2,054$, $\chi^2 = 5.66$, $P = 0.0174$; Figure 3B) and

the total length of all fruits ($N = 848$, $\chi^2 = 5.53$, $df = 1$, $P = 0.0187$; Figure 3C). Total fruit size is a reliable proxy for seed number in this species (Wadgymar et al. 2017).

In one environment in which we monitored transplants over multiple years (SCH-2016 in Colorado), we also tested whether herbivory in year t influences survival to year $t+1$. The overall effect of damage on over-winter survival was not significant in this experiment ($N = 1,219$, $\chi^2 = 1.30$, $df = 1$, $P = 0.2547$). Together, these data show that herbivory generally reduces fitness, operating primarily via fecundity selection.

1.3.3 *BCMA* causes fitness tradeoffs

We used field and greenhouse data to test whether *BCMA* experiences balancing selection attributable to frequency-dependence, spatial and temporal variation in selection, and/or pleiotropic effects on different functional traits and fitness components.

No evidence for frequency-dependent effects of *BCMA*

Using temporary arrays in which we manipulated *BCMA* allele frequencies, we tested whether the effects of *BCMA* on fitness are frequency-dependent. For example, if rare phenotypes confer greater defense, then natural selection can maintain multiple defense phenotypes within and among populations (Bergelson et al. 2001, Hodgins & Barrett 2006, Trotter & Spencer 2009). We found no effect of genotype frequency (Table 3) on either herbivore defense (Figure 5A) or survival (Figure 5B), two important

components of fitness. These results provide no support for negative frequency-dependent selection on *BCMA*.

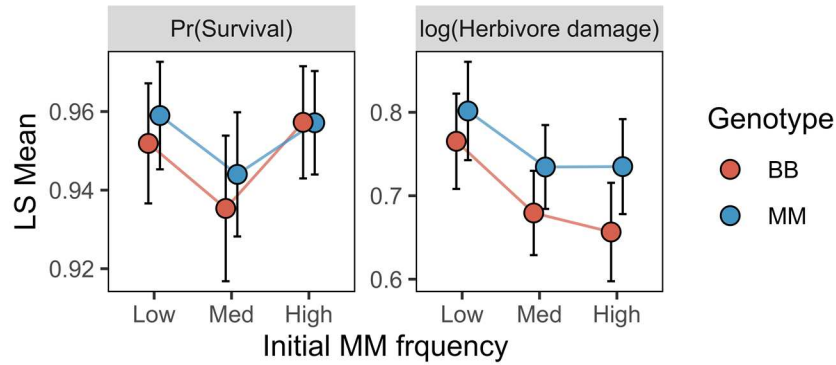


Figure 5. Genotype frequency does not alter the effect of *BCMA* on herbivore resistance or survival. In experimental arrays in which we manipulated the starting genotype frequency of the *BCMA* NIL homozygotes, there was no effect of genotype frequency on herbivore damage or survival.

Table 3. Effects of *BCMA* genotype on herbivore defense and survival in manipulative field arrays.

A) Herbivore defense (N = 5,193)						
Model term	Nparm	NumDF	DenDF	F	Pr(>F)	
<i>BCMA</i>	1	1	9.201	8.3990	0.0173	*
Environment	5	5	48.74	2.2721	0.0618	.
<i>BCMA</i> *Env	5	5	5149	2.3623	0.0376	*
Frequency [†]	2	2	49.07	0.7569	0.4745	
<i>BCMA</i> *Frequency [†]	2	2	5119	0.6312	0.5320	
B) Survival (N = 5,880)						
Model term	DF		χ^2		Pr(>ChiSq)	
<i>BCMA</i>	1		5.2690		0.02171	*
Environment	5		89.2772		$<2.2 \times 10^{-16}$	***
<i>BCMA</i> *Env	5		26.6144		6.8×10^{-5}	***
Frequency [†]	2		1.7201		0.4231	
<i>BCMA</i> *Frequency [†]	2		0.5318		0.7665	

[†]Refers to initial allele frequency category:

Low = 34% MM, Med = 50% MM, High = 66% MM.

Variation in herbivore defense across environments

Combining data from permanent field transplants, temporary manipulative field arrays, and a controlled laboratory environment, we tested for spatial and temporal variation in the effects of *BCMA* on insect resistance, a component of fitness. Together, these studies yielded data on >9,000 *BCMA* NIL replicates across 16 environments, and included both juvenile and mature adult plants. We found significant variation across environments in the effect of *BCMA* on insect resistance (Figure 6; Table 3A, Table 4A).

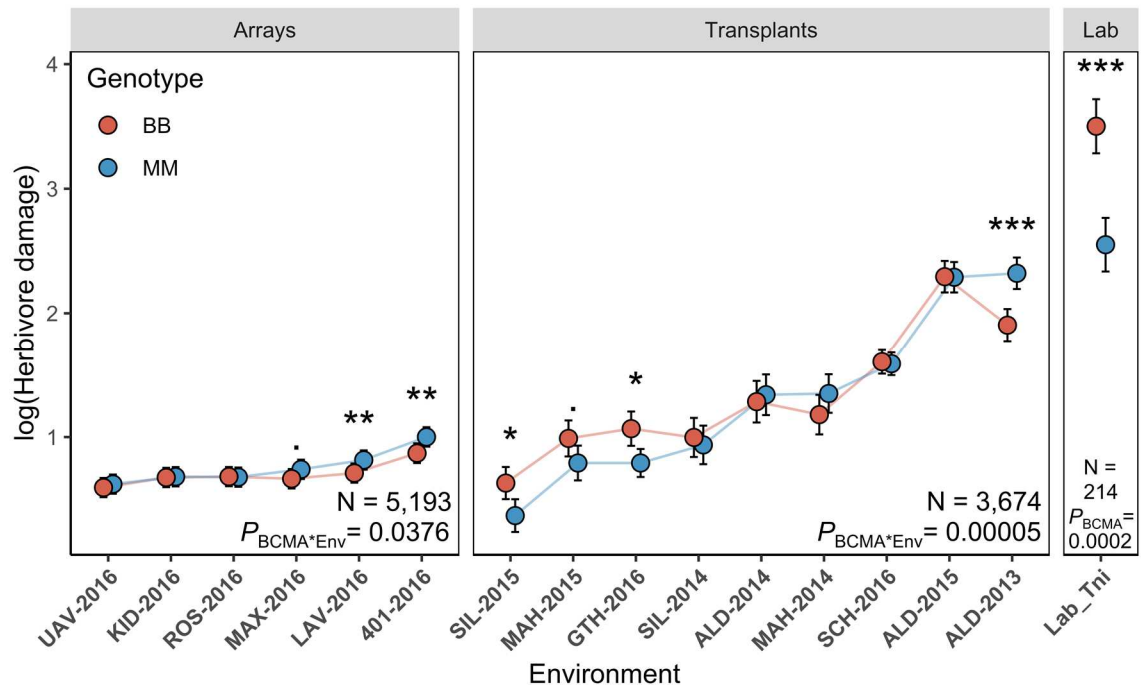


Figure 6. *BCMA* shows tradeoffs for defense across environments. In some environments, the branched-chain phenotype (*BB* NIL genotype) confers higher resistance, in others the methionine phenotype (*MM* NIL genotype) confers higher resistance, and in still others the strategies are functionally equivalent. Within each environment, symbols denote pairwise significant differences as follows: $\cdot = P < 0.10$; $* = P < 0.05$; $ = P < 0.01$; $*** = P < 0.0001$.**

Here, we see clear evidence of change in rank resistance (a component of fitness; see 1.3.2) of alleles across environments, the hallmark of antagonistic pleiotropy. Variable effects of *BCMA* alleles on resistance to natural herbivores in the field were also recapitulated in a controlled lab setting using a model insect herbivore, *Trichoplusia ni* (Table 4B).

Table 4. Effects of *BCMA* on herbivore defense across environments. A: Variation in damage across permanent field transplants. B: Variation in damage across genotypes within one lab environment.

A) Permanent transplants (N = 3,674)						
Model term	Nparm	NumDF	DenDF	F	Pr(>F)	
<i>BCMA</i>	1	1	15.77	0.0934	0.7639	
Environment	8	8	140.7	28.0465	<.0001	***
<i>BCMA</i> *Env	8	8	2835	4.1880	<.0001	***
B) Laboratory environment (N = 214)						
Model term	Nparm	NumDf	DenDF	F	Pr(>F)	
<i>BCMA</i>	1	1	6.195	57.6652	0.0002	**

Variation in survival across environments

While herbivory does influence fitness in this species by reducing reproductive output, herbivory alone appears to have modest effects on survival (see 1.3.2). However, we also used field data to test whether *BCMA* directly influences survival over the course of a growing season, regardless of how herbivore damage mediates that relationship. We found tradeoffs across environments in how *BCMA* influenced survival (Figure 7); the *BB* genotype had higher survival in one Idaho environment (SIL-2015)

and one Colorado environment (ROS-2016), while the *MM* genotype had higher survival in four Colorado environments (401-2016, KID-2016, GTH-2016, and SCH-2016).

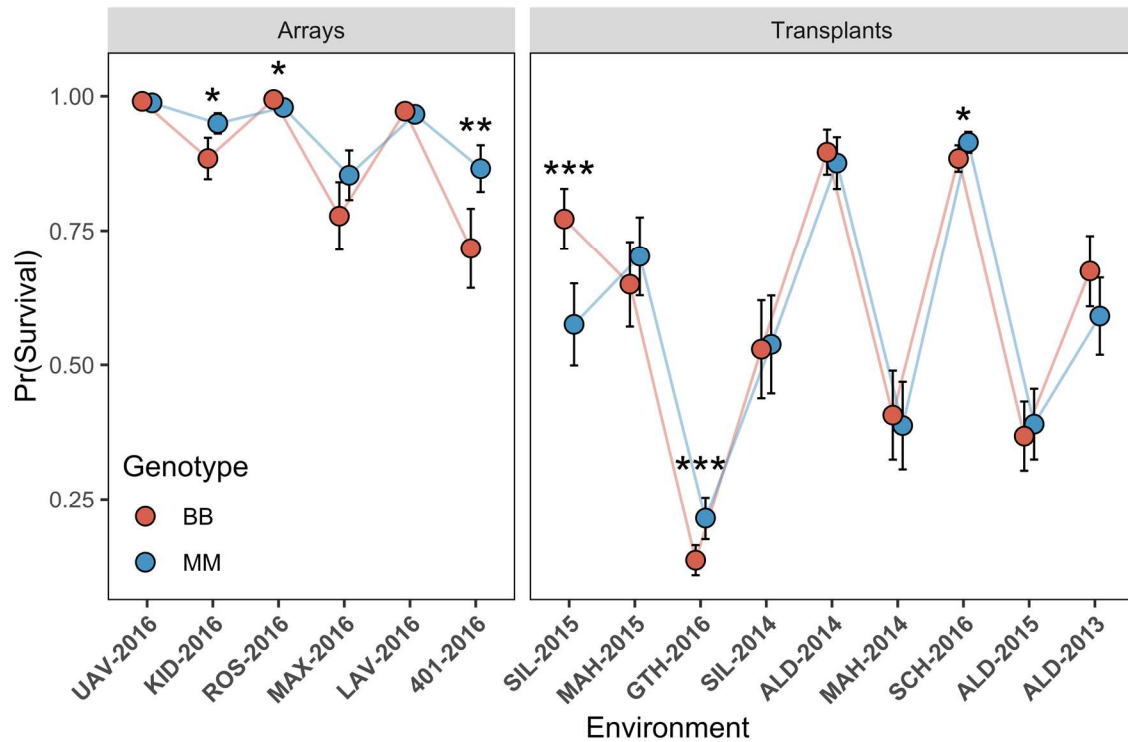


Figure 7. Genetic variation in survival in field environments. The effects of *BCMA* on survival varied depending on environment in temporary field arrays (left) and permanent transplants (right). Within each environment, symbols denote pairwise significant differences as follows: * = $P < 0.05$; ** = $P < 0.01$; * = $P < 0.0001$.**

These significant genotype $\times$ environment interactions (Table 3B, Table 5) and significant changes in rank survival provide additional evidence that *BCMA* confers genetic tradeoffs for fitness across environments.

Table 5. Effects of *BCMA* on survival in permanent field transplants.

Model term	DF	χ^2	Pr(>ChiSq)	
<i>BCMA</i>	1	3.1973	0.0738	
Environment	8	198.3991	<2.2×10 ⁻¹⁶	***
<i>BCMA</i> ×Env	8	49.9425	4.19×10 ⁻⁸	***

In our field experiments, the *MM* genotype had higher survival in environments in which overall survival was low (Figure 6), *i.e.*, in which viability selection was stronger. Thus, we also tested for genetic response to selection by regressing the final *MM* genotype frequency upon the population proportion mortality for the temporary array experiment, where we had employed replicated artificial populations. Consistent with models of survival probability at the individual level, we found a positive relationship between final *MM* genotype frequency and the strength of viability selection (Figure 8, Table 6). A significant mortality × frequency interaction (Table 6) revealed that response to selection was stronger in treatments where the *MM* genotype was initially rare than where it was common, and strongest in the mid-frequency treatment, which contained the most genetic variation.

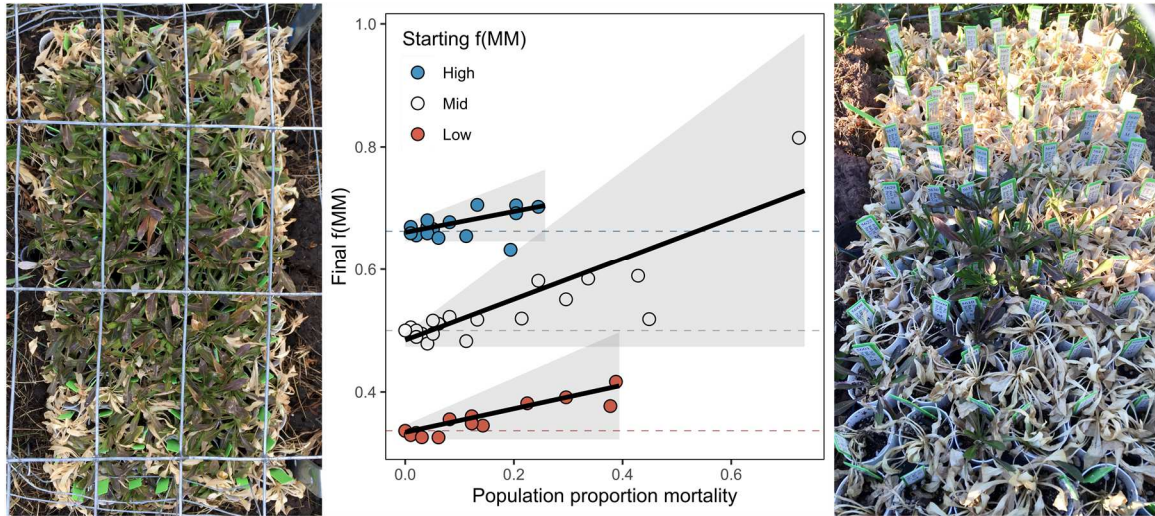


Figure 8. Drought response in field arrays. The *MM* genotype attained higher frequency in artificial populations that experienced greater mortality, showing a response to viability selection following a natural episode of drought. Dashed horizontal lines represent the starting $f(MM)$ in each treatment category. Solid black lines represent fitted predictions from a statistical model including a frequency $\times$ mortality interaction (Table 6), and shaded ribbons indicate ± 1 SE of the prediction. We observed a range of mortality levels across different arrays, with examples of low and high mortality shown to the left and right of the figure, respectively.

Table 6. Effects of viability selection on final *BCMA* genotype frequencies in temporary arrays.

Model term	DF	Sum Sq	<i>F</i>	Pr(> <i>F</i>)	
Proportion mortality	1	0.00230	3.6706	0.06122	.
Environment	5	0.00414	1.3221	0.27038	
Frequency [†]	2	0.48070	373.8690	$< 2.2 \times 10^{-16}$	***
Frequency [†] $\times$ prop. mortality	2	0.00529	4.2228	0.02032	*

[†]Refers to initial allele frequency category;

Low = 34% *MM*, Med = 50% *MM*, High = 66% *MM*.

Effects of *BCMA* on drought response

While we did not monitor or deliberately manipulate abiotic environmental conditions in our field experiments, we observed that high levels of mortality followed episodes of drought. This was particularly evident in the temporary arrays, where potted plants were confined to a smaller volume of soil moisture in each cell (Figure 7).

Table 7. Effects of genotype and morphology on water use under drought.

Model (N)	Model effect	DF	χ^2	P	
A (486)	<i>BCMA</i>	1	0.0943	0.7588	
	Initial height	1	52.9137	3.49×10^{-13}	***
	Final height	1	12.8290	0.0003	***
	<i>BCMA</i> × final height	1	0.5051	0.4772	
B (487)	<i>BCMA</i>	1	0.4522	0.5013	
	Total leaves	1	44.0913	3.13×10^{-11}	***
	<i>BCMA</i> × Total leaves	1	0.0190	0.8902	
C (477)	<i>BCMA</i>	1	0.0794	0.07781	
	Estimated LMA	1	22.4542	2.15×10^{-6}	***
	<i>BCMA</i> × LMA	1	1.4383	0.2304	
D (478)	<i>BCMA</i>	1	2.8861	0.08935	
	Starting height	1	25.9175	3.56×10^{-7}	***
	Final height	1	19.1444	1.21×10^{-5}	***
	Total leaves	1	1.0186	0.3128	
	Estimated LMA	1	3.8965	0.0484	*

We performed follow-up experiments in the greenhouse to explicitly test whether *BCMA* influences performance under drought, which may underlie observed effects on survival. We used a suite of models testing for the *BCMA* × trait effects on

water use under drought, and found that three traits (plant size, leaf number, and estimated leaf mass per area (LMA)) predicted water use, but were not genotype-dependent (Table 7A-C). We also used a single multivariate model to simultaneously test for the effects of all of these traits on water use under drought; here, we found that size and LMA still predicted water use under drought, but that total leaf number did

Table 8. Effects of *BCMA* on morphological responses to drought. Bold values denote $P < 0.05$.

Response variable (N)	Model effect (df)							
	Initial height (1)		<i>BCMA</i> (1)		Drought (1)		<i>BCMA</i> *Drought (1)	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Final height (954)	282.03	2.2×10⁻¹⁶	9.67	0.0019	9.13	0.0025	27.32	1.73×10⁻⁷
Total leaves (958)	413.63	2.2×10⁻¹⁶	7.10	0.0077	4.02	0.0451	8.52	0.0035
Estimated LMA (568)	7.38	0.0066	0.92	0.3363	3.01	0.0825	1.51	0.2197
Leaf width (951)	165.25	2.2×10⁻¹⁶	7.40	0.0065	6.13	0.0133	25.44	4.55×10⁻⁷
Growth rate (954)	215.28	2.2×10⁻¹⁶	9.67	0.0019	9.13	0.0025	27.32	1.73×10⁻⁷
Green leaves (959)	50.99	9.3×10⁻¹³	4.10	0.0428	4.74	0.0294	22.79	1.81×10⁻⁶
Leaf water content (569)	114.79	2.0×10⁻⁶	2.31	0.1285	95.97	2.0×10⁻¹⁶	3.14	0.0764

not, presumably due to correlations with other predictor variables (Table 7D). In the multi-trait model, *BCMA* had a marginal main effect on water use under drought ($P = 0.0894$), with the *MM* genotype showing very slightly reduced water use.

Although *BCMA* genotype did not directly affect water use under drought, we tested to see whether it influenced the way the morphological traits controlling water use responded to drought. In other words, we tested for whether morphologies driving water use were plastic across drought and well-watered treatments, and whether the magnitude and/or direction of plasticity was influenced by *BCMA* genotype. We found

that all traits driving water use (size, leaf number, and LMA) were plastic in response to drought. In all of these cases, the *MM* genotype conferred a significant reduction in that trait value under drought (Figure 9, lower panels). Conversely, trait values for the *BB* genotype were consistent across wet and dry treatments, or even increased under drought. This pattern was consistent across many traits measured (Table 8; Appendix E:

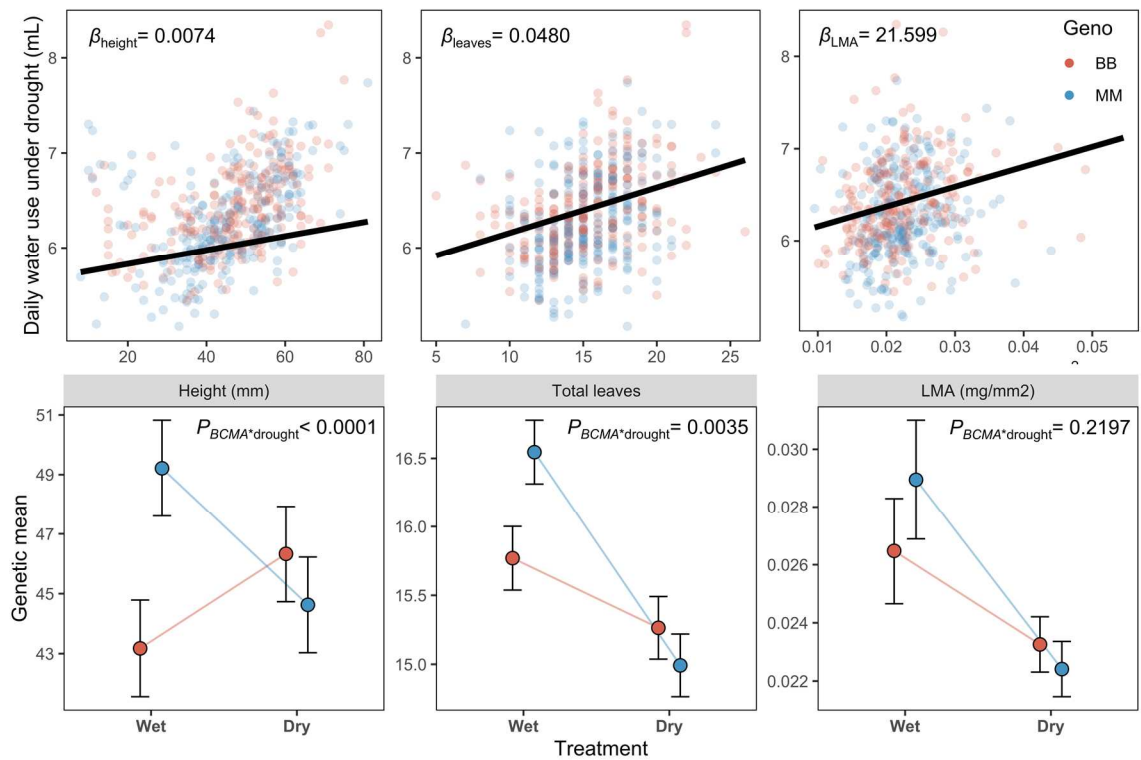


Figure 9. *BCMA* alleles confer differential response to controlled drought. While *BCMA* does not affect water use under drought directly, water use is predicted by a number of morphological variables, including height, number of leaves, and estimated leaf mass per area (LMA) (top). All of these morphological traits are plastic under drought (bottom), and two show significant *BCMA* × drought interactions, indicating genetic variation in phenotypic responses to drought. Slopes shown in top panels were estimated from separate models for each trait (Table 8A-C).

Figure E1, Table E2). This suggests that the *MM BCMA* genotype confers morphological plasticity under drought that reduces water use.

We also assessed response to controlled drought in a panel of genotypes collected from natural populations to determine whether the effects of *BCMA* on drought response were still observable in genetic backgrounds more diverse than our biparental NIL population. We found that, even in variable genetic backgrounds, *BCMA* significantly predicted water use under drought (Figure 10). Genotypes with low BC-ratio had higher leaf water content under drought ($F_{1,231} = 8.17$, $P_{\text{BC-ratio}} = 0.0046$, $N = 237$),

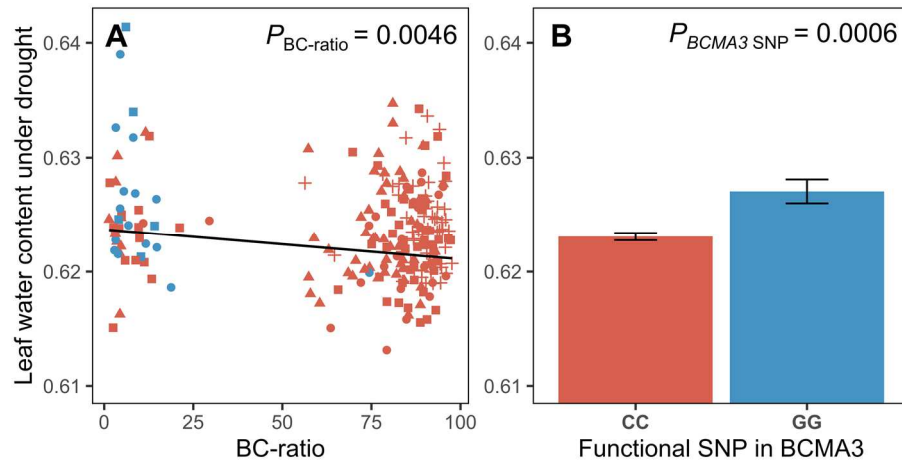


Figure 10. *BCMA* influences drought response in variable genetic backgrounds. Across a panel of >200 genotypes, genetic mean glucosinolate phenotype (A) and SNP identity in BC-ratio GWAS (B) both predict leaf water content under drought. In both panels, color indicates SNP identity, with red denoting genotypes homozygous for the LTM parental SNP, and blue denoting genotypes homozygous for the SAD12 parental SNP. Shapes in panel A indicate genetic groups (COL: circles; NOR: triangles; UTA: squares; WES: crosses) as described in Wang et al. (2019).

and SNP identity correlated with BC-ratio significantly predicted that response ($F_{1,220} = 0.12$, $P_{BCMA3\ SNP} = 0.0006$, $N = 225$).

1.3.4 Molecular signatures are consistent with balancing selection on *BCMA*

Our field and greenhouse data show effects of glucosinolate profile on functional traits (herbivore defense, drought response) and fitness components (survival, reproduction). Tradeoffs in these effects across environments may generate balancing selection on *BCMA*. If that is the case, evolutionary responses to long-term balancing selection should leave detectable molecular signatures in the genome and across the landscape. We used sequence analysis and phenotype–environment associations to test for these molecular signatures.

***BCMA3* has elevated nucleotide diversity**

We analyzed nucleotide diversity (π) within *BCMA3* and genes of similar size across a panel of 55 genotypes. Consistent with predictions for balancing selection, we found that the observed level of nucleotide diversity within this gene is in the extreme tail of the distribution of nucleotide diversity observed across other comparable genes (Figure 11). Nucleotide diversity in *BCMA3* exceeded that of 95.8% of 1,775 comparable loci.

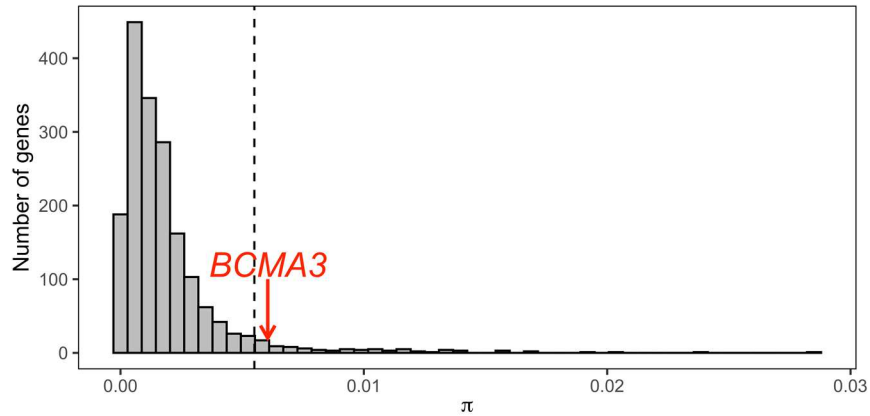


Figure 11. *BCMA* has high nucleotide diversity relative to comparable genes. The observed level of nucleotide diversity (π) in *BCMA3* (red arrow) exceeds the 5% tail (dashed line) of comparable genes in the *B. stricta* genome.

Drought in home environments is associated with BC-ratio

Our field and greenhouse experiments implicate pleiotropic effects of *BCMA* on multiple functional traits (herbivore defense and drought response) as an ecological mechanism influencing balancing selection. The impacts of glucosinolates on insect herbivores are well characterized; however, effects of glucosinolates on drought response have not been previously elucidated in this species. If drought stress in addition to insect herbivory has influenced glucosinolate evolution in *B. stricta*, we expect that response to selection might cause the distribution of glucosinolate phenotypes across the landscape to be correlated with drought-related environmental variables. Using a principal components analysis of long-term averages of climate data

at the source locations of 267 genotypes across the species range, we found that one axis of climate variation significantly predicted BC-ratio (Table 9; Figure 11, left).

Table 9. Geography and climate of origin predict BC-ratio.

Model Effect	DF	Sum Sq.	F	Pr > F	
Genetic group	3	12,593	4.64	0.00353	**
Latitude	1	947	1.05	0.3071	
Longitude	1	3,606	3.99	0.0469	*
Elevation	1	3,409	3.77	0.0533	.
Climate PC1	1	12,994	14.37	0.0002	***
Climate PC2	1	261	0.29	0.5916	
Climate PC3	1	948	1.05	0.3069	
Climate PC4	1	2,461	2.72	0.1003	
Climate PC5	1	9	0.01	0.9192	

We used axis loading to determine which raw climate variables were most strongly correlated with the axes predicting BC-ratio, and found that several were strongly correlated with PCA axis 1 ($|r| > 0.70$; Appendix H: Table H1, Figure H1). All of the climatic variables that were highly correlated with climate axis 1 were drought-related: temperature of the warmest month, annual precipitation, precipitation of the wettest month, precipitation of the driest month, precipitation of the wettest quarter, precipitation of the driest quarter, and precipitation of the coldest quarter (Table 10). We then performed univariate regressions of BC-ratio upon each of these 7 climatic variables to test the hypothesis that historical selection by abiotic climate variables has resulted in correlations between drought conditions and the current distribution of glucosinolate phenotypes across the landscape. We found that 5 of these 7 climatic

variables significantly predict BC-ratio (Appendix H: Table H2). All of these relationships show that Met-GS are more common in drier environments (Figure 12; Appendix H: Figure H2). These results corroborate our experimental data suggesting that the Met-GS *BCMA* alleles confer beneficial morphological and physiological responses to drought, which may drive patterns of survival in the field.

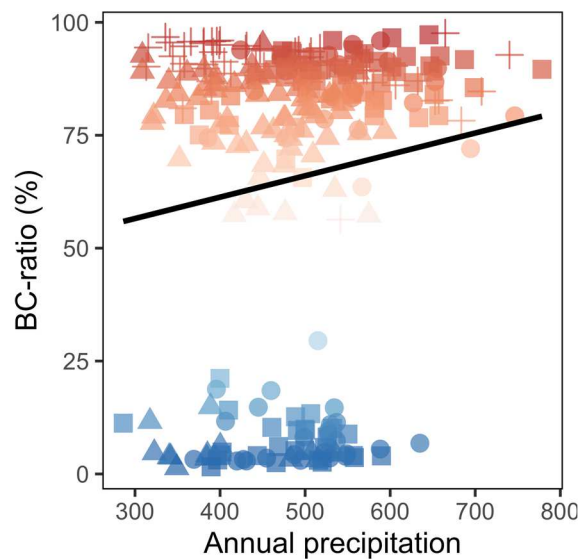


Figure 12. Glucosinolate phenotypes are correlated with drought conditions in source environments. Genotypes producing Met-derived GS are more common in drier environments, while those producing BC-derived GS are more common in wetter environments (Table 9). Symbols denote genetic groups as described in Wang et al. (2019). COL: circles; NOR: triangles; UTA: squares; WES: crosses.

Pleiotropy vs. tight linkage

A large portion of our results presented here utilize NILs generated from natural crosses of parental genotypes with contrasting GS phenotypes. These genetic tools confer distinct advantages, especially with regard to field experimentation, as they do not require special permitting to plant and manipulate outdoors. However, even in the

case of crosses generated with very fine-scaled mapping, the ability to attribute phenotypic variation with certainty to a particular gene is limited by other loci that may occur in tight linkage with the gene of interest. To address this, we used genotyping-by-sequencing on a panel of 65 NILs (Appendix A) to characterize the *BCMA* haplotype. Using the newly assembled SAD12 version_2 genome as a reference, we found that F_{ST} values between *BB* and *MM* genotypes were elevated in the region immediately surrounding *BCMA3*, as expected (Figure 13A). By assessing the patterns of F_{ST} across the chromosome, we also characterized the size of the broader *BCMA* haplotype: ~212 kb, spanning from position 11,737 to 11,949 kb on chromosome 7. The focal region in the genome extends 42 kb in the 5' direction and 167 kb in the 3' direction of *BCMA3* (Figure 13B).

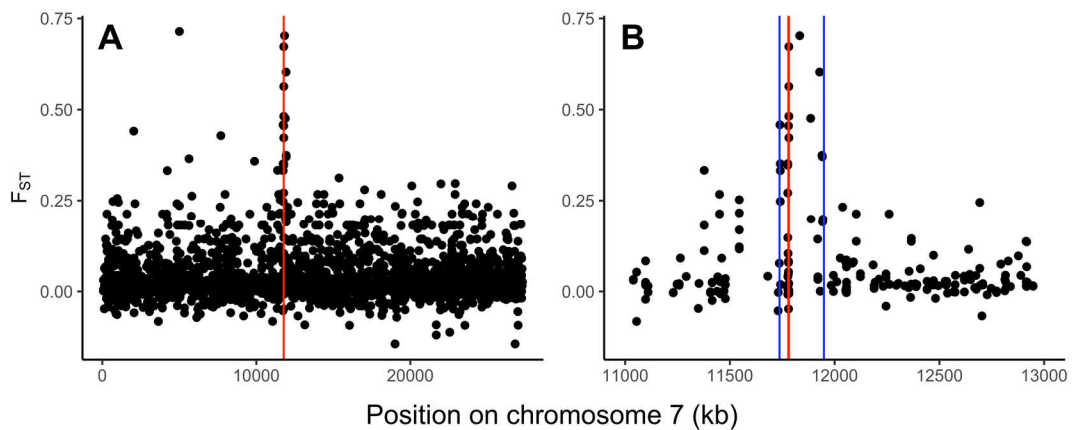


Figure 13. Characterization of the *BCMA* haplotype. A: Comparisons of genomic sequence data between *BB* and *MM* NIL genotypes show an F_{ST} peak around the *BCMA3* gene (red line). B: A finer-scale view of (A), with blue vertical lines delimiting the extent of the ~212 kb non-recombinant haplotype interval.

Sequencing of the NIL genotypes revealed 41 SNPs within these haplotype boundaries (Figure 13B). Phytozome annotation of the version_1.2 reference genome shows that nine other genes are present in this 212 kb isogenic region, all in the 3' direction from *BCMA3* (Table 10). A literature search revealed that, besides *BCMA3*, several of these genes either contribute to drought response or change expression in response to drought (Table 10).

Table 10. *B. stricta* genes in the non-recombinant BCMA haplotype, and their *Arabidopsis* homologs. All *B. stricta* gene IDs, positions, and annotations reflect the version_1.2 reference genome on Phytozome, and fall on Scaffold 0597. Shaded rows indicate genes whose expression is responsive to drought. Shaded rows with bold text indicate genes with impacts on drought response which have been validated with functional genetic studies.

<i>Boechera stricta</i> gene			<i>Arabidopsis thaliana</i> homolog		
kb from BCMA3	Gene ID	Annotation	Gene ID	Function or putative function	References
0	Bostr.0597s0001	Cytochrome P450 family 79, subfamily C	AT1G16410 (CYP79F1)	Pleiotropic effects on glucosinolate biosynthesis and drought response	Salehin et al. 2019
75,050	Bostr.0597s0002	Rapid alkalinization factor (RALF)-like protein	AT4G14020	Genes encoding RALF family proteins change expression patterns under drought stress and biotic stress	Atkinson et al. 2013 Sharma et al. 2016
81,962	Bostr.0597s0003	Selenium-binding protein 1	AT4G14030 (SBP1)	SBPs influence oxidative stress response; expression patterns change under drought	Dutilleul et al. 2008 & refs. therein
84,605	Bostr.0597s0004	Selenium-binding protein 2	AT4G14040 (EDA38)		
88,128	Bostr.0597s0005	Pentatricopeptide repeat (PPR) superfamily protein	AT4G14050 (MEF35)	Overexpression of at least one PPR, SOAR1, increases drought tolerance	Jiang et al. 2015
113,569	Bostr.0597s0006	Pathogenesis-related protein Bet v I family	AT4G14060	Bet v I proteins in birch trees contribute to human allergies and asthma; unknown biological function <i>in planta</i>	Wen et al. 1997 Sinha et al. 2014
132,776	Bostr.0597s0007	PFI1-like DNA helicase	AT3G51690	While not a close structural homolog (17.7% similarity), PIF1 in <i>Arabidopsis</i> is a transcription factor influencing chlorophyll biosynthesis	Huq et al. 2004
150,504	Bostr.0597s0008	Long-chain-fatty-acid ligase / Acyl-synthetase	AT4G14070 (AAE15)	Fatty acid elongation; possible membrane repair following stress. Translated at higher levels in senesced, dehydrated, or cold-stressed tissue	Koo et al. 2005
153,571	Bostr.0597s0009	Beta-1,3-glucanase related	AT4G14080 (MEE48)	Similar to O-Glycosyl hydrolase family 17 proteins, which influence carbohydrate metabolism	Henrissat et al. 1995
155,650	Bostr.0597s0010	None	AT4G14100	Uncharacterized; GO match to “transferases, transferring glycosyl groups”	The Arabidopsis Information Resource (TAIR)

Among natural accessions in the COL group, LD was low in the *BCMA* interval (Appendix I, Figures I1-I2), suggesting that observed variation in complex traits such as herbivore resistance, height, leaf number, LWC, and drought tolerance are unlikely to be attributable to other loci within the *BCMA* interval.

1.4 Discussion

Understanding how genetic variation in complex traits is maintained despite natural selection is a fundamental challenge in evolutionary biology. Here, we provide a suite of evidence documenting functional links between genetic variation in *BCMA*, chemical (glucosinolate) and physiological (drought tolerance) functional traits, and fitness in nature. Specifically, allelic variation within *BCMA3* alters herbivore defense and drought tolerance, yielding fitness tradeoffs across environments. These tradeoffs are consistent with balancing selection on *BCMA3*, allowing complex trait variation to be maintained across the landscape. This ecological evidence for balancing selection is corroborated by molecular analyses, which reveal that nucleotide diversity within *BCMA3* is elevated compared to genome-wide variation in this species, and that glucosinolate phenotypes are correlated with drought conditions in home environments. Finally, the multiple genetic variants underlying glucosinolate profiles are geographically widely distributed across the species range, suggesting that the *BCMA* polymorphism is old.

1.4.1 Evidence for balancing selection

The degree to which different evolutionary processes may explain the maintenance of polymorphism has been the subject of debate for decades (Hedrick et al. 1976, Hedrick 1986, Hedrick 1999). Particularly, while empirical studies often attribute persistent genetic variation to spatial variation in selection, temporal variation in selection, and genetic tradeoffs for fitness (antagonistic pleiotropy), the degree to which these expectations are supported by population genetics models is mixed. Generally, the mathematical conditions required (particular combinations of selection and dominance coefficients, heritability, gene flow, etc.) to predict stable polymorphisms under these scenarios tend to be restrictive, and are frequently discordant with empirical observations (Hedrick 2006).

However, theoretical models generally agree that among these processes, spatial variation in selection tends to be the most permissive, providing the widest ranges of parameter space under which polymorphism can be maintained. Further, conditions such as inbreeding, limited gene flow, and limited dispersal can make it easier for spatial variation in selection to maintain polymorphisms (Hedrick 1999). Our study system, a predominantly self-pollinating perennial wildflower with gravity-dispersed seeds, is exemplary of these conditions. Finally, the majority of population genetic models dealing with balancing selection focus on traits controlled by a single locus;

conditions for maintenance of genetic variation in complex traits may also be more permissive than those required when considering single loci (Turelli & Barton 2004).

Thus, the evidence we present here is compatible with the predictions of population genetic models. While we have emphasized the contributions of a gene of interest, *BCMA3*, to observed phenotypes, we ultimately aim to understand maintenance of variation in complex, polygenic traits such as insect resistance, drought tolerance, and fitness. The ecological and genetic signatures of selection we observe in the *BCMA* haplotype yield insights into the way tractable genes with large effects on some traits influence patterns of genetic variation in complex traits under selection in nature.

The conditions we characterize influencing selection on *BCMA* are in fact hallmarks of evolutionary mechanisms of balancing selection on complex traits. We find consistent evidence for changes in the sign of *BCMA* allelic effects on fitness across environments (Figures 6-7) (Turelli & Barton 2004, Mitchell-Olds et al. 2007). This is consistent with examples of QTL exhibiting contrasting effects on fitness under variable laboratory and field conditions (Oakley et al. 2014, Price et al. 2018). This is also consistent with recent work showing that pleiotropic effects correlated with individual SNPs across variable environments contribute to polygenic trait variation (Troth et al. 2018). Our case builds upon these other studies by offering explanations of tractable, functional consequences for allelic variation across environments on complex phenotypes that underlie fitness.

When discussing maintenance of polymorphisms under selection, the question of scale is also worth consideration. Despite mechanisms of balancing selection varying in how restrictive their conditions are, all are theoretically plausible, and most have received some level of empirical support. However, contrasting mechanisms of balancing selection have different implications for the spatial scale at which variation is maintained. Overdominance and frequency-dependent selection, for example, may maintain “local polymorphisms” within populations. Conversely, spatial variation in selection can maintain “global polymorphisms” across populations (Mitchell-Olds et al. 2007). Our data are consistent with the latter scenario; our diverse collection of accessions captures across-population phenotypic and genetic variation. Spatial variation in selection may also contribute to maintenance of polymorphism within populations, if the scale of gene flow (via mating and/or migration) is greater than the scale of environmental variation driving selection. While in general gene flow is thought to be low in *B. stricta*, as the species shows both high F_{IS} and high F_{ST} among populations (Song et al. 2006), the absolute spatial scale of gene flow and environmental drivers of selection are still unknown. We encourage future research into the implications of the patterns we describe here on within-population variation.

1.4.2 Environmental variation in the expression of tradeoffs

In the case of glucosinolate profile in *B. stricta*, we see clear evidence of functional trait and fitness tradeoffs conferred by contrasting *BCMA* alleles across environments, yielding balancing selection. However, we also observe conditional neutrality; in seven of the 15 tested field environments, contrasting *BCMA* alleles conferred no detectable difference in either insect resistance or survival. Furthermore, in many environments in which we documented fitness tradeoffs conferred by *BCMA* alleles, the magnitude of the effect was small. In such cases, experiments must deploy large sample sizes into many environments to have sufficient power to detect changes in allelic effects when they occur (Anderson et al. 2011, Anderson et al. 2013). This is important to consider from a practical perspective; the relative lack of empirical evidence for fitness tradeoffs for alleles underpinning complex trait variation is likely influenced by the problem of statistical power (Anderson et al. 2013). Thus, we posit that such patterns of balancing selection on complex traits may be more prevalent than is indicated by current empirical evidence.

Environmental fluctuations between allelic fitness differences of small effect and allelic fitness differences of no effect also likely have evolutionary consequences.

Population genetic models sometimes define conditions for the maintenance of polymorphism in terms of the strength of balancing selection relative to the strength of directional selection when the polymorphism is perturbed from equilibrium (Turelli &

Barton 2004). As long as fitness differences are expressed reasonably frequently and in a reasonable number of environments (to prevent drift to fixation), small effect sizes interspersed with conditional neutrality might aid in the maintenance of polymorphism. Such patterns of selection avoid strong negative temporal autocorrelation within environments, which, although favoring contrasting alleles in different environments, eventually erodes genetic variation (Hedrick 1986).

1.4.3 Dissecting effects of pleiotropy and linkage

Our results provide conclusive evidence that allelic variation in the *BCMA* haplotype confers tradeoffs across environments in both functional traits (herbivore defense, drought response) and fitness. However, there are some limitations to the precision with which we can attribute causality of these tradeoffs to our candidate gene, *BCMA3*; our results also revealed that there are several other genes that occur in the non-recombinant interval tested experimentally with our NILs (Table 10). However, outside of NILs, LD within and around the *BCMA* region is low across natural populations (Appendix I), suggesting that if *BCMA3* were not causal of the patterns we have observed, those patterns would not be recapitulated when utilizing genotypes with broad genetic backgrounds. In this case, we find that the *BCMA* region and the BC-ratio phenotype predict insect resistance and drought response traits both in NILs and in broad genetic backgrounds.

The causal effect of *BCMA3* on glucosinolate variation and herbivore resistance has been previously characterized using cloning and transgenic approaches (Prasad et al. 2012). However, effects of this gene on drought response and survival in the field were unknown prior to this study. Despite this, there is a growing body of evidence documenting the effects of glucosinolates on drought response in plants, making a scenario in which *BCMA3* has direct, pleiotropic effects on drought response highly plausible. Recent analysis of *Arabidopsis CYP79F1* and *CYP79F2* double knockout mutants (Tantikanjana et al. 2004), orthologs to *BCMA* in *Boechera*, found direct effects of these glucosinolate biosynthesis genes on drought tolerance; mutants lacking glucosinolate production were significantly less drought tolerant than wild-type genotypes producing glucosinolates (Salehin et al. 2019). This process was mediated by effects of glucosinolate degradation products on stomatal aperture, as has also been documented in earlier studies (Hossain et al. 2013).

In addition, there is evidence that glucosinolates may influence aquaporins (Martínez et al. 2015), and their amino acid precursors may function as osmolytes, regulating water balance at the cellular level (Mewis et al. 2012). Combined with widespread evidence of their role in mediating plant-herbivore and plant-microbe interactions (Hopkins et al. 2009), these studies paint an increasingly detailed portrait of glucosinolates as multi-functional biomolecules which, in conjunction with hormones such as auxin and IAA, help to coordinate organismal responses to both biotic and

abiotic stressors (Abuelsoud et al. 2016, Nguyen et al. 2016, Shani et al. 2017, Salehin et al. 2019).

In this case, allelic variation in our *B. stricta* *BCMA* NILs captures variation in glucosinolate profile rather than presence/absence or up-/down-regulation, which are common target phenotypes assessed in molecular and functional genomic approaches (in part because, unlike *Boechera*, *Arabidopsis* does not commonly express complex variation in Met- vs. BC-derived glucosinolate profiles). However, we suggest that allelic variation in glucosinolate molecular structure might elicit differential responses to drought, similar to findings in knock-out mutant or overexpression studies (Salehin et al. 2019). In addition, it may be possible that concentrations of free amino acids in the cell following glucosinolate biosynthesis by enzymes with different affinities may contribute to this response (Mewis et al. 2012). We encourage further investigation into differences in signaling responses that may be elicited by glucosinolates and breakdown products with contrasting molecular structures.

1.4.4 Interactions of biotic and abiotic drivers of selection

In addition to revealing the genetic basis of cross-environment fitness tradeoffs that drives balancing selection on *BCMA*, we identify two key drivers of selection on glucosinolate evolution in *B. stricta*: herbivore pressure and drought stress. The magnitude of selection exerted by each of these drivers likely varies, and perhaps

covary, in complex ways across the landscape. Concrete agents of selection on quantitative traits are rarely identified in evolutionary genetics studies, despite the importance of ecological interactions in driving evolutionary outcomes (Anderson et al. 2014). Because evolutionary responses to selection by biotic and abiotic agents may differ in their genetic architecture – traits experiencing selection by biotic drivers are often controlled by QTL of larger effect sizes than traits experiencing selection by abiotic drivers (Louthan and Kay 2011) – complex traits experiencing selection by both types may be prone to evolve genetic architectures with a mix of large- and small-effect loci contributing.

In addition, the role of interacting and covarying ecological drivers of selection has not yet, to our knowledge, been explicitly addressed in the context of balancing selection. For example, drought stress may alter herbivore pressure directly by changing arthropod abundance or metabolic rates, and/or indirectly by influencing plant growth and secondary metabolite concentration. This could yield complex, non-additive fitness landscapes selecting for different defense optima across environments. We encourage further research into the implications of interactions among selective drivers on the maintenance of polymorphism.

1.5 Conclusions

We present integrative ecological and molecular evidence demonstrating balancing selection on *BCMA* generated by biotic and abiotic selection. In addition to characterizing balancing selection on an ecologically important complex trait in natural habitats, we link this variation to a functional genetic basis. Although *BCMA* has large phenotypic effects on plant chemistry, it has subtle effects on complex traits such as herbivore resistance, drought tolerance, and fitness in nature. Approaches emphasizing tractable intermediary phenotypes have similarly helped to identify possible genetic bases of human metabolic variation (Kettunen et al. 2012). Thus, we demonstrate that, with appropriate resources and experimental design, it is possible to link ecological mechanisms of balancing selection to functional genetic variation underlying complex traits.

2. Maintenance of polymorphism under selection by multiple drivers

Lauren N. Carley, William F. Morris, and Tom Mitchell-Olds

2.1 Introduction

Local adaptation, evidenced by genotypes displaying higher fitness in their home environments than in foreign ones, can allow polymorphisms to persist across the landscape as different alleles best suited for local environments are selected for (Mitchell-Olds et al. 2007). As such, environmental heterogeneity is commonly cited as a force influencing the maintenance of genetic diversity (Hedrick 2006). Classical evolutionary approaches to testing for local adaptation include common garden and reciprocal transplant experiments, in which performance of genotypes or populations originating in different environments is measured in the same environment. Such experiments have yielded substantial insights into the prevalence of local adaptation among natural populations (e.g. Ågren & Schemske 2012, Wadgymar et al. 2017, Selby & Willis 2018, Briscoe Runquist et al. 2020, Hargreaves et al. 2020). However, even in well-designed common garden or reciprocal transplant experiments, specific environmental drivers that influence the magnitude and direction of selection across locations are often not identified (Anderson et al. 2014). While there are some excellent examples to the contrary (e.g. Campbell et al. 1994, Mauricio & Rausher 1997, Sletvold et al. 2010), a lack of understanding of ecological drivers of selection can obscure the specific traits and

underlying genetic loci that confer observed “home-site” advantages (Anderson et al. 2014). This limits the extent to which our understanding of adaptive evolution in nature is mechanistically grounded. Further, while driver-agnostic experiments can document the existence of local adaptation (or lack thereof, e.g. Anderson & Wadgymar 2020) at a given point in time, failure to identify drivers of selection precludes predictions of adaptive responses as environments change (MacColl 2011). This task is further complicated when traits experience selection by multiple ecological drivers, which themselves may interact and covary.

Conversely, elucidating environmental drivers of demographic performance is becoming an increasingly common goal in population biology (Doak & Morris 2010, Louthan et al. 2017, Sheth & Angert 2018, Benning et al. 2019) and community ecology (Clark et al. 2014, Nuñez et al. 2019), especially in the face of prevalent anthropogenic impacts on climate and land use (Morris et al. 2020). However, even demographic studies that leverage explicit environmental manipulations to identify drivers of population performance rarely use common garden or reciprocal transplant approaches to tease apart naturally occurring genotype-environment covariance (but see recent examples in Benning & Moeller 2019, Campbell 2019, and Anderson & Wadgymar 2020), or take phenotypic evolutionary change into account when predicting population responses to environmental change (but see Lachapelle & Bell 2012, and other examples of evolutionary rescue).

In this study, we combined evolutionary genetics and population ecological approaches to elucidate the impacts of biotic and abiotic environmental variation in selection on a focal trait: secondary chemical profile in *Boechera stricta*, a perennial wild relative of *Arabidopsis*. Previous work in this system identified both herbivore pressure and drought stress as drivers of selection upon this trait (Carley et al., *in prep*; section 1 of this document), implicating variation in these drivers as a cause of balancing selection on chemical phenotypes. This prior work offers an explanation for current patterns of phenotypic variation across the landscape that have been generated by past balancing selection—a snapshot view. However, little is known about how interactions among ecological drivers of selection are expected to play out in future scenarios, explicitly predicting whether and under what circumstances polymorphisms are expected to be maintained.

To address this, we utilized near-isogenic *B. stricta* genotypes (NILs) varying in a locus influencing secondary metabolite profile (Prasad et al. 2012) which experience selection by both herbivore pressure and drought (Carley et al. *in prep*). We manipulated both drivers of selection on glucosinolate variation and tracked their interactive effects on the fitness of 2,300 individuals in an experimental common garden for four years, capturing the full life span of the majority (~78%) of these short-lived perennial plants. From these longitudinal data, we built genetically explicit demographic models integrating the effects of varying selective drivers on lifetime fitness. We used these

models to predict the fate of different genetic lineages under varying combinations of selective drivers, addressing the following specific questions:

- (1) How do contrasting NIL genotypes respond to manipulations of biotic and abiotic agents of selection?
- (2) Under what ranges of environmental conditions do we expect to see polymorphic genotypes and phenotypes maintained?
- (3) How does inference about selection from longitudinal studies compare to that derived from “snapshot” approaches?
- (4) What are the implications of covariance among selective drivers on the maintenance of polymorphism?

2.2 Methods

2.2.1 Study system and focal trait

Boechera stricta (Brassicaceae) is a perennial wild relative of *Arabidopsis* native to the western USA that is a model system for evolutionary ecology and genetics (Rushworth et al. 2011). It is highly self-pollinating (F_{IS} : 0.74 – 0.97; Song et al. 2006), and natural accessions commonly exist as locally adapted inbred genotypes. Like other members of the Brassicaceae, *B. stricta* produces secondary metabolites called glucosinolates (GS), which mediate ecological interactions with herbivores and pathogens (Hopkins et al. 2009). GS profile in *B. stricta* is highly polymorphic across the

species range. One axis of GS variation, the proportion of GS derived from branched-chain amino acids vs. methionine (“BC-ratio”; Schranz et al. 2009, Prasad et al. 2012), has elevated variation that appears to be maintained by balancing selection driven by both herbivore pressure and drought stress (Carley et al., *in prep*; Section 1 of this document). A major gene controlling BC-ratio was previously elucidated (Prasad et al. 2012), and near-isogenic lines (NILs) have been generated via crossing, yielding genotypes with contrasting BC-ratio alleles in otherwise homogenous genetic backgrounds (Carley et al. *in prep*; see Section 1.2.1 and Appendix A). Here, we utilized NILs homozygous at the *BCMA* locus, which determines BC-ratio, with alleles conferring either branched-chain glucosinolates or methionine-derived glucosinolates. We refer to these lines as the “BB” and “MM” genotypes, respectively. Each NIL genotype was created using several independent crossing lineages to account for possible effects of unlinked loci outside of the focal *BCMA* region (Appendix A; Appendix J: Table J1).

2.2.3 Manipulative field experiment

Common garden

We tested for fitness consequences conferred by different glucosinolate phenotypes by monitoring experimental transplants of the BB and MM NIL genotypes in a common garden near the Rocky Mountain Biological Laboratory (RMBL) in Schofield Townsite, CO, USA for four years. The garden was located at 39.035636°,

-107.059488° at 3,145m elevation, and was fenced to prevent trampling and grazing by megaherbivores (*e.g.* elk, moose, mule deer, and domestic cattle).

As described in 1.2.4, we germinated seeds under laboratory conditions at Duke University and reared germinants for two months under greenhouse conditions before shipping plants to the field site. We transplanted 2,300 juvenile plants directly into the matrix of native vegetation in a randomized, complete block design containing 50 plants per block, spaced at constant density in 10 cm intervals (10 plants per row x 5 plants per column in each block; Appendix J: Figure J1). The two NIL genotypes were equally represented in each block at the time of transplanting, and the 25 replicate individuals per genotype per block were distributed evenly across independent crossing lineages. We transplanted the focal genotypes in two cohorts: 2015 (N = 1,500) and 2016 (N = 800) (Appendix J: Table J1).

Naturally occurring *B. stricta* seeds germinate in the springtime, following snowmelt; germinants live for one season as a vegetative rosette, and then may flower and fruit following vernalization (cold treatment) in the second or later year of the life cycle (Figure 14; Anderson & Wadgymar 2020). We transplanted juvenile rosettes in late September and early October of each planting year, allowing transplants to experience natural vernalization beginning shortly thereafter (first snowfall of second planting year: October 6, 2016; *pers. obs.*). We then monitored survival, growth, and reproduction at the end of each growing season beginning in the summer following transplanting.

Environmental drivers

In this subalpine system, the growing season is short and flanked by long winters with high snowpack. Hydrologic conditions during the growing season are driven primarily by snowmelt in the spring and monsoon rains in the mid-summer. This multi-year experiment spanned a range of ambient abiotic conditions, including substantial variation in both winter and summer precipitation dynamics (Table 11).

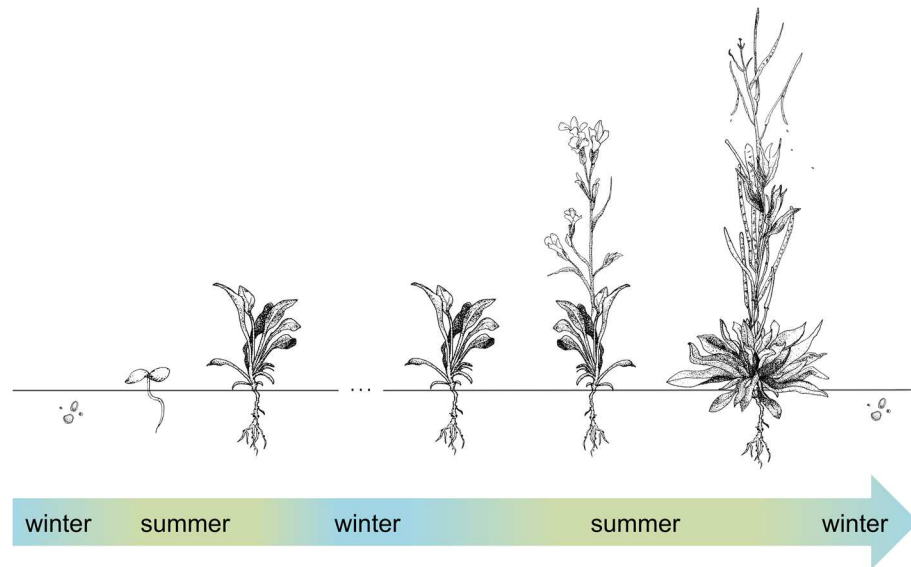


Figure 14. Life cycle of *Boechera stricta*. Left to right: Seeds germinate in early summer following snowmelt. Germinants that survive the first season establish as vegetative juvenile rosettes for at least one season. Following the next winter's cold cueing, vegetative rosettes may flower in early summer and produce fruits in mid-summer. Mature fruits dehisce and disperse seeds in late summer, after which seeds remain in the seed bank for at least one winter before germinating. Modified from Anderson & Wadgymar 2020. Artwork by Erin Fox.

Table 11. Ambient environmental conditions during the demographic common garden experiment.

Year	Winter precip. (cm) ¹	Max snow water eq. (cm) ²	Melt date (ordinal) ³	Average summer air temp. (°C) ⁴			Summer precip. (cm) ⁴	Summer soil moisture (m ³ /m ³) ⁵		
				min	mean	max		min	mean	max
2016	90.7	70.1	161	5.4	10.9	18.5	9.9	0.0424	0.1029	0.2128
2017	134.6	126.5	164	5.4	10.9	19.0	10.2	0.0552	0.1251	0.2274
2018	74.4	72.9	145	5.3	11.6	19.8	5.6	0.0437	0.0940	0.2010
2019	134.1	128.5	176	6.0	11.9	19.9	3.8	0.0406	0.0937	0.2596

¹Sum of daily precipitation between September 1 of the preceding year and the day of snowmelt in the current year. Data from SNOTEL sensor site #737, located 2.25 km SW of the experimental garden at 3261m elevation (USDA NRCS 2020).

²Maximum value of snow water equivalent (theoretical volume of water released if all snowpack melted simultaneously) occurring between September 1 of the preceding year and day of snowmelt in the current year (USDA NRCS 2020).

³Snowmelt date in Schofield Park, rounded to nearest ordinal day estimated in Anderson & Wadgyr 2020 (2016-2018) or via personal observation (2019).

⁴Daily air temperature (averages) and total precipitation (sum) occurring between the snowmelt date and September 1 of the current year (USDA NRCS 2020).

⁵Soil moisture data from Schofield Park taken using Decagon 5TM dataloggers deployed at 10cm depth from the date of snowmelt until September of the current year (J. T. Anderson, *unpub.*).

Environmental manipulations

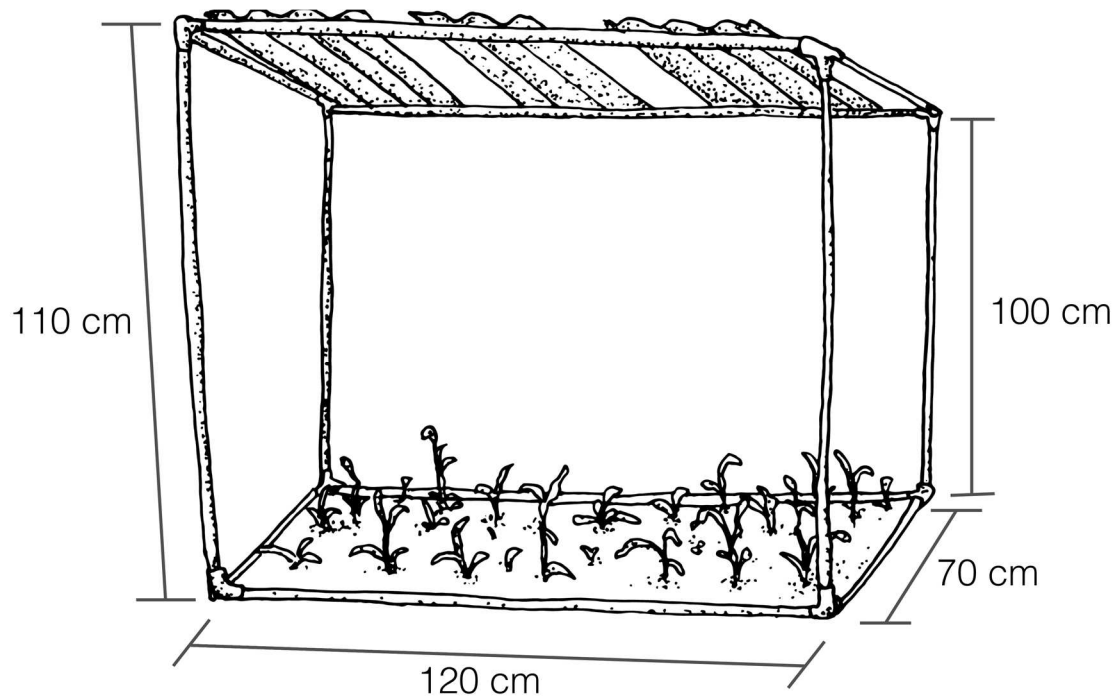
To test for fitness effects of variation in selective drivers, we manipulated both soil moisture and herbivore pressure during the growing seasons from 2017-2019. These manipulations spanned all field growing seasons for the 2016 planting cohort. The 2015 planting cohort experienced one year under ambient field conditions prior to the beginning of manipulations. An overview of the experimental design for manipulations is provided in Table 12.

Table 12. Experimental design for driver manipulations. Because manipulations began in year 2 of the study, plants which had already died prior to June 2017 (436 BB and 259 MM) were unmanipulated and experienced ambient conditions in the first year of life. This left 1,605 of 2,300 transplants to experience driver manipulations, across all treatments and genotypes. N_{BB} and N_{MM} refer to the number of replicate individuals of the homozygous branched-chain glucosinolate NIL and homozygous methionine glucosinolate NIL, respectively.

		Soil moisture manipulation	
		Ambient	Reduced
		(23 blocks)	(23 blocks)
Herbivory manipulation (individual plants)	Ambient	$N_{BB} = 180$	$N_{BB} = 176$
		$N_{MM} = 223$	$N_{MM} = 223$
	Reduced	$N_{BB} = 182$	$N_{BB} = 176$
		$N_{MM} = 227$	$N_{MM} = 218$

We manipulated soil moisture using rainout shelters, which intercept precipitation while minimally altering other environmental conditions such as temperature and light (Yahdijan & Sala 2002, Shriver 2015). Specifically, we constructed rectangular prism-shaped frames from 0.5" diameter PVC pipes and fitted them with clear corrugated polycarbonate slats on top (Suntuf®, Palram Americas Ltd., Kutztown, PA) to cover 50% of the surface area of each block. Each shelter covered one full randomized complete block of transplants and extended 10 cm beyond block perimeters in all dimensions as a buffer zone (Figure 15). To reduce lateral flow of moisture among blocks, we also installed strips of 6 mil thickness (~0.15 mm) polyethylene film (Moisture Barricade, Roberts Consolidated Industries Inc., Boca Raton, FL) vertically to 10 cm depth in narrow trenches around the perimeter of each rainout shelter. Because of

space constraints in the experimental garden, blocks were not randomly assigned to ambient or reduced rainfall treatments. Instead, rainout shelters were installed in a checkerboard design (Appendix J: Figure J2).



**Figure 15. Rainout shelter design. Shelters covered 100 x 50 cm garden blocks plus a 10 cm buffer on all sides. Narrow trenches lined with a plastic moisture barrier also extended 10 cm belowground to reduce lateral water flow among blocks (not shown).
Artwork by Erin Fox.**

Following shelter deployment in 2017 and 2018, we tracked the effectiveness of the reduced rainfall treatment by measuring soil moisture every 2-4 days at three points within each garden block for the duration of the growing season, using a Hydrosense™ handheld soil moisture measurement system (Campbell Scientific Inc., Logan, UT) with 12 cm probes. To monitor non-target effects of the shelters, we also measured soil temperature in the center of each garden block every 2-4 days during the 2017 and 2018

growing seasons using a probe thermometer. Finally, we measured light transmission at ground level in each garden block on three dates in 2017 using a handheld quantum sensor (MQ-200, Apogee Instruments, Logan, UT). In 2018, a year of low snowpack, early snowmelt, and low summer precipitation (Table 11), we also supplemented soil moisture to the control treatment by adding 5.7 L of water to each garden block approximately weekly for the month of June.

We manipulated herbivore pressure at the individual plant level. Because some plants had already died prior to the start of manipulations, we first censused survival early in the growing season (mid-June) in 2017 to determine the number of surviving individuals per genotype and per block (Table 12). Then, we assigned living individuals to ambient or reduced herbivory treatments. Specifically, we summed survivors per genotype within each of the 46 garden blocks, and assigned 50% of surviving individuals of each genotype within each block to the treatment or control group using a random number generator. As such, the number of replicates of a genotype in the herbivory manipulation treatment group was approximately equal to the number of controls within each block, even if the total number of replicates per genotype was uneven within or across blocks due to initial differences in survival (Table 12; Appendix J: Table J2). We marked all individuals in the reduced herbivory treatment with a brightly colored plastic toothpick to easily identify treatment assignments within the randomized garden blocks throughout the growing season.

Weekly throughout the growing seasons in 2017, 2018, and 2019, we applied insecticide (Thuricide® BT Caterpillar Control, Southern Ag, Rubonia, FL) to each individual plant assigned to the reduced herbivory treatment, according to the manufacturer's instructions. Specifically, we used flexible plastic transparency sheets to create a cone around each target plant and applied insecticide using a spray bottle, focusing application within the plastic cone to minimize drift onto non-target plants. Using the same methods, we sprayed an equal volume of water weekly onto all control plants. The insecticide used has a general mechanism of action and is advertised to deter and/or kill a variety of insect grazers including larval butterflies, moths, beetles, and flies. We monitored the efficacy of herbivory manipulations by estimating herbivore damage to aboveground tissue of surviving individuals at the end of each growing season (see *Plant measurements* below).

<i>Inferring unobserved data</i>

To build driver-sensitive demographic models, we sought to include estimates of both soil moisture and herbivore pressure as predictors of survival, growth, and reproduction during each year of the experiment. However, due to logistical constraints, we were unable to measure soil moisture in garden blocks during the 2019 growing season. Therefore, we combined our garden-specific measurements of soil moisture from 2017 and 2018 with similar data from a nearby field site 0.5 km NW (J. T. Anderson,

unpubl. data) to predict unobserved block-level measurements of soil moisture in 2016 and 2019, as well as on unmeasured days in 2017 and 2018.

To fit a predictive relationship between off-site garden mean soil moisture and block-specific values in our garden, we focused on data from the 2018 field season, which had the best temporal resolution and range in our garden. For off-site data, we first calculated a daily garden mean value from continuous datalogger measurements taken at 15-minute intervals in 5 locations at 10 cm depth under ambient environmental conditions (Decagon 5TM sensors, now ECH20 5TM, Meter Environment, Pullman, WA; J. T. Anderson, *unpubl. data*). For our direct in-garden measurements, we calculated block-level means from three measurements per block on each observation day. We then merged the off-site and block-level means based on observation day. We used a linear model to regress our observed daily block-level means upon the off-site daily means, accounting for a fixed effect of garden block and the block $\times$ off-site mean interaction. This approach yielded block-specific slope and intercept coefficient estimates that relate block-level treatment and microhabitat conditions in our experimental garden to off-site mean values (Appendix K). Off-site means significantly predicted in-garden values ($F = 3,703.88$, $P < 2.2 \times 10^{-16}$, $N = 1,180$), and in-garden blocks varied significantly both in the intercepts (block identity main effect: $F = 10.33$, $P < 2.2 \times 10^{-16}$) and slopes (block $\times$ off-site mean interaction: $F = 1.07$, $P < 2.2 \times 10^{-16}$) of that relationship. The full model explained

approximately 80% of the variance in the observed in-garden block level measurements (multiple $R^2 = 0.8001$, adjusted $R^2 = 0.7834$).

Because we directly measured soil moisture in our experimental garden in 2017 as well, we tested the accuracy of our 2018-parameterized predictive model against observed data in that season. A univariate linear model regressing observed upon predicted values revealed a strong positive correlation among the two ($r = 0.7654$, $F = 908.55$, $P < 2.2 \times 10^{-16}$), although visualizing the relationship revealed that the model tended to over-predict block-level soil moisture (Appendix K). Adding a constant value (Int_y) to the intercept equal to the growing season mean VWC deviation from the 2018 growing season mean centered the observed vs. predicted values around the 1:1 line (Appendix K). As such, we ultimately predicted daily unobserved values using the following general formula, for each block b in year y :

$$VWC_{b,y} = Int_y + Int_b + Mean \times Slope_b$$

where *Mean* is the off-site daily mean VWC.

We used the same formula to predict unobserved values during the 2016 growing season; however, in that season, we added back a constant value to blocks assigned to the rainout treatment equal to the mean difference in VWC among rainout and control plots in the 2017 and 2018 observations. In this way, we estimated block-level variation in soil moisture prior to the start of the environmental manipulations, which began in 2017.

This approach yielded daily estimates of block-level VWC variation that were concordant with observed values, but at higher and more even temporal resolution, and expanded to include estimated values for the 2016 and 2019 growing seasons (Figure 16). From these predicted values, we calculated the block-level mean VWC across the growing season (spanning from snowmelt date through September 1; Table 11). We used these block-specific annual growing season means as a drought-related environmental driver in our demographic models.

<i>Assessing impacts of manipulations</i>

For soil moisture, soil temperature, and light availability (all measured at the block level), we tested for the effects of our manipulations using linear models ('lm' function in R v. 3.6.0 (R Core Team 2019)) accounting for census year, rainout treatment, and their interaction. For herbivore damage (measured at the individual plant level), we assessed treatment impacts in two ways. First, we modeled the probability of herbivory in response to insecticide treatment, drought treatment, census year, and their interactions using generalized linear models ('glm' in R) with a binomial distribution and logit link function. Second, among plants that received any herbivory, we modeled the amount of herbivory in response to insecticide treatment, drought treatment, census year, and their interactions using a linear model ('lm' in R) with a Gaussian distribution. We log-transformed the response variable to improve model residuals. For all analyses

of manipulation efficacy, we focused on the subset of data spanning 2017-2019, during which manipulations were implemented.

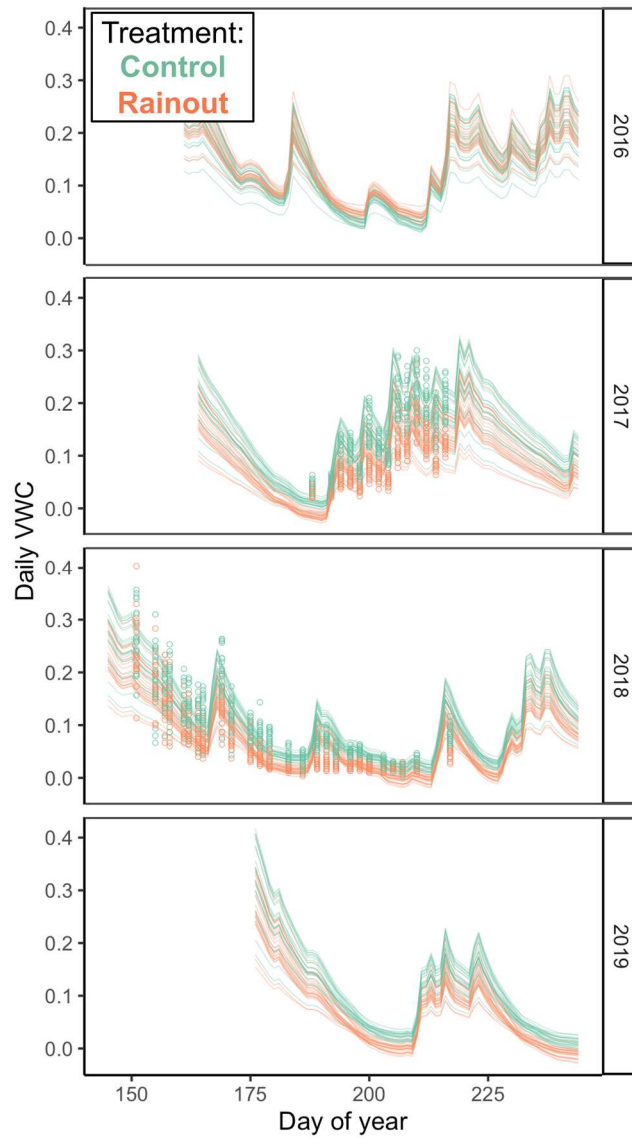


Figure 16. Seasonal variation in block-level soil moisture. Solid lines show daily predicted values and open circles show measured values during four growing seasons (date of snowmelt through September 1). Rainout shelter manipulations spanned 2017-2019.

To describe the main effect of each treatment on driver variation, we extracted model-predicted least-squares means and standard error values within treatment groups using ‘emmeans’ in R v. 3.6.0 (Lenth 2019). For linear models (abiotic variables and amount of herbivory), we tested significance of model effects using ANOVA. For the logistic regression (probability of herbivory), we tested significance of model effects using likelihood ratio tests (‘lrtest’ function from the ‘lmtest’ package in R; Zeileis & Hothorn 2002) comparing the full model to nested reduced models, dropping one effect at a time.

Plant measurements

We conducted full plant censuses at the end of the growing season (late July through late August) in each year. During full censuses, we scored each individual as alive or dead, and measured the size of each living plant. We scored living plants for their phenological stage, which we binned into reproductive (had produced flowers or fruits) or non-reproductive (no flowers or fruits) categories. For plants with fruits, we recorded the number and average length of fruits to estimate total reproductive output.

Our NILs contained genetic material from a distant ID genotype as well as a more local CO parent (see Section 1.2.1). Because we used NIL crosses rather than natural local genotypes, our research permit specified that we could not allow experimental plants to mature and disperse seeds. To estimate reproductive output in

the demographically relevant unit of total seeds per plant, we used direct counts of mature seeds per plant from a different *B. stricta* common garden experiment conducted at a nearby field site 0.5 km NW of our garden (Anderson & Wadgymar 2020) and measurements of fruit number and length from the same individuals (J. T. Anderson, *unpubl. data*). We modeled seed number in response to total fruit length (the sum of the lengths of all fruits) using a univariate linear model ('lm' in R v. 3.6.0; R Core Team 2019). Total fruit length was correlated with seed number in this location ($r = 0.742$; $P = 1.03 \times 10^{-12}$, $N = 66$). We used the fitted regression to predict seed number per plant in our own experimental transplants using the following equation, when fruit length is measured in cm:

$$\text{Seed \#} = -0.3984 + 10.2584 \times \text{total fruit length}$$

We also measured herbivory on aboveground tissue by counting the total number of leaves and the number of damaged leaves per plant and estimating the average percent damage per damaged leaf in 5% bins. We used these raw data to estimate total proportion leaf area removed (LAR) by herbivores as follows:

$$\% \text{ LAR} = \frac{\# \text{ damaged leaves}}{\# \text{ leaves}} \times \text{average \% damage per damaged leaf}$$

2.2.3 Demographic analyses

Vital rate regressions and model selection

To construct our demographic models, we first estimated 9 different vital rates (Figure 17). We modeled five of these as size-dependent: growth, adult survival, the probability of bolting, the probability of reproducing, and reproductive output. We modeled the remaining four transitions as size-independent: the probability of germination, the proportion of seeds which remain dormant in the seed bank, the size of new germinants, and the probability of survival of new germinants in the first year of life. Because growth patterns varied substantially depending on discrete state transitions (whether an individual bolted in the current year as well as the previous year), we modeled the probability of bolting and the continuous growth regressions within state-specific categories (Appendix L).

Given the results of prior work in this system, we hypothesized that manipulating soil moisture and herbivore pressure would influence fitness of *B. stricta* GS NILs. However, whether these fitness effects are consistent across years and/or life stages was unknown. Thus, we began our demographic modeling approach by fitting a global model for each of the size-dependent life history transitions. These global models fit each vital rate in response to fixed main effects of size, soil moisture, herbivory, and all possible 2- and 3-way interactions. We modeled binary probabilistic transitions (adult survival, the probability of bolting, and probability of reproducing) using generalized linear models with a binomial distribution and logit link function ('glm' function in R v.

3.6.0). We modeled continuous responses (growth and amount of reproductive output) using linear models with a Gaussian distribution ('lm' function in R v. 3.6.0).

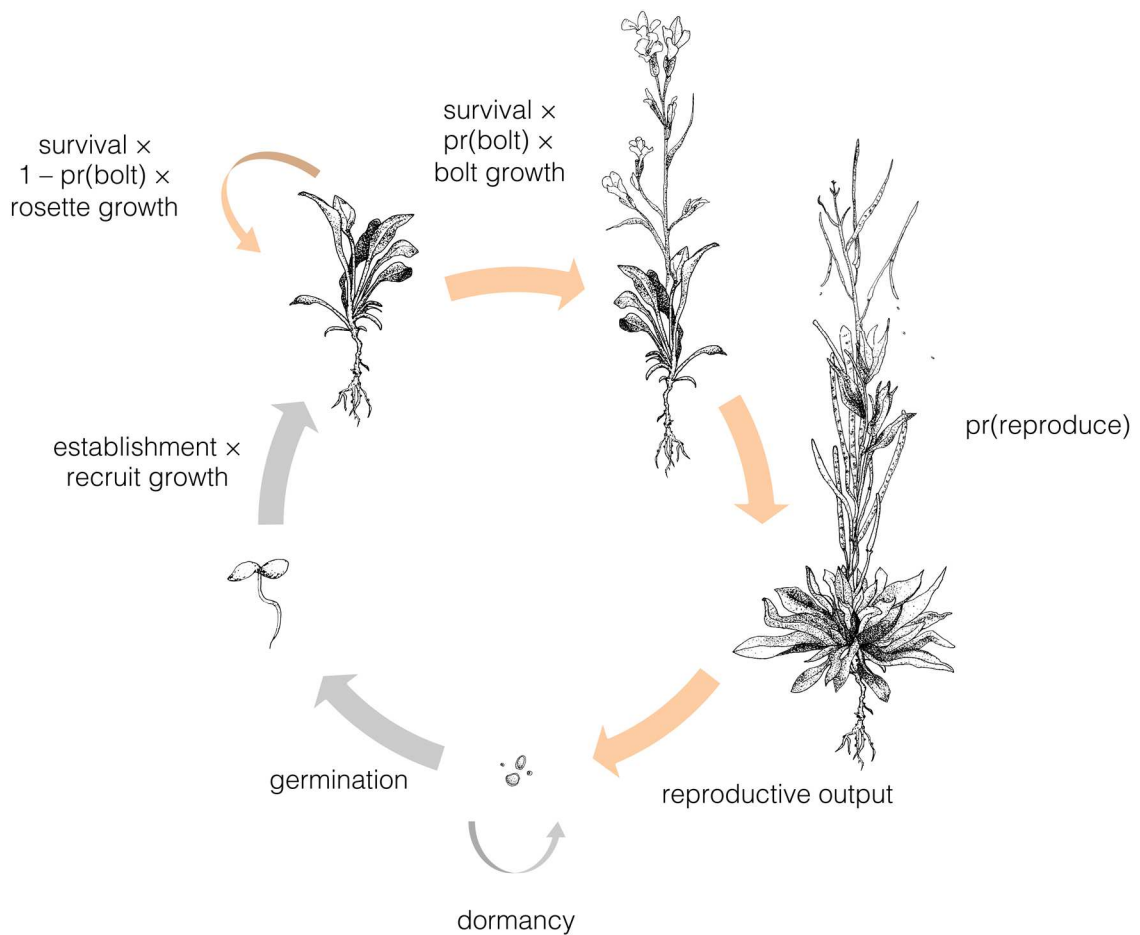


Figure 17. Visual model of demographic analyses. All orange arrows represent vital rates which we modeled as size- and driver-dependent. All gray arrows represent vital rates which we modeled as size- and driver-independent. We modeled all driver-dependent vital rates, as well as driver-independent recruit size, separately for each genotype to incorporate effects of genetic variation on fitness. Artwork by Erin Fox.

We then used model selection ('dredge' function in the MuMIn package (Bartón 2019)) to compare the global model to all possible reduced models containing subsets of

the size, driver, and interaction effects. We selected the suite of coefficients from the models with the best support (lowest AICc score) that retained, at a minimum, an intercept, even if they did not retain driver effects. We constructed and analyzed vital rate regressions separately for the two NIL genotypes since we had no *a priori* expectation that they ought to respond to driver manipulations in the same way; thus, the effects of drivers on vital rates were allowed to differ in magnitude and direction across genotypes, as well as in whether or not the driver was retained at all (*i.e.*, presence or absence of an effect of any magnitude or direction). Additional details regarding model selection for size-dependent vital rates can be found in Appendix M. In Table 13 we summarize the vital rate regressions used in this study.

We employed data from a combination of sources to estimate size-independent vital rates predicting recruitment of new individuals. As we were required to prevent incursion of non-local genotypes through seed dispersal, we did not estimate germination rates directly in our experiment. However, a similar study on *B. stricta* demography conducted in a common garden 0.5 km NW of our study site reported germination rates under similar ambient environmental conditions (Anderson & Wadgymar 2020). Because we did not measure germination directly under different environmental manipulations, we modeled germination as a fixed probability across all driver combinations and genotypes, using the local values reported in Anderson & Wadgymar (2020). Similarly, we modeled the dormancy rate as a fixed proportion of all

seeds produced regardless of genotype or environment, as reported in Anderson & Wadgymar (2020). We encourage future research investigating the effects of genotype and environment on these early life-history transitions.

Because we transplanted juvenile plants at the end of growing seasons, we could not directly measure germinant establishment under field conditions. Thus, we modeled the probability of establishment as a fixed value reflecting the establishment rate of local genotypes in a nearby field experiment, as reported in Anderson & Wadgymar (2020). For propagules that successfully germinated and established, we assigned new recruits to juvenile size values drawn from genotype-specific normal distributions. We parameterized these distributions by calculating the mean and standard deviation of plant size for juvenile transplants at the time of planting (approx. 2 months old, similar to the age of new recruits at the end of the first growing season following spring germination).

Table 13. Vital rate regressions retained after model selection. Driver- and size-sensitive models may include effects of size (z), herbivory (h), soil moisture (w), and interactions, or not, depending on model selection criteria. Vital rate functions that were not driver-sensitive were estimated either from genotype-specific summary statistics (recruit size) or published values for this species under comparable field conditions (all others). For functions dependent on bolting, 0 and 1 represent “did bolt” and “did not bolt”, respectively.

IPM kernel	Life history transition	Vital rate function	NIL geno.	Best supported model
P	Adult survival	$S(z, h, w)$	BB MM	$surv_{t+1} \sim z_t + w_t$ $surv_{t+1} \sim z_t + w_t + h_t + z_t \times w_t + h_t \times w_t$
P, F	Probability of bolting	$B0(z, h, w)$ $B1(z, h, w)$	BB MM BB MM	$bolt_{t+1} \mid bolt_t = 0 \sim z_t + w_t + z_t \times w_t$ $bolt_{t+1} \mid bolt_t = 0 \sim z_t$ $bolt_{t+1} \mid bolt_t = 1 \sim z_t + w_t + h_t + z_t \times w_t$ $bolt_{t+1} \mid bolt_t = 1 \sim z_t + w_t + h_t + z_t \times w_t$
P	Growth*	$G00(z', z, h, w)$ $G01(z', z, h, w)$ $G10(z', z, h, w)$ $G11(z', z, h, w)$	BB MM BB MM MM BB	$z_{t+1} \mid bolt_t = 0 \ \& \ bolt_{t+1} = 0 \sim z_t + w_{t+1} + h_{t+1} + h_{t+1} \times w_{t+1}$ $z_{t+1} \mid bolt_t = 0 \ \& \ bolt_{t+1} = 0 \sim z_t + w_{t+1} + h_{t+1}$ $z_{t+1} \mid bolt_t = 0 \ \& \ bolt_{t+1} = 1 \sim z_t + w_{t+1} + h_{t+1}$ $z_{t+1} \mid bolt_t = 0 \ \& \ bolt_{t+1} = 1 \sim z_t + h_{t+1} + z_t \times h_{t+1}$ $z_{t+1} \mid bolt_t = 1 \ \& \ bolt_{t+1} = 0 \sim w_{t+1}$ $z_{t+1} \mid bolt_t = 1 \ \& \ bolt_{t+1} = 0 \sim z_t$ $z_{t+1} \mid bolt_t = 1 \ \& \ bolt_{t+1} = 1 \sim z_t + w_{t+1} + h_{t+1} + h_{t+1} \times w_{t+1} + h_{t+1} \times z_t$ $z_{t+1} \mid bolt_t = 1 \ \& \ bolt_{t+1} = 1 \sim w_{t+1} + h_{t+1} + h_{t+1} \times w_{t+1}$
F	Probability of reproduction	$Pr(z, h, w)$	BB MM	$pr_{t+1} \mid bolt_{t+1} = 1 \sim z_{t+1}$ $pr_{t+1} \mid bolt_{t+1} = 1 \sim z_{t+1} + w_{t+1} + h_{t+1} + h_{t+1} \times w_{t+1} + w_{t+1} \times z_{t+1}$
	Repro. amount	$R(z, h, w)$	BB MM	$seeds_{t+1} \mid Pr_{t+1} = 1 \sim z_{t+1} + w_{t+1}$ $seeds_{t+1} \mid Pr_{t+1} = 1 \sim z_{t+1} + w_{t+1} + h_{t+1} + h_{t+1} \times z_{t+1} + w_{t+1} \times z_{t+1}$
	Dormancy	$D()$	both	0.032
	Germination**	$g()$	both	-2.76
	Germinant survival**	$s()$	both	0.514
	Recruit size	$C(z')$	BB MM	$N(\mu = 4.17, \sigma = 0.88)$ $N(\mu = 4.40, \sigma = 0.72)$

*We also estimated variance in growth by drawing from a normal distribution around each predicted z' , with $\mu = z'$ and σ = the standard deviation of the residuals of the particular growth function retained in each state-specific model.

**Fixed estimates for germination and germinant survival are coefficients are estimated from logistic regressions, and thus presented on the logit scale.

Integral projection modeling

To assess effects of driver variation on lifetime fitness, we built integral projection models (IPMs) to calculate the growth rate (λ) for each NIL genotype under each of the two model selection scenarios. λ is a measure of the average lifetime fitness among a group of individuals with the same vital rates (Caswell 1989). Most often, this is measured at the population level, although it is also an applicable metric of lifetime fitness of individual organisms (McGraw & Caswell 1996). Here, we apply this integrated measure of lifetime fitness to self-pollinating, inbred genetic lineages to assess the persistence of genotypes under different environmental conditions.

Following Merow et al. (2014) and Ellner & Rees (2006), we incorporated all vital rates (Table 13) into an IPM using two kernels. The survival/growth kernel, P , assesses the number of individuals of size z' predicted to occur at time $t+1$ given the growth and survival rates modeled for individuals of size z present in the population at time t . Because we modeled growth as state-dependent, our survival/growth kernel also accounted for the probability of bolting (b), which causes discrete state transitions (Appendix L). In addition, because we also modeled growth and survival dependent on herbivory (h) and soil moisture (water, w) variation, the general form our survival/growth kernel was as follows:

$$P(z', z, h, w) = S(z, h, w)B(z, h, w, b)G(z', h, w) \quad (\text{Eq. 1})$$

Our fecundity kernel, F , integrated life history transitions regarding recruitment: the probability of bolting (B), the probability reproducing given bolting (Pr), the number of seeds produced given reproduction (R), the germination rate (g), the dormancy rate (D), the germinant survival rate (s), and the size distribution of established recruits (C). The first three transitions were size- and driver-dependent, while the latter four were size- and driver-independent. Thus, the kernel was defined as:

$$F(z', z, h, w) = [B(z, h, w, b) \Pr(z, h, w) R(z, h, w) + \Pr(z, h, w) R(z, h, w) [(1 - g()) D()]] \times g() s() C(z') \quad (\text{Eq. 2})$$

In other words, the net recruitment of new individuals at time $t+1$ is modeled as a function of all seeds produced plus seeds that remain viable in the seed bank (bracketed portion of Eq. 2) multiplied by the probability of germinating and surviving the first year of growth. Following Anderson & Wadgymar (2020), we allow seeds to persist in the seed bank for one year. Germinants that survive and establish are assigned a size using the genotype-specific normal distribution $C(z')$.

Here, we provide general definitions for the P and F kernels, assuming that size-dependent vital rates were also sensitive to both environmental drivers (h and w). However, depending on genotype and vital rate, model selection revealed that some regressions were not sensitive to both drivers. In those cases, we included zeroes as coefficient estimates for those model terms, removing their effects from the life history transition while allowing us to use generalized forms for defining vital rate functions and IPM kernels. Similarly, these general descriptions describe the logic of life history

transitions across all discrete state spaces, but because we used state-specific functions for bolting and growth, the drivers retained differed across particular vital rate regressions. Full details about discrete state transitions and integration with continuous drivers are provided in Appendix L.

We evaluated the kernels over size ranges for z and z' spanning from $0.9\times$ the minimum size (L) through $1.1\times$ the maximum size (U) values observed in the longitudinal dataset. We defined the number (n) of kernel meshpoints as 100, yielding a meshpoint width (d) of approximately 0.6 cm. In other words, while our vital rate regressions were parameterized using continuous data, we evaluated model outputs at discretized intervals for individuals in ~ 0.6 cm size bins.

Similarly, we evaluated each IPM over a biologically relevant range of driver values. We defined a vector of herbivory values (h) ranging from 0 to 100% aboveground tissue removal by herbivores. We defined a vector of soil moisture (water, w) values ranging from 0 through $1.25\times$ the maximum observed soil moisture value across the 3-year experiment. We set the length of each driver vector equal to 20 and populated the vectors with equally spaced values ranging from the minimum to maximum driver value in increments of $1/20^{\text{th}}$ of the maximum value. We parameterized vital rate regressions with un-transformed driver values. However, to assess variation in lifetime fitness across environmental space at a resolution most applicable to observed driver values in the field, we assigned breaks to the herbivory vector on the log scale, since the

distribution of herbivore damage observed is skewed toward small values (Appendix L: Figure L4). At this resolution, we evaluated our IPM kernels in a total of $20 \times 20 = 400$ environmental combinations. Within each environmental combination, we calculated the full IPM kernel K as the sum of P and F , and calculated λ as the dominant eigenvalue of K .

Assessing outcomes

We assessed the effects of driver variation on fitness components by comparing vital rate regressions among the two NIL genotypes. We assessed net selection by both drivers by regressing lifetime fitness (λ) upon herbivory, soil moisture, and their interaction using a linear model.

Further, we assessed variation in lifetime fitness (λ) across environmental combinations to explore ecological scenarios in which polymorphism might be maintained. Specifically, we plotted λ onto driver space, and identified the region(s) in which $\lambda > 1$ for both genotypes, *i.e.*, where both genotypes can persist and increase when rare. This is similar to “niche modeling”, which is typically performed at the species level, but has also been applied to genotypic variants within species (e.g. Birch 1953, D’Aguillo et al. 2019). Finally, we calculated the ratio of $\lambda_{BB}:\lambda_{MM}$ across driver space to approximate the relative abundance of the NIL genotypes under different environmental scenarios.

2.3 Results

2.3.1 Environmental manipulations increase variation in driver space

Our environmental manipulations significantly altered the magnitude of both environmental drivers influencing selection on *B. stricta* chemical profiles. Our rainout shelters reduced soil moisture by 3.1%, a relative reduction of 27% compared to control blocks (main effect of drought treatment during the three manipulated seasons: $F = 80.3481$, $P = 2.626 \times 10^{-15}$, $N = 138$; Figure 18A). Importantly, implementation of rainout shelters did not have off-target effects on soil temperature ($F = 0.1251$, $P = 0.7236$, $N = 1,824$) or light availability ($F = 0.3790$, $P = 0.5392$, $N = 138$) (Appendix J: Figure J3).

Our insecticide treatment also significantly altered herbivore damage, reducing the probability of herbivory by 6.8%, a relative reduction of 9.6% (main effect of herbivory treatment during the three manipulated seasons: $\chi^2 = 13.639$, $P = 0.0002$, $N = 2,474$; Figure 18B) and the amount of herbivory by 0.33%, a relative reduction of 12.6% (percentages transformed from least-squares means of log-transformed model estimates back to data scale; $F = 12.4199$, $P = 0.0004$, $N = 1,729$; Figure 18C).

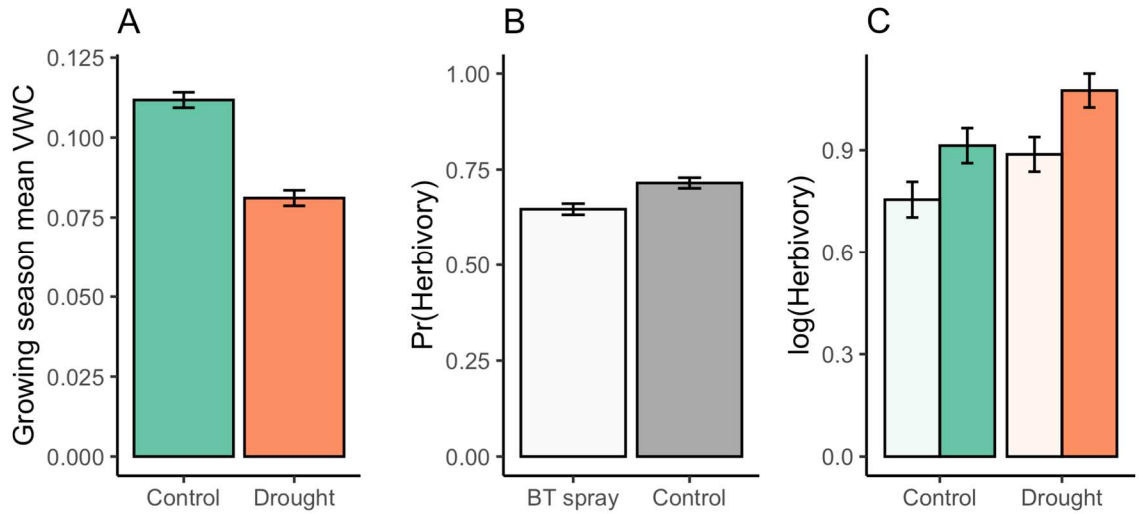


Figure 18. Experimental manipulations significantly altered environmental drivers of selection on glucosinolates.

Analyses across categorical treatments also revealed evidence of driver covariance (Figure 18C); among the three field seasons during which drivers were manipulated, the drought treatment increased the amount of herbivory received by damaged plants ($F = 9.1996$, $P = 0.0025$), but not the probability of damage occurring ($\chi^2 = 2.7072$, $P = 0.0999$).

We also investigated correlations among block mean values of VWC and herbivory across years. These block-level results, spanning both manipulated and unmanipulated environmental variation, corroborate the individual level results. Soil moisture was not correlated with the proportion of plants receiving herbivore damage ($F = 0.3342$, $P = 0.5642$, $N = 137$; Figure 19A), but seasonal mean soil moisture was significantly correlated with the average amount of herbivore damage received, with plants in drier plots receiving more herbivore damage ($r = -0.2375$, $F = 7.8935$, $P = 0.0057$, $N = 134$; Figure 19B).

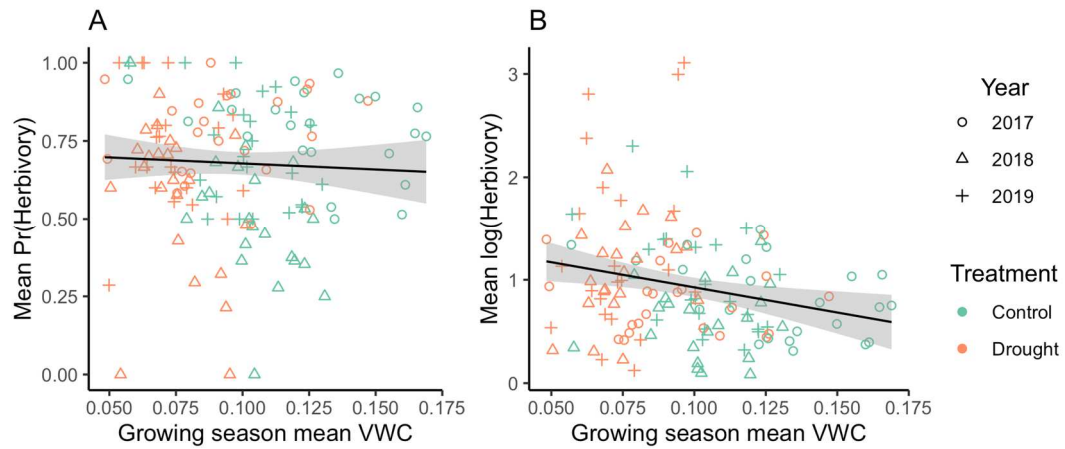


Figure 19. Covariance among selective drivers. Soil moisture was not correlated with the probability of herbivore damage (A), but plants in plots with lower soil moisture received greater amounts of herbivore damage (B).

2.3.2 Genetic variation in sensitivity to environmental drivers

Prior work has posited that two key ecological drivers, herbivory and drought stress, influence selection on glucosinolate variation in *B. stricta*. However, the gold standard for confirming that particular drivers influence fitness is *in situ* manipulation of those drivers, and measurement of fitness effects under ensuing manipulated conditions (Anderson et al. 2014). Our longitudinal, manipulative field study revealed variable and interacting effects of these two drivers on fitness.

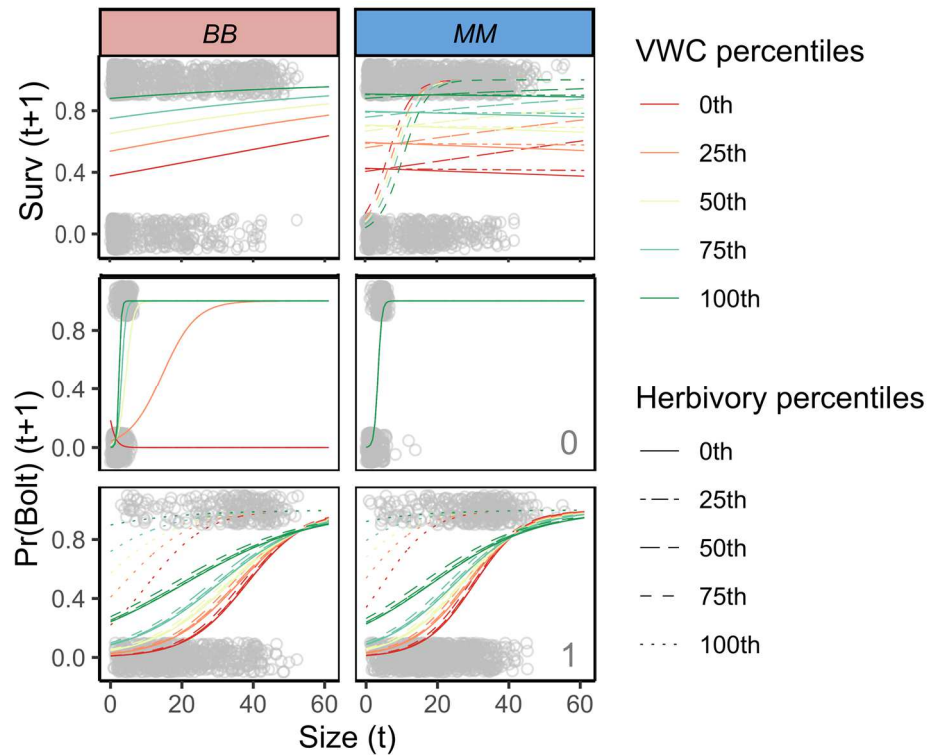


Figure 20. Effects of herbivory and drought on survival (upper) and the probability of bolting (lower). For bolting, the two rows indicate state-specific vital rates; $\text{bolt}_t = 0$ is shown in the center row, and $\text{bolt}_t = 1$ is shown in the bottom row. Columns show genetic variation in vital rates among NILs; *BB* = branched-chain glucosinolate homozygote; *MM* = methionine glucosinolate homozygote.

The survival model (Figure 20, upper) revealed that the *MM* genotype was sensitive to herbivory, while the *BB* genotype was not. Both genotypes generally had reduced survival under low soil moisture, although drought stress and herbivory had interactive effects on survival for *MM*. Counterintuitively, the probability of survival for *MM* was increased at higher levels of herbivory, at most size values, as evidenced by dashed lines (sparser dashes indicating greater herbivore damage) generally having

steeper positive slopes within each set of colored lines (colors indicating VWC quantiles).

Bolting transitions also revealed genetic variation in drier responses. Among individuals which had not bolted previously (Figure 20, middle), the *MM* genotype's probability of bolting was driven solely by rosette size, and was not sensitive to either herbivory or soil moisture. Conversely, the probability of bolting for *BB* was reduced by drought stress. Among individuals that had previously bolted (Figure 20, lower), genetic differences in the probability of bolting were subtler; both genotypes were generally less likely to bolt under increasing drought stress, and, counterintuitively, both generally showed higher probabilities of bolting at higher levels of herbivory. At large sizes, however, the negative effect of drought on bolting was reversed for the *MM* genotype.

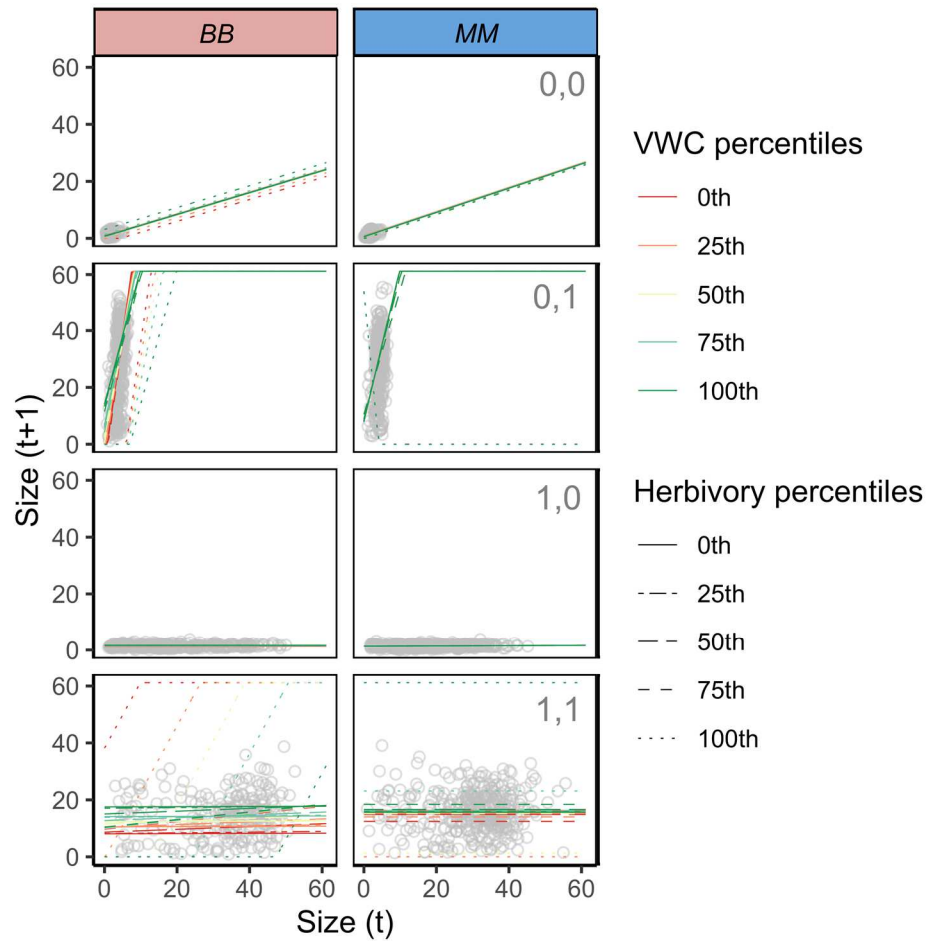


Figure 21. Effects of herbivory and drought on survival and growth. Rows indicate state-specific vital rates; gray numbers within each row indicate discrete bolting state in time t and $t+1$, respectively. Columns show genetic variation in vital rates among NILs; *BB* = branched-chain glucosinolate homozygote; *MM* = methionine glucosinolate homozygote.

Our vital rate regressions also revealed complex patterns in growth. First, growth was strongly influenced by discrete state transitions, including both bolting in the present year and bolting in the previous year (Figure 21, rows). Among rosettes that remain rosettes (bolting history = 0, 0), growth patterns were similar across genotypes, although high levels of herbivory revealed a suppressive effect of drought on growth for

the *BB* genotype (separation of colored lines when dashed), while drought had no effect on *MM*. Among rosettes that bolted (bolt history = 0, 1), *BB* individuals were sensitive to both herbivory and drought, with high levels of either generally reducing growth. Drought x herbivory interactions, however, revealed reversal of negative effects of low soil moisture at high levels of herbivory on *BB* individuals (reversal of color order among dashed vs. solid lines). *MM* rosettes that bolted were not sensitive to drought, but showed reduced growth under higher herbivory, especially at very high levels (negative slope for dotted line). Among individuals that bolted previously but not again (bolt history = 1, 0), growth patterns were similar across genotypes, in that $size_{t+1}$ for individuals in this state was relatively constant. *BB* growth was size-insensitive, but the predicted mean $size_{t+1}$ increased slightly with higher soil moisture; *MM* growth was slightly positively correlated with $size_t$, but insensitive to both drivers. Finally, among individuals that bolted in two consecutive years (bolt history = 1, 1), low soil moisture reduced growth for both genotypes at low to moderate levels of herbivory, although the magnitude of the effect of drought on growth was greater for *BB* than *MM* individuals. High levels of herbivory also intensified the effects of drought on growth (separation among colors among dotted vs. dashed and solid lines), but in opposite directions; high soil moisture at high herbivory levels increased growth for *MM* individuals, but suppressed growth for *BB* individuals. Growth for *MM* individuals in this state was size-insensitive.

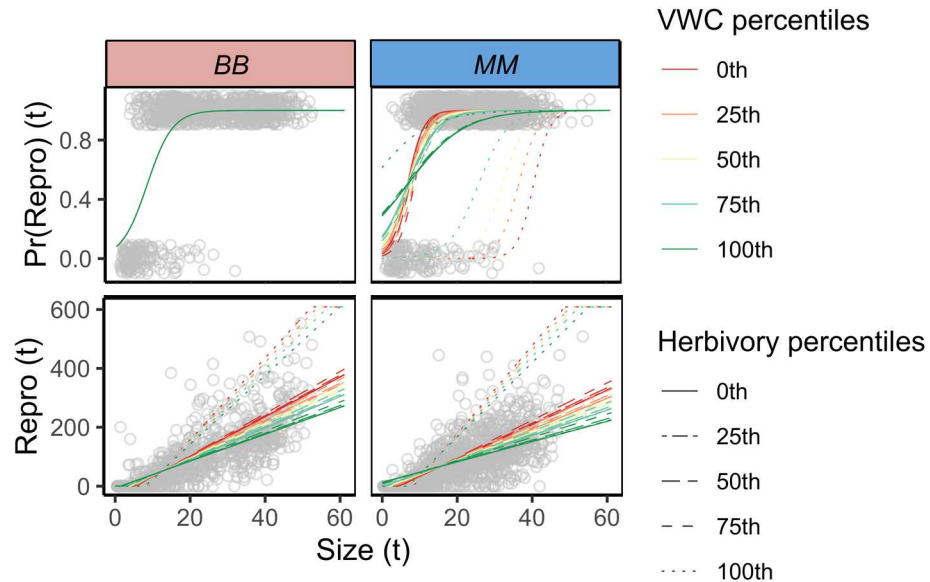


Figure 22. Effects of herbivory and drought on reproduction. Upper row shows probability of reproducing, conditional upon bolting. Lower row shows reproductive output (estimated number of seeds), conditional upon reproducing. Columns show genetic variation in vital rates among NILs; *BB* = branched-chain glucosinolate homozygote; *MM* = methionine glucosinolate homozygote.

Finally, we assessed genetic variation in the effects of drought and herbivory on reproduction (Figure 22). The probability of reproduction among *BB* individuals did not vary depending on either herbivory or drought stress. Conversely, the probability of reproduction among *MM* was influenced by both drivers, as well as their interactions. At small sizes, drought reduces the probability of reproduction, but that effect is reversed at larger sizes (reversal of colored lines across sizes). High levels of herbivory on large individuals, however, reversed that effect again, with probability of

reproduction reduced under drought when herbivory is very high (reversal of colors in dotted vs. dashed and solid lines).

2.3.3 Net effects of environmental variation on fitness

Our vital rate regressions revealed considerable variation in the effects of environmental drivers on fitness components, both across genotypes and across components within each genotype. We also explored the net effect of driver variation on lifetime fitness by regressing predicted λ values from all combinations of environmental parameter space upon both drivers in a multiple regression. This model revealed that both soil moisture and herbivory drive variation in λ for both genotypes (Table 14), indicating that variation in these ecological factors do influence selection on glucosinolate profiles.

Table 14. Effects of driver variation on λ across driver space.

Genotype	Model effect	Coefficient (SE)	Sum Sq.	DF	F	P	
<i>BB</i>	VWC	9.27 (0.14)	87.042	1	4455.9	<0.0001	***
	Herbivory	0.007 (0.0005)	4.476	1	229.1	<0.0001	***
	VWC $\times$ herbivory	-0.101 (0.004)	12.905	1	660.6	<0.0001	***
<i>MM</i>	VWC	6.93 (0.13)	48.584	1	3023.8	<0.0001	***
	Herbivory	0.004 (0.0004)	1.540	1	95.8	<0.0001	***
	VWC $\times$ herbivory	-0.084 (0.004)	8.839	1	550.1	<0.0001	***

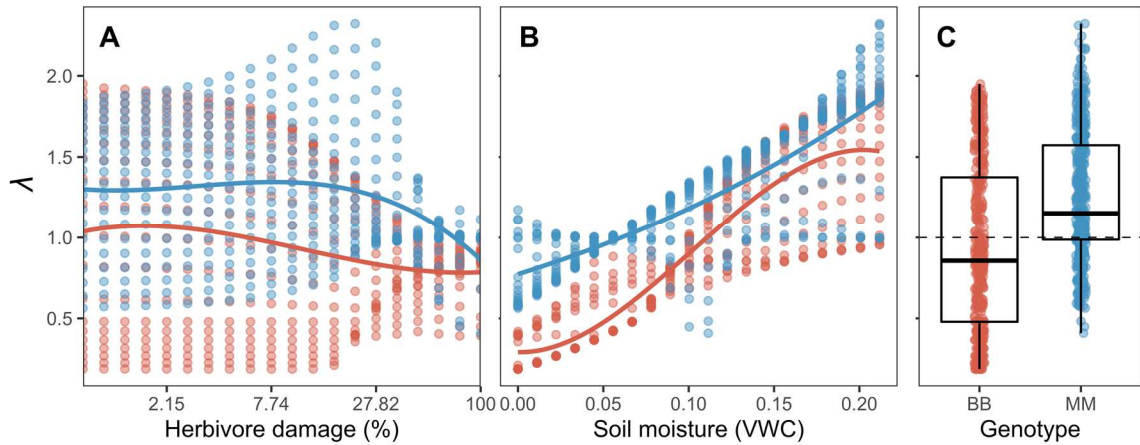


Figure 23. Net effects of herbivory (A), soil moisture (B), and genotype (C) on lifetime fitness. In A, note that breaks along the x-axis are shown on a log scale. Curves overlaid in A and B are cubic splines. In C, dashed line indicates $\lambda = 1$.

On average, the *MM* genotype had higher lifetime fitness across the range of modeled environments, but both genotypes had a lifetime fitness of $\lambda \geq 1$ in a number of environmental combinations (Figure 23C). Unsurprisingly, increased soil moisture yielded higher fitness in both genotypes, although the effect was more strongly positive for *BB* than for *MM* (Table 14; Figure 23B). Counterintuitively, the main effect of herbivore damage on lifetime fitness was also positive, although the magnitude of the effect was very small (Table 14). However, a significant negative interaction between the two drivers indicates that ultimately, the weakly positive effect of herbivore damage on fitness became increasingly negative as soil moisture increased (Table 14). Ultimately, interactions among herbivory and soil moisture result in complex fitness landscapes for both genotypes (Figure 24).

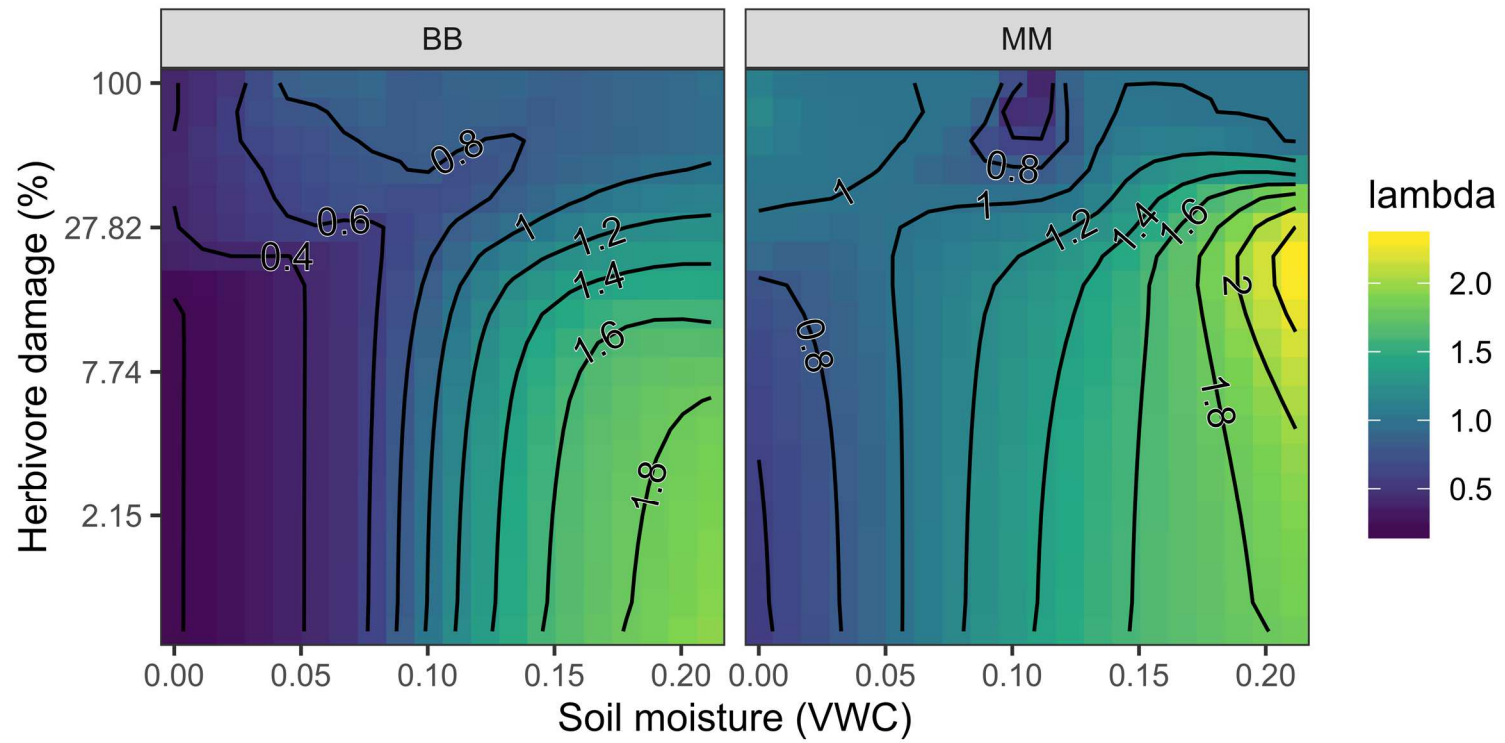


Figure 24. Figure 25. Variation in lifetime fitness across driver space. Heatmaps show genotype-specific predicted λ values (brighter = higher lifetime fitness) across bivariate environmental variation in soil moisture and herbivore damage. Note that breaks on y-axis are shown on a log scale.

2.3.4 Maintenance of polymorphism

We further assessed variation in λ across driver space to consider under which environmental scenarios, if any, we may expect to see both genotypes persist. We explored this in two ways. First, we visualized genotype-specific λ estimates across all combinations of environmental parameter space, and identified the regions of environmental parameter space in which $\lambda \geq 1$ (Figure 25). We found that the *MM* genotype was predicted to exceed replacement in a greater range of environmental space than was *BB* (67% vs. 39% of 400 modeled environmental combinations, respectively). However, because the more restricted range of environmental conditions supporting *BB* was nested completely within the permissible conditions for *MM*, we still observed a sizable amount of environmental combinations (39%) that could potentially support polymorphic populations.

In general, higher soil moisture environments seem most likely to promote maintenance of polymorphism, although high levels of herbivory even in wet environments could drive populations to *MM* monomorphism, or to decline to local extinction. The ranges of natural (unmanipulated) variation in soil moisture and herbivory across all four years of the experiment fell within this potentially polymorphic environmental space (Figure 25, white crosses), suggesting that the environmental conditions promoting polymorphism are biologically reasonable. However, observed driver values in drier years (2018, 2019) were close to the $\lambda \geq 1$ boundary for *BB*.

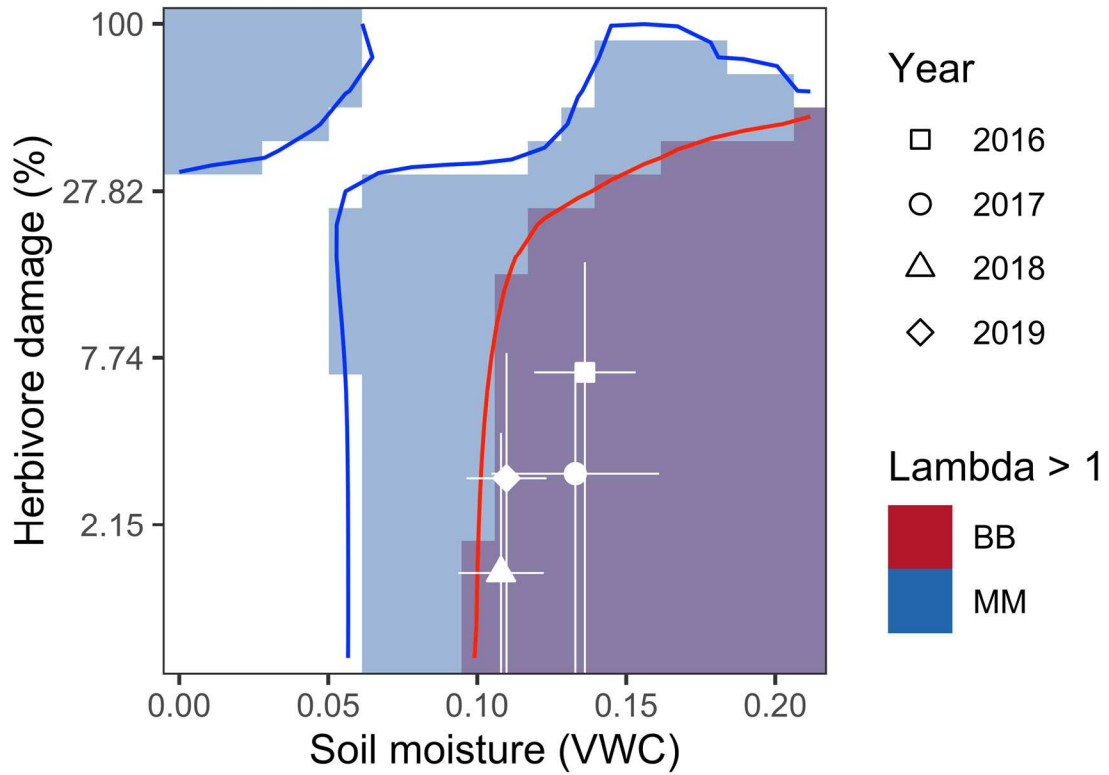


Figure 26. Environments which may maintain polymorphisms. Fitness landscape heatmaps and contours are simplified to show overlaid regions in which $\lambda \geq 1$ for both genotypes. White crosses show mean and standard deviation of driver values in unmanipulated plants or blocks during each year of the experiment. Note that breaks on the y-axis are shown on a log scale.

Second, to assess the relative fitness of the two genotypes under different environmental combinations, we also visualized the ratio of $\lambda_{MM} : \lambda_{BB}$ across driver space (Figure 26). We found that, while this ratio was often expected to exceed 1 due to higher average lifetime fitness of the *MM* genotype (Figure 24C), there were several regions of environmental parameter space in which both genotypes are predicted to persist with similar lifetime fitness. The largest region in which $\lambda_{MM} : \lambda_{BB}$ was close to or equal to 1

was that spanning high soil moisture and low to moderate herbivory (Figure 26), nested within the polymorphic space. We also found smaller regions of equivalent or similar lifetime fitness at high soil moisture and high herbivory, and at moderate soil moisture and high herbivory. However, these environmental combinations fell within regions where $\lambda < 1$ for both genotypes, and were farther from observed combinations during the span of the experiment (Figure 25).

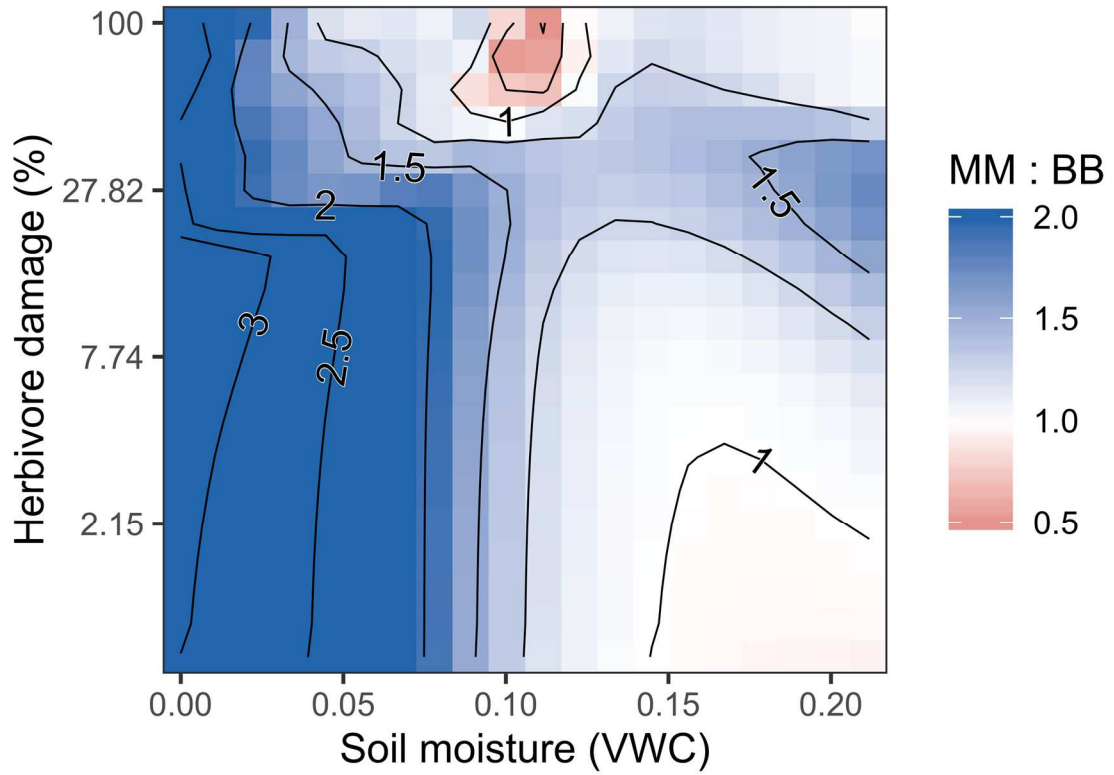


Figure 27. Ratio of $\lambda_{MM} : \lambda_{BB}$ across environmental space.

2.4 Discussion

2.4.1 Demographic approaches to understanding selection and variation

While it is generally acknowledged that the effects of a single locus can have antagonistic effects on fitness across different environments (Mitchell-Olds et al. 2007), varying effects on suites of fitness components expressed sequentially throughout the life cycle are often more difficult to assess. Studies that have investigated such pleiotropic life-history tradeoffs have found that failure to account for selection acting on genotypes and/or phenotypes across multiple life stages can yield erroneous conclusions (Schluter et al. 1991), for example by inferring directional rather than stabilizing selection (Mojica & Kelly 2010). Alternative approaches that take into account the contingency of subsequent fitness components across life-history have been developed (Geyer et al. 2007, Shaw et al. 2008), but are not well equipped to consider continuous in addition to probabilistic state transitions (*e.g.* growth/shrinkage/stasis). Demographic models offer a robust approach for synthesizing variation in the expression of phenotypic traits and life-history transitions into an integrated metric of lifetime fitness (λ), and integral projection models specifically offer flexibility when accounting for continuous, size-dependent transitions (Merow et al. 2014).

Here, we parameterized an IPM with two continuous environmental drivers known to affect performance and fitness components in *B. stricta*: herbivory and drought. Past work has documented “snapshot” effects of variation in both of these

drivers on individual fitness components (*e.g.* survival, reproduction) and phenotypes that underlie them (*e.g.* herbivore defense and drought response). We expand upon prior studies and synthesize the long-term effects of driver variation on the lifetime fitness of two NIL genotypes varying in secondary metabolic profile. Thus, we provide a means to simultaneously assess the effects of fitness tradeoffs across environments, as well as within environments across life history, on the maintenance of contrasting glucosinolate genotypes and phenotypes in populations.

Our results reveal interesting patterns that illuminate the differences between approaches that emphasize fitness components vs. integrated lifetime measurements. Our vital rate regressions show substantial variation in genotypic responses to driver variation, particularly for survival, the probability of bolting among rosettes, juvenile growth, and the probability of reproduction (Figures 20-22). In general, these patterns are often consistent with a previously characterized herbivore defense / drought response tradeoff; for example, the *MM* genotype, which appears to be more water use conservative under drought (Carley et al., *in prep*), is insensitive to drought in many vital rates (probability of bolting, juvenile growth), or in some cases is stimulated by drought when the *BB* genotype is not (probability of reproduction). However, these vital rate regressions also reveal complex interactions among herbivory and drought that are more difficult to detect in snapshot studies. For example, high herbivory often alters the direction of the effects of drought on fitness components; sometimes this

counterintuitively predicts highest fitness when herbivory is very high (*e.g.* survival for the *MM* genotype; adult probability of bolting for both genotypes). Conversely, for other vital rates, high herbivory exacerbates the negative effects of drought on fitness (*e.g.* probability of reproduction for the *MM* genotype).

This complex genetic variation in the sensitivity of vital rates to herbivory and drought demonstrates the importance of assessing integrated lifetime fitness when considering selection on genotypes and phenotypes of perennial organisms. Ultimately, complex and sometimes opposing effects of both drivers on individual vital rates yields variable fitness landscape when integrated into net effects on λ (Figure 24). At the lifetime fitness scale, we still see evidence that *MM* may indeed be more drought-tolerant, as it experiences weaker net selection by soil moisture (Table 14, Figure 23B) and is predicted to persist above replacement in environmental combinations spanning much lower values of soil moisture (Figure 25). However, the possibility of a straightforward drought tolerance / herbivore defense tradeoff is also complicated somewhat when considered across the full life span. For example, the environmental conditions supporting *MM* exceeds those of *BB* on the herbivory axis, as well (Figure 25), although there are localized areas of moderate soil moisture and high herbivory in which the lifetime fitness of *BB* is highest (Figure 24).

Despite this, our models predict that both genotypes are able to maintain sufficiently high fitness to persist under a substantial portion (39%) of the biologically

relevant driver combinations that we assessed. Thus, genetic differences expressed at the fitness component level do not necessarily preclude the possibility of contrasting genotypes and phenotypes persisting within populations (Figure 25), potentially at reasonably common frequencies (Figure 26).

2.4.2 Limitations and assumptions

Ultimately, to understand the fate of genetic polymorphisms within interbreeding populations, factors such as (random or non-random) mating, the scale and prevalence of migration and dispersal, and the rates of mutation and genetic drift must be considered in addition to fitness effects of alleles in different environments (Hedrick 1986). Furthermore, dominance patterns of alleles and subsequent fitness of heterozygotes also influence the maintenance of polymorphism (Hedrick 1999).

Unfortunately, this study was unable to explicitly characterize any of these factors; further research assessing population genetic parameters in the context of selection on glucosinolate variation and underlying genes is encouraged. However, demographic approaches also offer distinct benefits over standard population genetics models, including straightforward interpretation of integrated fitness effects in perennial organisms with overlapping generations. In addition, leveraging regression-based approaches facilitates inference about the effects of continuous environmental drivers on

fitness, rather than net fitness differences across discrete environments (Merow et al. 2014).

While λ offers a general, integrated measurement of lifetime fitness, its direct demographic interpretation is the population, or here genotypic, growth rate. In some cases, the persistence of genetic lineages forward through time may not be biologically relevant or interesting without consideration of other factors such as mating, dominance of alleles, and selection on heterozygotes. However, our study organism, *B. stricta*, is highly self-pollinating and rarely heterozygous (F_{IS} : 0.74 – 0.97; Song et al. 2006). Thus, characterizing λ is useful both because of its ability to integrate variable and sometimes contrasting effects of selection across life-history stages, and because it is a reasonable biological reflection of the persistence of inbred genetic lineages under different environmental conditions.

Finally, while we argue that modeling λ is useful given both our research questions and the biology of our study organism, our demographic approach also has limitations. Particularly, we provide density-independent estimates of λ that also do not account for conspecific or heterospecific competition or facilitation. Given the experimental design in our common garden, with genotypes planted in randomized, complete-block designs at constant density, this is not unreasonable. However, we acknowledge that density-dependent effects and neighbor interactions are likely to contribute to more complicated selection and population dynamics in the wild.

2.4.3 Genetic variation, environmental change, and driver covariance

The persistence of genetic variation for adaptive traits is of considerable interest both theoretically and practically. Particularly, whether the amount of standing genetic variation in populations is sufficient to permit adaptation to future environmental changes has become an increasing concern, especially in the light of rapid anthropogenic environmental change (Etterson & Shaw 2001). Variation in traits underlying ecological interactions such as herbivore defense can also have bottom-up effects on ecological communities (van Loon et al. 1992, Poelman et al. 2008, Sun et al. 2009, Johnson et al. 2009, Reznick 2013), making understanding patterns of selection and response to selection equally important from ecological and evolutionary perspectives (Thompson 1998).

Here, we show that despite variable and often contrasting effects of drought and herbivory across individual fitness components, both glucosinolate strategies are predicted to persist at or above replacement ($\lambda \geq 1$) in many of the environmental combinations that we assessed. This suggests that, in the case of *BCMA*, the environmental conditions required to maintain polymorphic glucosinolate phenotypes and genotypes may not be uncommon. However, the natural conditions observed in our garden fell close to boundary of predicted polymorphic environment space, suggesting that increasing drought across years, and/or increasing variance in soil moisture across

microhabitats within years, may push some populations into monomorphic environment space (Figure 25).

Our manipulative field study also revealed non-independence of driver variation and their effects. Experimental manipulation of drought stress also increased herbivory (Figure 19B), and these drivers often had negative interactions on fitness components (Figures 20-22) and λ (Table 14). Correlations among climate variables and herbivore pressure are frequently documented, especially across broad latitudinal (Salazar & Marquis 2012) and altitudinal gradients (Rasman et al. 2014). Our results, reflecting manipulated abiotic variation at the scale of meters within a single garden, also demonstrate the capacity of abiotic and biotic drivers of selection to covary significantly at the microgeographic scale. Thus, while soil moisture ultimately had greater effects on fitness than herbivory (Figure 23), changes in drought stress over time may also elicit concomitant shifts in herbivore feeding (*e.g.* via changes in abundance, composition, and/or metabolism of arthropods; Chown et al. 1999 Agrawal et al. 2006, Iason et al. 2011) and/or its effects on plant fitness (*e.g.* via direct effects of drought on the expression of resistance; Nguyen et al. 2016, Pezzolla et al. 2017). If changes in soil moisture do indeed induce concordant changes in herbivore pressure, increasing drought may reduce the likelihood of polymorphism being maintained, at least in environments that are similar to our common garden. This is consistent with previous research showing that abiotic environmental change has the capacity to drive selection

via both direct and indirect ecological effects, as has been posited elsewhere (Louthan et al. 2017).

2.5 Conclusions

Many studies of natural variation and adaptation fail to identify the particular traits conferring fitness benefits, and those that do often fail to concretely identify environmental drivers of selection acting upon them. Here, we leveraged a genetically tractable system under manipulated field conditions to demonstrate that glucosinolate profile does indeed experience selection caused by herbivory and drought, as evidenced by significant driver effects on fitness components (vital rate regressions), and variation in the magnitude and direction of those effects across genotypes. We also estimate the integrated lifetime effects of selection by herbivory and drought, revealing that, despite variation in selection across genotypes, contrasting glucosinolate strategies should both be capable of persisting under a reasonable range of environmental parameter space. While there are some limitations to the inferences that can be drawn from demographic models without consideration of other population genetic processes, we provide a transferrable framework for assessing effects of environmental variation on the maintenance of genetic and phenotypic diversity within and across populations.

Conclusions

The two data chapters presented offer contrasting and complementary perspectives on the question of natural variation. Together, they provide evidence suggesting that our focal trait, glucosinolate variation in *Boechera stricta*, (1) has elevated variation due to past balancing selection, and (2) may persist polymorphically under a range of environmental conditions.

Generally, we demonstrate that leveraging genetically and experimentally tractable tools into natural habitats can help to elucidate mechanistic links between genotype, environment, and fitness, in a context that is relevant to selection on natural populations. Further, we emphasize that a synthesis of tools and perspectives from across ecological and evolutionary subfields can yield complementary insights into the causes and consequences of natural variation.

Appendix A. Genetic tools

Generation of CFR-NILs

Crossing design

We generated near-isogenic lines isolating genetic variation as narrowly as possible around *BCMA1/3* as determined by naturally occurring closest flanking recombination breakpoints (CFR-NILs). These CFR-NILs (simply referred to in this document as NILs) were generated by crossing the SAD12 inbred genotype (maternal parent) with the LTM inbred genotype (paternal parent) and repeatedly selfing offspring. Using genome-wide markers (Schranz et al. 2009), we identified a single F4 individual heterozygous near *BCMA3* and largely homozygous elsewhere. We selfed this F4 *BCMA* heterozygote and screened offspring in the F5 generation for recombinants using PCR markers.

Screening F5 offspring

Specifically, we grew 5,213 F5 plants in the greenhouse (substrate: 75% Fafard 4P and 25% MetroMix 200; 24° C daytime temperature; 16 h day length) at Duke University in Durham, NC, USA for 10 weeks. We then collected leaves from each plant (~0.1 – 0.2 g fresh weight per sample), flash froze them in liquid N₂, and stored samples at -80° C until sample processing.

DNA extraction

We isolated genomic DNA from frozen leaves as described in Dellaporta (1983), using 96-well plates for high-throughput processing. We ground frozen leaves in tubes containing 3 or 4 stainless steel ball bearings by shaking in a GenoGrinder 2000 (Spex Centriprep, NJ, USA) for 2 min at 1,000 strokes per minute. We flash-froze ground tissue again in liquid N₂ and then centrifuged plates briefly to collect ground material at the bottom of each tube. We extracted DNA by adding 100 µL of extraction buffer (100 mM Tris pH 8.0, 50 mM EDTA pH 8.0, 500 mM NaCl, 10 mM β-mercaptoethanol, 2.0% sodium dodecyl sulphate) to ground tissue and incubating in a 65° C water bath with gentle mixing for 20 min. We added 30 µL of potassium acetate (5M) to lysed tissue and incubated mixtures on ice for 15 min. We clarified precipitated solutions by centrifugation at 3,500 rpm for 10 min at air temperature. We then transferred 85 µL of supernatant to new tubes containing 100 µL of isopropanol, mixed tubes gently, and incubated on ice for 20 min. We pelleted precipitated DNA by centrifuging at 4,000 rpm for 10 min, washed pellets twice with 100 µL of chilled 70% ethanol, and then air dried them for 30 min. We resuspended pellets in 50 µL of Tris-HCl buffer (pH 8.0) containing RNase A (~100x dilution of 100 mg/mL) by incubating tubes at 65° C for 10 min with gentle agitation. We cooled resuspended DNA to room temperature, and used this for PCR screening.

PCR screening.

We performed initial screens for recombinants in the genomic regions surrounding *BCMA* (Schranz et al. 2009) using primer pairs specific to three markers close to *BCMA3*: *BstES0012* (*At5g33340*) and *At4g14310* and *At4g15960* (Table A1). Specifically, we used 2 µL of DNA from each F5 offspring as PCR template, and amplified markers using 1 unit of Taq polymerase (Bioline, MA, USA), 2 pmol of each primer, and 5 nmol of dNTPs, along with 1x buffer supplemented with MgCl₂ in a final reaction volume of 25 µL. We performed PCR as follows: a hot start of 2 min at 94° C; 30 amplification cycles including a 30 s anneal at 55° C and 1 min elongation at 72° C; and a final extension of 10 min at 72° C. We scored each offspring for amplified markers in two ways. For *At4g14310* amplicons, we directly scored amplicon size polymorphism directly by running PCR products on a 2% agarose gel. For *BstES0012* (*At5g33340*) and *Atg15960* amplicons, we performed restriction digests (using 8 µL of PCR product with *BclI* and *BglII* enzymes, respectively, according to the manufacturer's instructions) and separated digested amplicon fragments on a 1.5% agarose gel (BioRad, CA, USA).

Table A1. Primer sequences used in screening F5 offspring for recombination in the *BCMA* region.

Target gene	Direction	Sequence
<i>At4g14310</i>	Forward	5'-GCTCTTGCTCCTCTACCCAATA-3'
	Reverse	5'-GGTGCGGGTGAGAGTTWGAG-3'
<i>BstES0012</i> (<i>At5g33340</i>)	Forward	5'-GTCAGAGTTGTGCCCCGAAT-3'
	Reverse	5'-CCTCAAATGGCGGTGAATA -3'

<i>At4g15960</i>	Forward	5'-CTCAACGTCGGAACAGGAAC -3'
	Reverse	5'-GTTCTCACAGCCCCAAACAC -3'

Selection of CFR genotypes.

Using the PCR-based method described above, we identified 209 F5 individuals that were recombinant in the *BCMA3* region. We selfed these plants, and scored the F6 progeny using 13 tightly-linked polymorphic markers to identify homozygotes with recombination near *BCMA3* (Prasad et al. 2012). We chose two F6s showing recombinants close to *BCMA3* (subsequently verified by genotyping-by-sequencing, see section 1.2.6), which we refer to as closest flanking recombinants (CFRs).

Resulting CFR-NIL genotypes

We crossed the two CFR genotypes to generate an F1 progeny (“KAS-38”), and selfed that to generate F2s. We identified multiple independent homozygous *BB* and *MM* F2 genotypes, which we call “family lines”, using PCR marker BSTES0008 (Prasad et al. 2012) and HPLC to determine glucosinolate profiles. By retaining multiple independent family lines with homozygous *BB* and *MM* genotypes, we control for possible confounding effects of variable loci elsewhere in the genome. We bulked seeds from each family line via selfing, and used F3 or later selfed progenies in all experiments.

Assessing genetic variation in the BCMA region

We used genotyping-by-sequencing to characterize additional genetic variation outside of focal genes in the *BCMA* region. Full methods are described in 1.2.6.

We performed GBS on a total of 67 individuals representing 23 independent family lines of the two *BCMA* NIL genotypes (Table A2).

Table A2. *BCMA* NIL genotypes used for genotyping-by-sequencing.

Genotype (Parental allele; BC-ratio)	Family #	Generation	N: GBS	N: HPLC
BB (LTM; high)	9	F5	0	10
	10	F5	3	10
	13	F4	1	6
		F5	2	4
	14	F5	3	10
		F6	3	10
	15	F5	3	10
	19	F6	2	4
	24	F6	0	10
	35	F5	1	4
		F6	1	4
	36	F6	3	10
	48	F4	2	6
		F5	1	4
	62	“LATEST” (F6)	1	6
		F5	2	4
MM (SAD; low)	8	F5	3	10
	20	F5	2	4
	21	F5	3	10
	23	F6	3	10
	25	F5	3	8
	27	F3	1	2
	31	F6	3	10
	38	F6	3	10
	39	F6	3	10
	41	F6	3	10
	49	F5	3	10
	56	F5	3	10
TOTALS:			67	216

We identified unique samples during multiplexed genotyping-by-sequencing using 34 barcoded adapters (Table A3) in unique combination with two indexed primers (Table A4).

Table A3. Sequences of barcoded adapters used in multiplexed GSB sequencing.

Adapter	Barcode	Adapter	Barcode
B2Y	CTGATT	B29Y	GATCAA
B4Y	CGCTGT	B31Y	GTGGAA
B5Y	GTCTCT	B33Y	GGGTAA
B6Y	ATCTCC	B34Y	CGTCAA
B8Y	TATAGT	B35Y	GTTGCA
B9Y	CAGCAT	B36Y	TCCATA
B10Y	TAATCC	B38Y	ACTCGA
B12Y	CCTGAA	B39Y	GTTCGA
B14Y	AACTTA	B40Y	ATATAC
B15Y	G TTCCT	B41Y	TTAGTA
B16Y	CTAATA	B42Y	TCGCTT
B17Y	CCGAAT	B43Y	TCGTCT
B18Y	CTTGTA	B44Y	CAATAT
B19Y	CCCTCG	B45Y	TCATGG
B20Y	ACTGAC	B46Y	CAGGAA
B24Y	GTGAGA	B47Y	ATCGAC
B27Y	CTCTCA	B48Y	TTGCGA

Table A4. Indexed primers used in multiplexed GBS sequencing.

Index	Sequence
FC1_IND1	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTC*T
FC1_IND2	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGCTC*T

Appendix B. Allelic variation in *BCMA3*

Table B1. Prevalence of different *BCMA3* structural variant combinations in a subset of genotypes characterized with Sanger sequencing. Note that the 8 bp deletion is only observed in accessions that also possess the premature stop codon in *BCMA3*. Genetic groups reflect assignments in Wang et al. (2019).

Genetic Group	Total N	Structural variant			
		Complete exons		Premature stop	
		+ no deletion	+ 8 bp deletion	+ no deletion	+ 8 bp deletion
COL	36	16	0	4	16
NOR	17	17	0	0	0
UTA	18	14	0	1	3
WES	21	21	0	0	0
Admixed	0	NA	NA	NA	NA

Appendix C. Details on field and lab herbivory experiments.



Figure C1. Implementation of temporary field arrays. We deployed 10 racks containing 98 juvenile plants into each of six field sites in Colorado during the 2016 growing season. While individuals remained contained in plastic cones during this experiment, the racks were sunk flush to the soil surface (A) to facilitate integration into the surrounding environment and minimize obstacles preventing herbivore access to plants. We excluded megaherbivores using wire cages (B), but insects and small mammals could browse freely.



Figure C2. Evidence of drought-induced mortality in temporary arrays. Signs of drought stress, such as tissue browning and death, were observed from low (A) to high (B) frequencies across individual arrays, depending on local environment.

Within each array, individuals were randomly assigned to positions, distributing edge effects equally across genotypes.

Table C1. Sample size in field transplant experiments by genotype and family.

Genotype (Parental allele; BC-ratio)	Family #	N per environment								
		ALD- 2013	ALD- 2014	MAH- 2014	SIL- 2014	ALD- 2015	MAH- 2015	SIL- 2015	GTH- 2016	SCH- 2016
<i>BB</i> (LTM; high)	10	80	40	60	50	45	30	35	0	0
	13	80	40	60	50	45	30	35	150	150
	14	80	40	60	50	45	30	35	0	0
	15	80	40	60	50	45	30	35	150	150
	19	0	0	0	0	45	30	35	150	150
	35	0	0	0	0	45	30	35	150	150
	36	0	0	0	0	45	30	35	0	0
	48	0	0	0	0	45	30	35	0	0
	91	0	0	0	0	45	30	35	0	0
<i>MM</i> (SAD; low)	8	80	40	60	50	45	30	35	150	150
	20	80	40	60	50	45	30	35	150	150
	21	80	40	60	50	45	30	35	150	150
	23	0	0	0	0	45	30	35	150	150
	25	80	40	60	50	45	30	35	150	150
	27	80	40	60	50	45	30	35	0	0
	38	0	0	0	0	45	30	35	0	0
	39	0	0	0	0	45	30	35	0	0
	41	0	0	0	0	45	30	35	0	0
	49	0	0	0	0	45	30	35	0	0
TOTALS:		720	360	540	450	855	570	665	1,350	1,350

Table C2. Sample size in field array experiment by genotype and family.

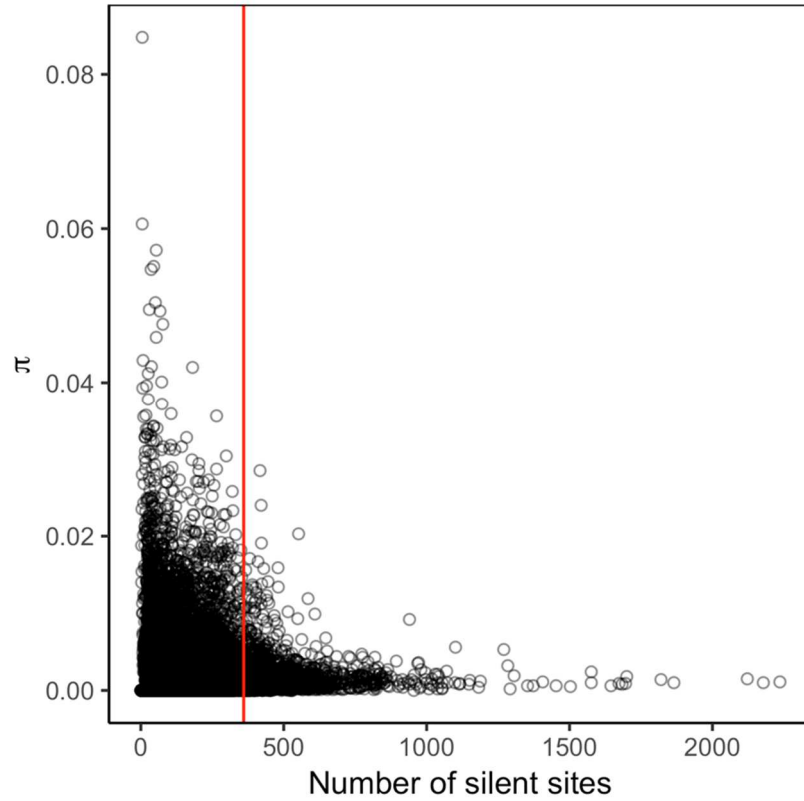
Genotype (Parental allele; BC-ratio)	Family #	N: Field arrays
<i>BB</i>	19	588
(LTM; high)	35	588
	36	588
	62	588
	91	588
<i>MM</i>	31	588
(SAD; low)	38	588

39	588
41	588
56	588
TOTAL:	5,880

Table C3. Sample size in lab herbivory assay by genotype and family.

Genotype (Parental allele; BC-ratio)	Family #	N: <i>T. ni</i> insect resistance
<i>BB</i> (LTM; high)	19	25
	35	25
	36	25
	62	25
<i>MM</i> (SAD; low)	31	25
	38	25
	39	25
	41	25
	56	25
TOTAL:		225

Appendix D. Details on genomic filtering.



Supplemental Figure D1. Nucleotide diversity is negatively correlated with gene size. Each point represents one gene in the *B. stricta* genome for which π data was calculable and greater than 0 among a subset of 55 genotypes (see Methods).

Levels of nucleotide diversity (π) are highest for genes with few silent sites. To account for this, we compared π_{BCMA} to π of genes across the genome with a similar number of silent sites. *BCMA* contains 364 silent sites, so our threshold was 360 (indicated by red line).

Appendix E. Details on drought experiments.

Table E1. Replication of genotypes and families across greenhouse racks during the dry-down experiment (implemented in a completely randomized design).

Rack #	<i>BB</i>						<i>MM</i>					
	replicates per family					Total	replicates per family					Total
	19	35	36	62	91		31	38	39	41	56	
1	1	3	1	5	4	14	5	5	3	1	4	18
2	2	2	2	5	5	16	6	3	4	1	2	16
3	3	2	6	3	4	18	3	1	2	2	6	14
4	2	4	4	2	8	20	3	4	2	1	2	12
5	3	2	5	8	0	18	2	2	3	4	3	14
6	4	4	3	3	3	17	3	2	3	7	0	15
7	5	3	2	2	1	13	5	3	6	2	3	19
8	8	0	4	2	3	17	4	2	4	2	3	15
9	3	3	4	0	3	13	6	2	2	1	8	19
10	6	4	3	3	2	18	3	1	4	3	3	14
11	2	5	4	5	3	19	1	4	3	2	3	13
12	3	4	4	1	3	15	4	4	2	4	3	17
13	4	2	2	3	5	16	0	5	3	3	5	16
14	4	5	1	3	4	17	1	4	6	1	3	15
15	3	2	2	2	3	12	5	4	3	5	3	20
16	6	5	4	3	3	21	1	2	1	5	2	11
17	5	4	2	2	5	18	3	3	5	2	1	14
18	1	1	5	2	0	9	7	1	7	5	3	23
19	4	3	1	2	3	13	4	3	2	5	5	19
20	2	4	3	4	2	15	6	2	4	3	2	17
21	3	6	3	1	5	18	3	3	5	1	2	14
22	2	5	3	3	2	15	3	5	1	5	3	17
23	2	1	3	3	3	12	4	3	5	4	4	20
24	6	1	3	5	3	18	2	2	3	7	0	14
25	4	3	6	6	4	23	1	1	3	3	1	9
26	3	1	2	6	3	15	1	5	4	4	3	17
27	0	7	4	1	4	16	2	5	3	3	3	16
28	3	3	5	3	3	17	3	5	0	4	3	15
29	0	4	3	4	2	13	1	6	0	5	7	19
30	3	4	4	3	4	18	2	4	3	0	5	14
31	2	3	2	3	2	12	6	4	3	4	3	20
32	1	0	0	2	1	4	0	0	1	1	2	4
TOTALS:	100	100	100	100	100	500	100	100	100	100	100	500

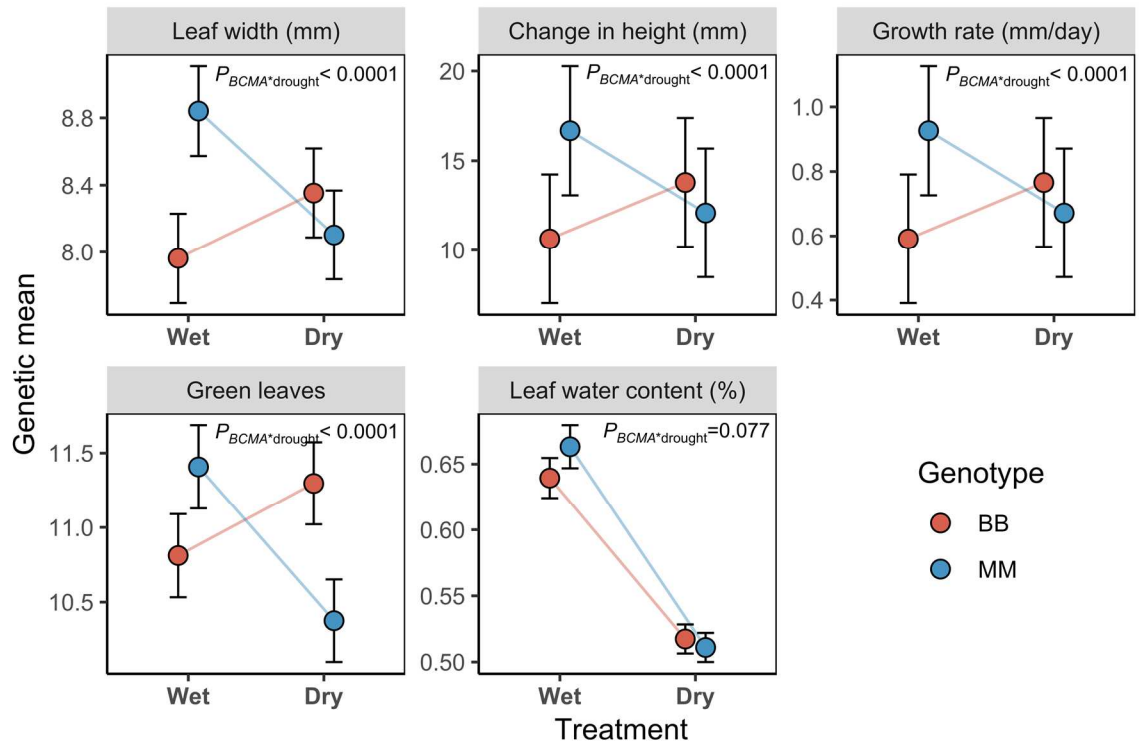


Figure E1. Genetic variation in norms of reaction to drought stress. *BCMA* alleles in the NIL background confer contrasting response to morphological traits such as leaf size and number as well as physiological traits such as growth. Both genotypes reduce leaf water content under drought, but genetic differences in this response were only marginally significant.

Table E2. Pairwise genetic differences of *BCMA* NILs in morphology and physiology under the controlled greenhouse drought experiment. Least squared mean and standard error values for each trait were estimated from statistical models explained in 1.2.5. Within each row, means not connected by the same letter are significantly different as determined using a Tukey HSD post-hoc test ($P < 0.05$).

Phenotype (N)	LS mean $\pm$ 1SE			
	Wet		Dry	
	<i>MM</i>	<i>BB</i>	<i>MM</i>	<i>BB</i>
Final height (954)	49.21 $\pm$ 1.61 ^A	43.18 $\pm$ 1.61 ^C	44.63 $\pm$ 1.60 ^{BC}	46.33 $\pm$ 1.60 ^{AB}
Total leaves (958)	16.55 $\pm$ 0.23 ^A	15.77 $\pm$ 0.23 ^B	14.99 $\pm$ 0.23 ^C	15.26 $\pm$ 0.23 ^{BC}
Estimated LMA (568)	0.025 $\pm$ 0.012 ^A	0.026 $\pm$ 0.001 ^A	0.022 $\pm$ 0.001 ^B	0.022 $\pm$ 0.001 ^B
Leaf width (951)	8.84 $\pm$ 0.26 ^A	7.96 $\pm$ 0.26 ^B	8.10 $\pm$ 0.26 ^B	8.35 $\pm$ 0.26 ^{AB}
Change in height (954)	16.67 $\pm$ 1.61 ^A	10.63 $\pm$ 1.61 ^C	12.09 $\pm$ 1.60 ^{BC}	13.79 $\pm$ 1.60 ^{AB}
Growth rate (954)	0.93 $\pm$ 0.09 ^A	0.59 $\pm$ 0.09 ^C	0.67 $\pm$ 0.09 ^{BC}	0.77 $\pm$ 0.09 ^{AB}
Green leaves (959)	11.41 $\pm$ 0.22 ^A	10.81 $\pm$ 0.22 ^{AB}	10.38 $\pm$ 0.22 ^B	11.30 $\pm$ 0.22 ^A
Leaf water content (569)	0.66 $\pm$ 0.02 ^A	0.64 $\pm$ 0.02 ^A	0.51 $\pm$ 0.01 ^B	0.52 $\pm$ 0.01 ^B

Appendix F. Assembly of the *B. stricta* version_2 reference genome

Methods

Sample materials

We spread seeds from focal *B. stricta* genotypes (LTM [MT] and SAD12 [CO]) in 55 mm petri dishes with 2 pieces of filter paper immersed in ddH₂O and stored them in a refrigerator at 4° C until germination. We transplanted germinants into potting soil and grew them in a growth chamber set to 22° C with a 16 h day length.

High molecular weight DNA extraction

For the LTM (MT) genotype, we extracted DNA according to Mayjonade et al. (2018) with some modifications. Briefly, we ground ~3.5 g of etiolated young leaves to powder in liquid N₂. We added lysis buffer containing RNase A (Qiagen, 100 mg/mL) to tissue powder and incubated at 50° C for 30 minutes. We then added a volume of 5 M potassium acetate equal to 1/3 the lysis buffer volume. We mixed these solutions, incubated the mixture at 4° C for 30 minutes, and centrifuged tubes at 5,000 g for 10 min. We added one volume of phenol:chloroform:isoamyl alcohol (25:24:1, v/v, Thermo Fisher, USA) to the clarified supernatant. We inverted the solutions and centrifuged tubes at 8,000 g for 15 minutes, twice. We collected the supernatants and added one volume of binding buffer containing pre-washed Ampure beads (Beckman Coulter,

USA) to each tube. We incubated these mixtures for 10 minutes, placed the tubes on a magnet, and removed the supernatant. We then washed the Ampure beads with 70% ethanol. Subsequently, we added 100 μ L TE buffer (pH 8.0) to the mixture, and incubated tubes incubated at 37° C for 20 minutes. Finally, we placed tubes on a magnet for 1 h and eluted high molecular weight DNA in 1x TAE buffer.

For the SAD12 (CO) genotype, viscous metabolites hampered DNA extraction, so we used a modified extraction approach. First, we isolated nuclei according to Gendrel et al. (2005). We then filtered supernatants through pre-equilibrated anion exchange tips (Genomic-tip 20/G, Qiagen, USA), and performed subsequent extraction steps according to the manufacturer's instructions. Finally, we dissolved high-molecular-weight DNA in TE buffer (pH 8.0).

Library preparation and sequencing

We end-repaired genomic DNA using NEBNext FFPE Repair Mix and NEBNext End repair / dA-tailing Module (New England Biolabs, Ipswich, MA, USA), and then ligated sequencing adapters using NEBNext Quick Ligation Module (New England Biolabs), adapter mix and ligation buffer (SQK-LSK109 Ligation Sequencing Kit 1D, ONT, England). We cleaned library mixtures using 0.4x volume of AmPure beads (Beckmann Coulter, USA) and eluted cleaned libraries into 15 μ l of elution buffer (SQK-LSK109). We performed Oxford Nanopore Technology (ONT) MinION sequencing

according to the manufacturer's guidelines using R9 flow cells (FLO-MIN106, ONT). We performed basecalling using Guppy v2.3.5 (available on ONT community website, <https://community.nanoporetech.com>).

Genome assembly and quality evaluation

We removed adapters from the raw sequencing file with Porechop (<https://github.com/rrwick/Porechop>). We then used the correction function in Canu (Koren et al. 2017) to correct high-noise Nanopore sequences. For LTM (MT) and SAD12 (CO), reads shorter than 1 kb and 3 kb, respectively, were filtered out in this correction step. Next, we assembled reads into contigs using Smartdenovo (<https://github.com/ruanjue/smartdenovo>) with the k-mer set from 16 to 31. We assessed assembly quality using Quast (Mikheenko et al. 2018), and selected the genome assembly with the lowest contig number and highest N50 value for further analyses. We polished the best-quality assembly using Pilon (Walker et al. 2014) with the Illumina reads (NCBI Sequence Read Archive SRR396758).

Genome annotation

We used both transcriptome-based and *ab initio* prediction for genome annotation. We downloaded paired-end RNAseq reads of *B. stricta* LTM (MT) from JGI (JGI Project ID: 405127) (Nordberg et al. 2014). We used HISAT2 (Kim et al. 2015) to

align the transcriptome to the polished LTM (MT) Nanopore genome, and used StringTie (Pertea et al. 2015) to perform reference-based transcriptome assembly. We imported the assembled transcriptome into TransDecoder (Haas et al. 2013) to predict candidate coding regions with UniProt (UniProt Consortium 2019) as a reference. For *ab initio* prediction, we used AUGUSTUS (Stanke & Morgenstern 2005) to predict genes directly from the reference genome. We imported all results into EVidenceModeler (Haas et al. 2008) to obtain a weighted consensus gene structure. We searched the final annotation against the UniProt database by using Blast2GO (Götz et al. 2008). According to BUSCO, the completeness of the annotation was 96.5% (LTM (MT) genome v2.0) (Samão et al. 2015).

Repeat analysis and genome comparison

We performed *de novo* repeat identification in both LTM (MT) and SAD12 (CO) genomes. We constructed a repeat library was using RepeatModeler (Smit & Hubley 2015) and RepBase (Bao et al. 2015), and annotated the repeats in the combined repeat library with RepeatMasker (Smit et al. 2018).

To compare the assembly of *B. stricta* reference genomes version_2 and version_1.2 (Lee et al. 2017), we first broke the version_1.2 scaffolds at positions with single or multiple nucleotide N. Those long sequences (now without nucleotide N) were then treated as “long reads” and mapped onto the version_2 genome with minimap2 (Li

2018). We used custom R codes to identify the region of the version_2 genome not covered by the version_1.2 reads. These regions therefore represented locations that were either not assembled or treated as nucleotide N in the version_1.2 genome.

To identify rRNA gene locations, we downloaded *Arabidopsis thaliana* 5S (AJ307346.2) and 35S (X52320.1) rDNA from GenBank and performed blast search against the v2.2 genome.

Ordering and orienting scaffolds into linkage groups

In order to group scaffolds of LTM (MT) into chromosomes, we downloaded Illumina paired-end reads of 199 LTM (MT) × SAD (CO) recombinant inbred lines (RILs) from NCBI Sequence Read Archive (NCBI Sequence Read Archive SRR3947175~SRR3947373). We trimmed low-quality bases using Trimmomatic (Bolger et al. 2014) and mapped paired-end reads to the version_2 LTM (MT) genome (contigs only, at this stage) with the Burrows-Wheeler Aligner (Li & Durbin 2009). We used the Genome Analysis Toolkit (Van der Auwera et al. 2013) to create a variant call format (vcf) file containing both parents and 199 RILs. We used VCFtools (Danecek et al. 2011) to filter out indels and non-biallelic SNPs, and then filtered and transformed the vcf file as described in Lee et al. (2017). Briefly, SNPs were filtered by their depth and nucleotide calls, polarized as parental alleles, and the “marker genotype” of each 100 kb-window was determined by the proportion of parental reads in the window, resulting in

LTM (MT) homozygote, SAD12 (CO) homozygote, and heterozygote windows. Also, during this process, several RILs with high heterozygosity (likely due to sample contamination) were removed, leaving 179 RILs left for further analyses. Next, the genotype table was imported into MSTmap (Wu et al. 2008) to build genetic linkage map, resulting in seven linkage groups. Finally, the output of MSTmap was imported into ALLMAPS (Tang et al. 2015) to connect scaffolds into chromosomes.

***BCMA* locus identification and phylogeny**

We searched *BCMA3* gene sequence (GenBank JQ337907) against the LTM (MT) and SAD12 (CO) genomes using BLAST+ (Camacho et al. 2009). We drew dot plots of the *BCMA* region using Gepard (Krumsiek et al. 2007). We used MEGA7 (Kumar et al. 2016) to align the putative coding regions of *BCMA* copies and perform phylogenetic reconstruction of the order of gene duplication events. We constructed a phylogenetic tree with 1,000 bootstrap resamples using a maximum likelihood algorithm that allowed some sites to have an invariable evolution rate, and also allowed evolution rate differences among sites.

Natural variation of *BCMA* copy number

We downloaded the whole genome sequences of 486 *Boechera stricta* accessions, including 157 COL accessions, 105 NOR accessions, 126 UTA accessions, 81 WES

accessions and 17 admixtures from NCBI SRA, to investigate the copy number of *BCMA* in natural *B. stricta* populations. Specifically, we downloaded all of the SRA numbers archived from Wang et al. (2019) as well as SRA number SRR396758 for the MT (LTM) accession. To ensure that all *BCMA*-related reads were mapped onto the *BCMA3* copy in the CO accession, we created a new reference genome by masking ± 10 kb region of *BCMA2* in CO genome. We mapped the whole genome sequences of 486 accessions to the new reference genome with BWA after cutting adapters and checking quality (Li & Durbin 2009, Martin 2011). We used samtools to calculate the read depth of the *BCMA3* gene with mapping quality > 30 for each accession (Li 2011). We then divided the *BCMA3* sequencing depth of each accession by that accession's genome-wide average sequencing depth, excluding repetitive regions identified previously; this yields a "read depth ratio" that is indicative of copy number of the *BCMA* locus. Because SAD12 (CO) is known to have only a single copy of *BCMA* on chromosome 7 (*BCMA3*) in addition to the single copy on chromosome 2 (*BCMA2*) (see 1.2.1), we expect SAD to have a read depth ratio of approximately 2 according to this analysis.

Results

***Boechera stricta* version_2 reference genome**

We used the Oxford Nanopore long read technology to assemble new reference genomes of *Boechera stricta* accessions LTM (MT) and SAD12 (CO). A total of four

Nanopore flow cells were used for LTM, generating 31.4 Gb of data. After quality filtering, adaptor sequence removal, and excluding reads shorter than 1 kb, 25.8 Gb of data (approximately 140-fold of the genome) in 5.9 M reads with N50 at 15.3 kb were used for assembly. The genome was assembled into about 190 Mb, with 34 contigs and a BUSCO score at 98.5% (Table F1). Almost all of the assembly (189 Mb) was successfully placed on seven chromosomes using linkage map information, with the entire chromosome 4 was assembled into one contig (utg4). While the total assembly size was similar between this version_2 and the previous version_1.2 (Lee et al. 2017), genome version_1.2 contains a large proportion of nucleotide N (12.94%, Table F1), reducing the assembly size with informative nucleotides down to 164.84 Mb. On the other hand, none of our 190-Mb LTM genome contains nucleotide N, and almost all of the assembly can be placed on chromosomes.

Table F1. Comparisons between *Boechera stricta* LTM (MT) genome from version_1.2 and version_2 assemblies.

Attribute	version_1.2	version_2
Assembly size (Mb)	189.35	189.86
Amount of nucleotide N (Mb)	24.51	0
Scaffold number*	1990	34
Scaffold N50 (Mb)	2.19	13.80
Largest scaffold (Mb)	8.34	22.91
BUSCO score (%)	96.40	98.50
Scaffolds on chromosome	208	27
Size on chromosome (Mb)	183.72	188.87

*For version_1.2, scaffolds refer to the group of contigs connected together by mate-pair Illumina sequences. For v2, scaffolds refer to the contigs directly assembled from Nanopore reads.

Further, we generated three versions of assembly. Genome version_2.0 contains the original 34 contigs without chromosome information. Genome version_2.1 connected 27 contigs (some with known location but uncertain orientation on chromosomes) into chromosomes, and kept the 7 other scaffolds independent. Genome version_2.2 connected 19 contigs with well-supported location and orientation into chromosomes, while keeping the 15 other contigs independent. We performed gene and repeat annotation for all three versions. In total, the annotation pipeline incorporating both *ab initio* prediction and RNAseq evidence predicted 38,328 genes in genome version_2.0, with 26,002 of them having BLAST hits in the Uniprot protein database.

Dissecting the *BCMA1/3* haplotype

The long-read assembly not only resolves repeat-rich regions containing transposons, rDNA genes, and telomeres, it also greatly facilitates the dissection of a crucial quantitative trait locus, *BCMA1/3*, that controls insect resistance and fitness in the field (Prasad et al. 2012). We successfully assembled this locus for LTM MT and SAD CO, two parental accessions used to investigate the effect of *BCMA1/3* locus on glucosinolate composition (Prasad et al. 2012). Consistent with previous results, CO contains only one gene, *BCMA3*, in this locus (Figure 3A in main text). In MT, this locus spans about 200 Kb full of transposons and tandem repeats. We identified at least 12 *BCMA* gene copies in this region, with six of them containing only partial coding regions

and apparently having lost their functions. Among the other six that retained most of the coding regions, four of them have deletions or long insertions of various sizes

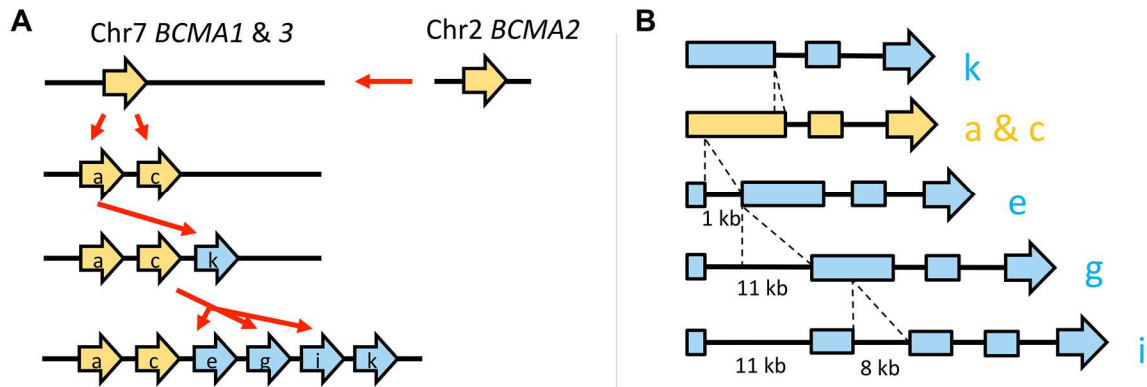


Figure F1. Inferred evolutionary history of BCMA duplications in *B. stricta*. A: Following the initial duplication event from chromosome 2 to chromosome 7, BCMA1/3 was copied several more times, with both BCMAa and BCMAc yielding progeny copies. B: Sequence alignment reveals insertions and deletions that convert most gene copies into non-functional pseudogenes.

(Figure F1), likely also disrupting their gene structure. The two other copies, BCMAa and BCMAc, have intact coding regions and are confirmed to be previously cloned genes BCMA3 and BCMA1, respectively (Prasad et al. 2012). Our results therefore showed a birth-and-death process of BCMA genes in this locus, with 12 copies in total and 10 as pseudogenes. Despite such a high proportion of pseudogenization, the survival of two copies with rapid evolution of differential amino acid activity results in the glucosinolate profile diversity and differential local adaptation across landscapes (Prasad et al. 2012).

Phylogenetic reconstruction revealed the evolutionary history of these copies (Figure 3B in main text). Consistent with the previous results, the first duplication event between the ancestral *Cyp79F1* gene (also called BCMA2) in chr2 and the BCMA1/3 locus

in chr7 happened after the divergence between *Boechera* and *Arabidopsis*. *Boechera retrofracta*, the sister species of *B. stricta*, has only one gene in the BCMA locus and is the outgroup of all *B. stricta* paralogs in the locus, suggesting the rampant gene duplication happened within *B. stricta*. The gene tree also showed that the duplication between *BCMA1* and *BCMA3*, the two functionally validated genes in this locus, happened before the differentiation between accessions MT (*B. stricta* subspecies WEST) and CO (*B. stricta* subspecies EAST). Therefore, CO had a secondary loss of *BCMA1* (Figure F2).

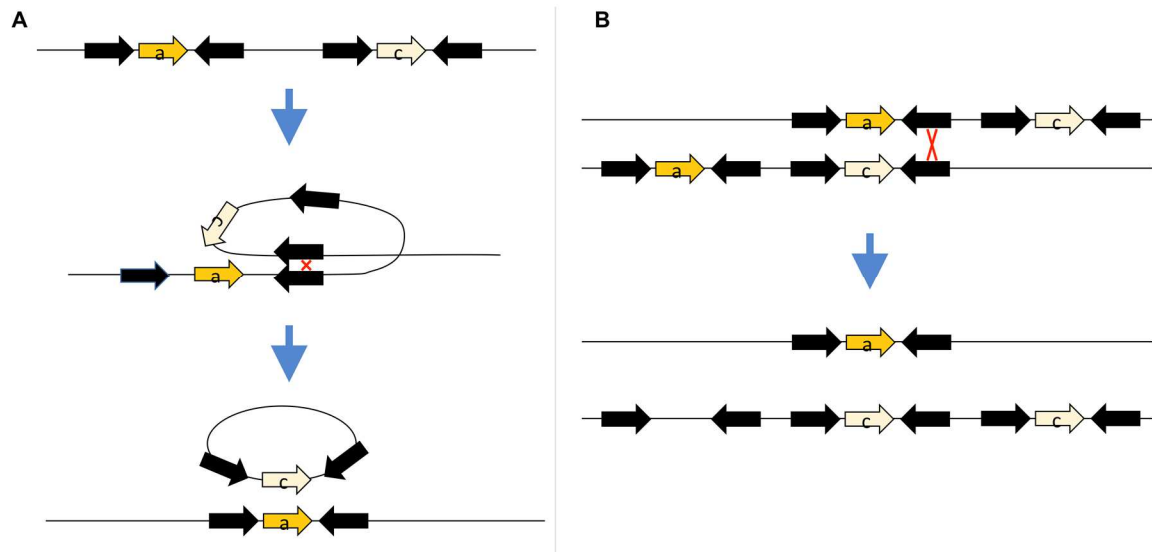


Figure F2. Proposed mechanisms causing secondary loss of *BCMA1*. Due to the prevalence of repetitive elements in the *BCMA* region on chromosome 7, it is possible that recombination events among inverted repeats excised *BCMA1* from some genetic backgrounds, either within a chromosome (A) or during crossing-over among chromosomes (B). Scenario B could also contribute to the proliferation of *BCMA* pseudogenes and flanking elements present in some lineages (Figure 3 in main text).

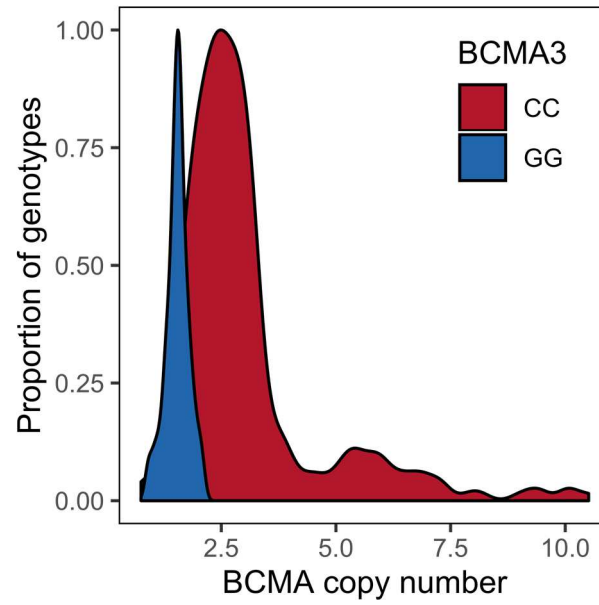


Figure F3. *BCMA* copy number variation by *BCMA3* genotype. Most genotypes with high *BCMA* copy number also possess the SNP already known to predict BC-ratio: the C allele at position 10,419,878, present in the LTM (MT) NIL parent.

Appendix G. Characterization of glucosinolate variation via HPLC

Overview of aliphatic glucosinolates produced in *B. stricta*

Boechera stricta produces glucosinolates (GS) from a variety of amino acid precursors (Windsor et al. 2005). Of particular interest, this species is able to synthesize GS from several branched-chain (BC) amino acids (Val, Ile, Leu; Schranz et al. 2009, Prasad et al. 2012) in addition to the ancestral methionine biosynthetic activity displayed in *Arabidopsis* and other close relatives (Windsor et al. 2005). Met- and BC-derived GS are all aliphatic GS, meaning that they are non-aromatic in structure. Conversely, other species in the Brassicaceae, including some *Boechera*, also produce indolic (Trp-derived) and aromatic (Phe- or Tyr-derived) GS (Windsor et al. 2005). Here, we focus on aliphatic compounds commonly produced in *B. stricta* leaves (Table G1), as have previously been well characterized (Schranz et al. 2009, Prasad et al. 2012).

Table G1. List of known aliphatic glucosinolates (GS) in *B. stricta* and internal standards in HPLC, with retention times used to call peaks in each experiment. Chemical names and amino acid (AA) precursors *sensu* Windsor et al. (2005).

Abbrev.	Full name*	AA precursor	Retention time(s) used in HPLC (min)		
			Broad panel	ID field	CO field
2OH1ME	ds-hydroxymethylethy-GS	Valine	1.16	NA	2.70 – 2.90
5MSOP	ds-5-methylsulfinylpentyl-GS	Methionine	2.47	NA	NA
<i>Sinabin</i> **	ds-4-hydroxybenzyl-GS	Tyrosine	2.81	NA	NA
<i>Sinigrin</i> **	ds-2-propenyl-GS	Methionine	NA	4.173 – 4.523	3.80 – 4.00
6MSOH	ds-6-methylsulfinylhexyl-GS	Methionine	NA	11.528 – 11.724	11.30 – 11.60
1MP	ds-1-methylpropyl-GS	Isoleucine	3.96	12.48 – 12.667	12.20 – 12.40
1ME	ds-1-methylethyl-GS	Valine	NA	7.229 – 7.934	7.00 – 7.30

*ds = desulfo- (all sulfur groups removed during GS extraction)

**internal standard used in particular HPLC runs; not endogenously expressed in *B. stricta*

Aliphatic glucosinolate production occurs via two main stages: (1) GS core biosynthesis and (2) side chain modification. Stage 1 controls the amino acid precursors that are utilized in GS biosynthesis, their elongation, and the elongated (or not) molecule's conversion into the "glucosinolate core". Stage 2 controls functional modification of amino acid side chains ("R-groups") via oxidation, hydroxylation, and/or esterification. Together, these processes result in >100 known specific GS compounds produced by presence/absence of and allelic variation in biosynthetic genes regulating different steps in the pathway (Windsor et al. 2005, Sønderby et al. 2010). *Arabidopsis* *CYP79F1/2* genes regulate the first step in core synthesis, converting elongated amino acid products to aldoximes (Sønderby et al. 2010), and the *BCMA3* ortholog in *Boechera stricta* determines enzyme affinity for. Val-derived compounds vs. (sometimes elongated) Met-derived compounds at this step (Prasad et al. 2012).

HPLC methods

Geographic variation across the species range

For the panel of 337 genotypes, we extracted and purified GS from approximately 5-15 mg of freeze dried plant tissue per individual, as described previously by Burow et.al. (2006), with modifications to facilitate high-throughput analyses. Briefly, we extracted GS in 1 mL 80% methanol (v:v) containing 50 μ M sinalbin (as an internal standard) on a shaker at 150 rpm at room temperature for 30 min. We

clarified extracted mixtures by centrifuging samples at 3,200 g for 10 min and adding supernatant (700 μ l) to a pre-equilibrated (80% methanol; v.v) DEAE Sephadex ion exchange column (28mg per sample, Sigma-Aldrich). We washed the column once with 0.5 mL 80% methanol (v:v), two times with 1 mL of water, and once with 0.5 mL 0.02 M MES buffer, pH 5.2. After washing, we added 30 μ l of Sulfatase from *Helix pomatia* (280 units/mL) solution (Sigma-Aldrich) on top of each column, and incubated samples at room temperature for 16 h. We eluted desulfo-glucosinolates from the columns in 0.5 mL of water.

We performed high-pressure liquid chromatography (HPLC) with a diode-array detector using an Agilent 1100 system with a 96-well-plate autosampler. We separated glucosinolates from extracted mixtures on a C-18 column (Zorbax Eclipse XDB-C18, 4.6 x 50 mm, 1.8 μ m) using a water ("solvent A") and acetonitrile ("solvent B") solvent gradient at a flow rate of 1.1 mL min⁻¹ at a temperature of 25° C. The solvent gradient during each 7.5 min run progressed as follows: 2% to 14% (v/v) B (0-4 min); 14% to 100% (v/v) B (4-4.1 min); 100% (v/v) B (4.1-5 min); 100% to 2% (v/v) B (5-5.1 min); and 2% (v/v) B (5.1-7.5 min). We monitored eluents by diode array detection between 190 and 360 nm (2 nm intervals). We identified the GS identify of specific eluents by retention time as described in Table G1, as well comparison of UV absorption spectra at A229 nm to those of isolated standard compounds. Furthermore, we confirmed the identity of ds-GS was using liquid chromatography - electrospray mass spectrometry (LC-ESI-MS) on a Bruker

Esquire 6000 ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). We report results as $\mu\text{mol (g dry weight)}^{-1}$ calculated from the peak areas at 229 nm relative to the peak area of the internal standard using the relative response factors 2.0 for all aliphatic glucosinolates (Burow et al. 2006).

Confirming phenotypes in *BCMA* NILs

We analyzed leaf samples from NIL transplants in common gardens both in ID and CO to confirm the expression of expected BC-ratio phenotypes under field environments. We collected a single leaf (~20 – 30 mg) from each sampled individual early in the field season (~June; to avoid possible effects of induced response to herbivore damage, which occurs later in the growing season) and placed it immediately in a vial of 1.7 mL 70% MeOH. We stored leaves in sealed MeOH vials for a minimum of one month to allow leaching of GS from leaf tissue into the solvent. We extracted desulfo-GS from leachates and quantified GS profile as described in Wagner & Mitchell-Olds (2018).

We collected samples from 202 replicate individuals spanning four environments to validate HPLC phenotypes (Table G2). Our HPLC phenotyping revealed that, consistent with what has been previously observed under greenhouse conditions (Prasad et. al 2012), the *BB* genotype produces glucosinolates predominantly from branched-chain amino acid precursors, yielding high BC-ratio, and the *MM* genotype

produces glucosinolates predominantly from methionine, yielding low BC-ratio (Figure G1).

Table G2. Sample sizes used to characterize BC-ratio under field conditions.

Genotype	Family	Idaho gardens			Colorado garden
		JAM-2015	MAH-2015	SIL-2015	SCH-2017
<i>MM</i>	10	10	2	4	0
	13	3	9	6	0
	14	0	7	5	11
	15	3	5	5	0
	35	0	0	0	11
	36	0	0	0	11
	91	0	0	0	9
<i>BB</i>	8	1	9	4	0
	20	9	9	2	0
	21	9	3	2	0
	23	0	0	0	10
	25	3	3	1	12
	27	4	2	2	0
	31	0	0	0	6
	38	0	0	0	9
TOTALS:		42	49	31	79

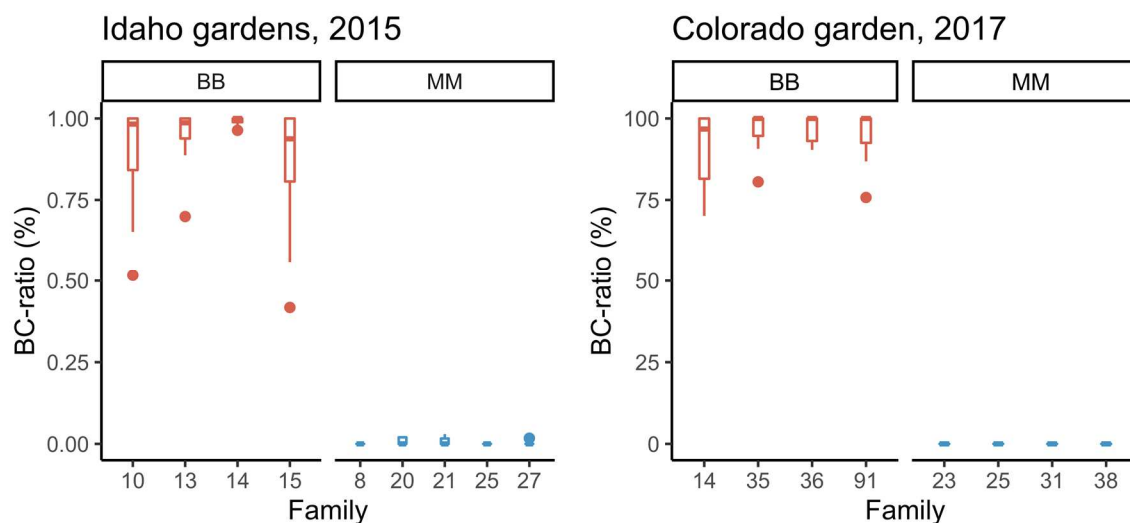


Figure G1. Confirmation of *BCMA* NIL glucosinolate phenotypes under field conditions. As expected, *BB* NIL genotypes produce glucosinolates derived predominantly from Val and Ile, while *MM* genotypes produce glucosinolates derived predominantly from Met.

Appendix H. Multivariate climate analyses.

Table H1. Summary of first five principal components for climate variation at the location of origin of 267 genotypes of *B. stricta*. Bold values are highly correlated ($|r| > 0.70$) with climate PCA axis 1, which significantly predicts BC-ratio.

Climate Variable		Climate loading onto axis (cumulative % climate variation explained)				
BioClim	Full name	PC1 (34.13)	PC2 (59.96)	PC3 (74.28)	PC4 (84.46)	PC5 (91.10)
bio1_12	Mean annual temperature	-0.47533	0.74863	0.34955	0.08348	0.16297
bio2_12	Mean diurnal range (Mean of monthly (max temp – min temp)	-0.47273	-0.53774	-0.42057	0.4082	0.14261
bio3_12	Isothermality (BIO2/BIO7)*100	-0.07828	-0.04657	-0.82176	0.13027	-0.11517
bio4_12	Temperature seasonality (standard deviation*100)	-0.44514	-0.55469	0.51228	0.25182	0.32884
bio5_12	Max temperature of warmest month	-0.77262	0.28873	0.27942	0.35608	0.18586
bio6_12	Min temperature of coldest month	-0.01356	0.93603	0.16778	-0.18644	-0.15878
bio7_12	Temperature annual range (BIO5-BIO6)	-0.554	-0.64758	0.05084	0.43221	0.28203
bio8_12	Mean temperature of wettest quarter	-0.58922	0.01368	-0.07646	-0.54778	0.32606
bio9_12	Mean temperature of driest quarter	0.46992	0.18212	0.40343	0.6091	-0.20075
bio10_12	Mean temperature of warmest quarter	-0.57529	0.52761	0.54418	0.12445	0.23998
bio11_12	Mean temperature of coldest quarter	-0.14404	0.93221	0.12736	-0.11033	-0.05687
bio12_12	Annual precipitation	0.93879	0.09011	0.11628	0.09803	0.24512
bio13_12	Precipitation of wettest month	0.74973	0.41854	-0.27485	0.12527	0.38988
bio14_12	Precipitation of driest month	0.72742	-0.39843	0.47696	-0.0829	-0.03451
bio15_12	Precipitation seasonality (coefficient of variation)	-0.09323	0.59063	-0.61698	0.15262	0.33418
bio16_12	Precipitation of wettest quarter	0.78343	0.39987	-0.1859	0.19675	0.36797
bio17_12	Precipitation of driest quarter	0.76389	-0.40664	0.39251	-0.1498	0.019
bio18_12	Precipitation of warmest quarter	0.38032	-0.32389	0.08558	-0.56375	0.53466

bio19_12	Precipitation of coldest quarter	0.84784	0.18329	-0.02377	0.44462	0.02827
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Table H2. Effects of individual climate predictors on glucosinolate profile.
Bold values indicate significant effects of climate on BC-ratio.

Model	Climate predictor	Model effect	Sum Sq	df	F	P
A	bio5 (max temp. in warmest mo.)	Climate	2,786	1	2.80	0.0953
		Genetic group	59,566	3	19.98	1.08×10 ⁻¹¹
B	bio12 (annual precip.)	Climate	14,778	1	15.59	0.0001
		Genetic group	66,467	3	23.37	1.95×10 ⁻¹³
C	bio13 (preip. in wettest mo.)	Climate	12,506	1	13.07	0.0004
		Genetic group	45,096	3	15.71	2.00×10 ⁻⁹
D	bio14 (precip. in driest mo.)	Climate	4,906	1	4.98	0.0265
		Genetic group	61,011	3	20.63	4.94×10 ⁻¹²
E	bio16 (precip. in wettest quarter)	Climate	12,945	1	13.56	0.0003
		Genetic group	49,340	3	17.22	3.08×10 ⁻¹⁰
F	bio17 (precip. in driest quarter)	Climate	6,542	1	6.68	0.0103
		Genetic group	62,949	3	21.42	1.93×10 ⁻¹²
G	bio19 (precip. in coldest quarter)	Climate	3,524	1	3.56	0.0604
		Genetic group	55,642	3	18.72	4.96×10 ⁻¹¹

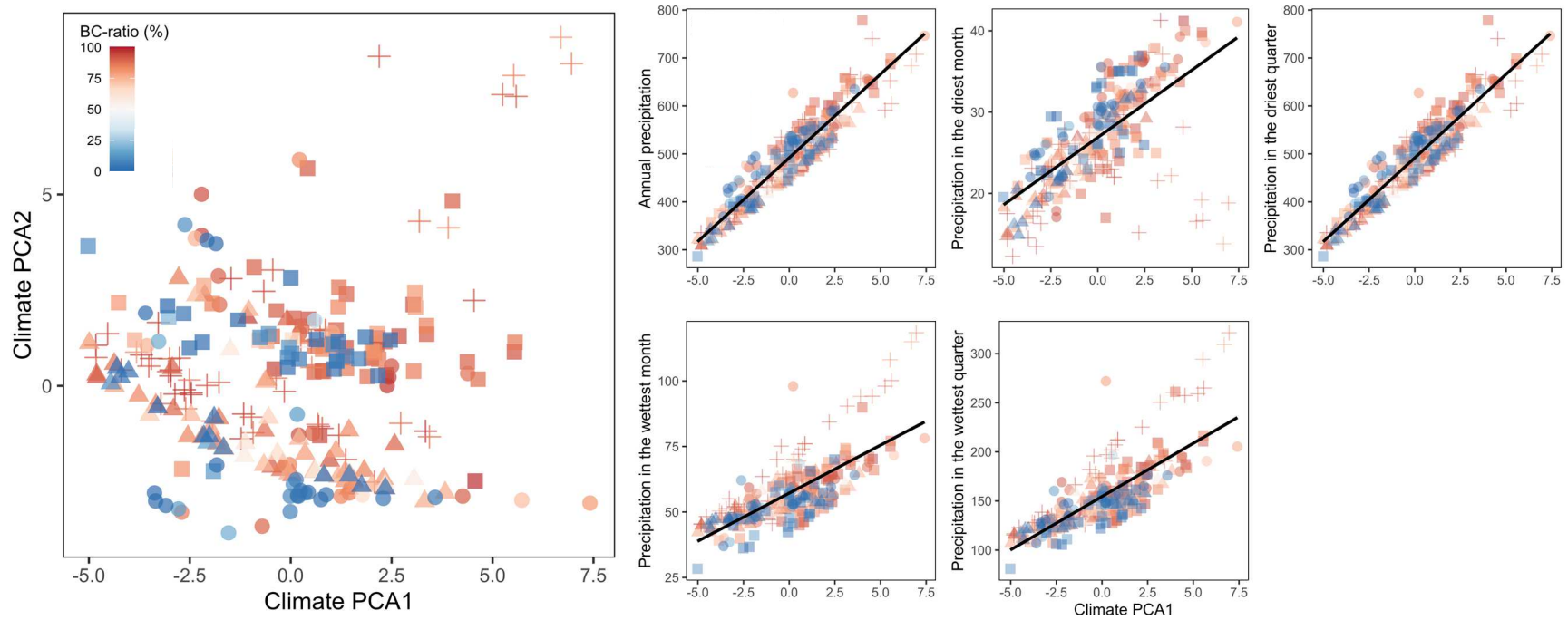


Figure H1. Drought-related climate variables are correlated with multivariate climatic predictors of BC-ratio. Axis 1 of a PCA of climate data, as well as five raw climate variables that loaded heavily onto it, significantly predict BC-ratio. Symbols denote genetic groups as described in Wang et al. (2019). COL: circles; NOR: triangles; UTA: squares; WES: crosses.

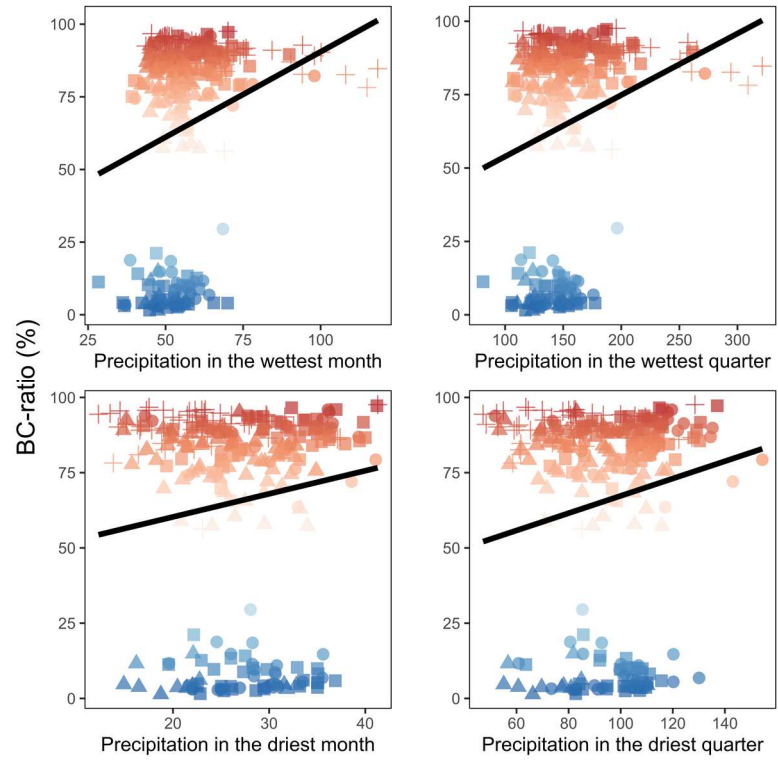


Figure H2. Raw drought variables predict BC-ratio. Met-GS are more common in drier environments. Shapes denote genetic groups as in Figure H1.

Appendix I. Linkage disequilibrium near *BCMA*

Methods

For a panel of 233 genotypes from the COL and UTA genetic groups, we mapped Illumina reads to the SAD12 version_2 reference genome using BWA, and called SNPs using GATK following Wang et al. (2019). We located the *BCMA3* gene in the version_2 reference using BLAST; it spans positions 11,779 – 11,782 kb. We also located the limits of the non-recombinant *BCMA* haplotype using FST analysis (see 1.2.6), which spans from 11,737 – 11,949 kb. We analyzed LD among SNPs within 250 kb of this interval, spanning 11,487kb – 12,199 kb on chromosome 7. We calculated LD among SNPs with minor allele frequency > 0.05 and missing rate < 0.6 in two groups of accessions: (1) a subset of 157 COL genotypes and (2) the full panel of 233 COL+UTA genotypes. We implemented all other SNP filtering thresholds as described in Wang et al. (2019).

These methods retained 2,229 SNPs in the COL group (1), and 2,460 SNPs in the COL+UTA group (2). To characterize the general extent of LD around *BCMA*, we estimated pairwise LD (r^2) between each pair of SNPs within the 712 kb interval using *plink* (Purcell et al. 2007). Our filtering thresholds retained one SNP within the *BCMA3* gene (minor allele frequency = 0.21, missing rate = 0.28). To determine whether *BCMA3* specifically was in LD with other loci, we also calculated LD between the *BCMA3* SNP and each other SNP in the interval.

Results

Within a genetic group (COL), the broad region of chromosome 7 around *BCMA* shows low LD (Figure I1A). When assessed in a pooled sample of COL and UTA accessions, LD in this region is higher, reflecting LD caused by population structure across a wide geographical scale (Figure I1B).

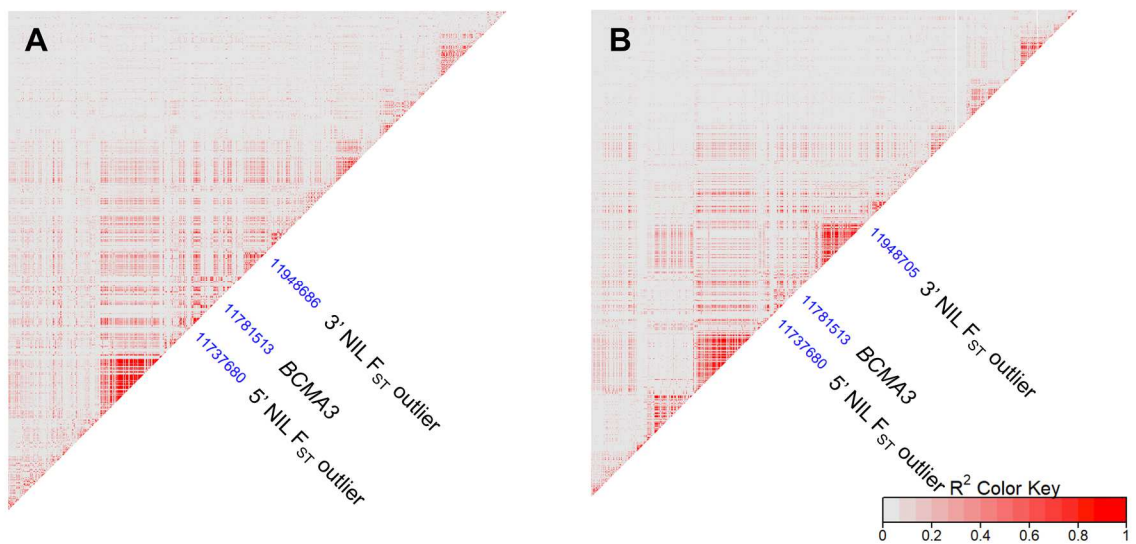


Figure I1. Heatmap showing linkage disequilibrium (r^2) in a 712 kb interval surrounding *BCMA3*. . A: Pairwise LD among 157 COL genotypes. B: Pairwise LD among 233 COL+UTA genotypes. Blue numbers indicate the position on chromosome 7, in bp, of the *BCMA3* gene and the closest SNP to the limits of the surrounding non-recombinant haplotype region in NILs.

We also found that the intermediate frequency SNP within *BCMA3* was in low LD with other SNPs in the surrounding region (Figure I2). This might be influenced by reads from both *BCMA1* and *BCMA3* genes mapping to the *BMCA3* region.

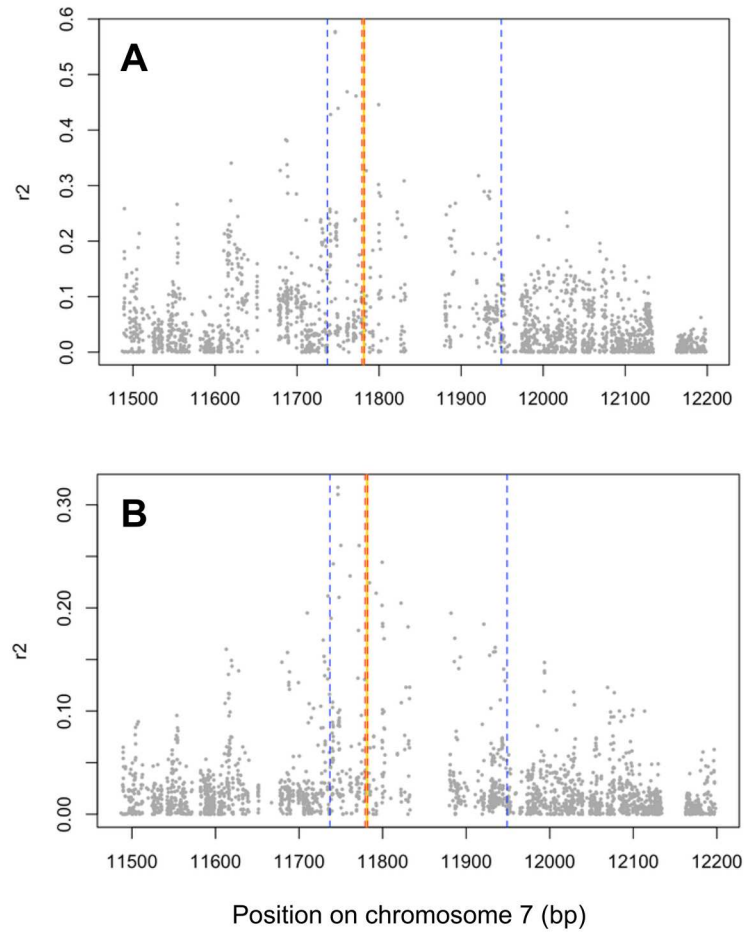


Figure I2. Linkage disequilibrium (r^2) among the *BCMA3* SNP and other nearby intermediate-frequency SNPs. A: 157 COL genotypes. B: 233 COL+UTA genotypes. Red dashed lines mark the start and stop location of the *BCMA3* gene, and the yellow line marks the position of the retained SNP within this gene. Blue dashed lines mark the boundaries of the non-recombinant *BCMA* interval in the NILs.

Appendix J. Demographic field experiment



Figure J1. Experimental transplants in October 2016. Individuals are transplanted in 50-plant randomized, complete blocks at constant density within the matrix of naturally occurring vegetation. Each experimental transplant is marked with a uniquely labeled white plastic tag.



Figure J2. Deployment of rainout shelters in June 2017. Shelters were implemented in a checkerboard rather than randomized pattern due to space constraints in the garden.

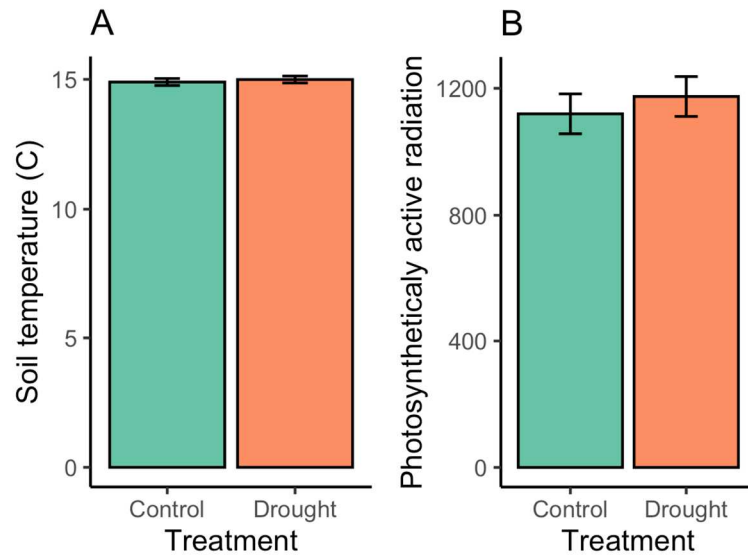


Figure J3. Experimental manipulations of soil moisture did not alter soil temperature (A) or light availability (B).

Table J1. Summary of genotypes and family lines used in the demographic field experiment.

Genotype	Family	Cohort		Total
		2015	2016	
BB	9	150	0	150
	13	150	0	150
	14	0	100	100
	15	150	0	150
	19	150	0	150
	35	150	100	250
	36	0	100	100
	91	0	100	100
MM	8	150	0	150
	20	150	0	150
	21	150	0	150
	23	150	100	250
	25	150	100	250
	31	0	100	100
	38	0	100	100
TOTALS:		1,500	800	2,300

Table J2. Number of replicates per genotype per treatment included in the demographic experiment.

Planting cohort	Garden block	Drought manipulation	Herbivory manipulation				Not manipulated (died before June 2017)	
			BT spray		Control			
			BB	MM	BB	MM	BB	MM
2015	1	Rainout	6	3	5	5	13	18
	2	Control	9	7	8	7	9	10
	3	Rainout	11	11	12	11	3	2
	4	Control	8	12	8	12	8	2
	5	Control	7	9	7	8	12	7
	6	Rainout	10	11	11	11	3	4
	7	Control	11	10	11	9	7	2
	8	Rainout	7	11	8	11	6	7
	9	Rainout	8	7	7	7	11	10
	10	Control	9	9	10	9	5	8
	11	Rainout	11	13	11	12	2	1
	12	Control	8	11	9	11	9	2
	13	Control	5	6	5	6	14	14
	14	Rainout	11	11	10	12	5	1
	15	Control	12	11	11	11	5	0
	16	Rainout	8	10	8	10	6	8
	17	Rainout	11	8	11	8	6	6
	18	Control	8	11	8	11	6	6
	19	Rainout	7	11	8	11	11	2
	20	Control	7	11	7	11	10	4
	21	Control	8	12	7	12	7	4
	22	Rainout	10	7	8	8	10	7
	23	Control	8	10	8	11	10	3
	24	Rainout	5	7	6	6	13	13
	25	Rainout	11	6	9	9	7	8
	26	Control	8	12	8	11	7	4
	27	Rainout	5	9	6	8	12	10
	28	Control	4	5	5	5	18	13
	29	Rainout	6	11	6	11	9	8
	30	Control	8	8	8	7	13	4
2016	1	Control	10	10	10	11	9	0
	2	Rainout	10	10	10	10	6	4

3	Control	9	12	8	10	5	6
4	Rainout	8	13	8	14	7	0
5	Rainout	7	11	7	11	10	4
6	Control	6	10	5	10	15	4
7	Rainout	4	11	5	11	11	8
8	Control	9	8	8	8	13	4
9	Control	6	10	6	10	14	4
10	Rainout	5	7	5	7	16	10
11	Control	6	13	7	12	10	2
12	Rainout	8	11	8	12	9	2
13	Rainout	4	11	4	11	16	4
14	Control	8	10	8	10	7	7
15	Rainout	3	8	3	7	19	10
16	Control	8	10	8	11	12	1

Appendix K. Inferring unobserved soil moisture

While we only directly measured soil moisture during two of the four growing seasons during this experiment, we used data from a similar nearby experimental garden (J. T. Anderson, *unpub.*) to predict unobserved soil moisture values at the block level. This approach was motivated by the finding that, among the two years of soil moisture data that we collected directly, block-level growing season means were highly correlated across years ($r = 0.846$, $P < 0.0001$, $N = 46$ blocks; Figure K1).

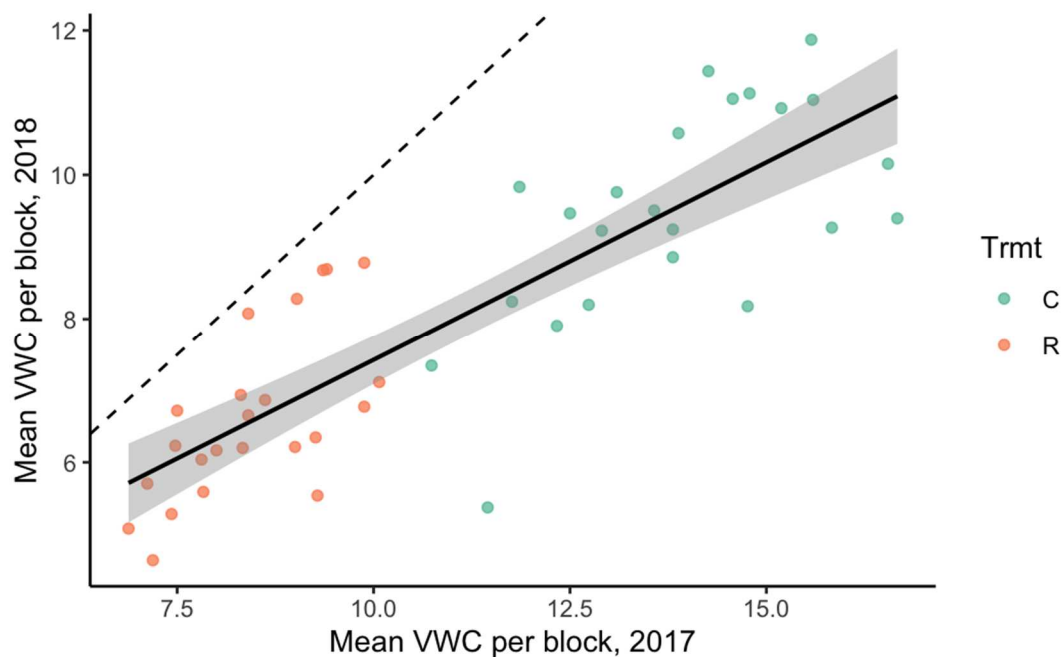


Figure K1. Justification for predicting unobserved values. Within the experimental garden, individual blocks retained similar rank dryness across years, even if overall mean VWC differed (*e.g.*, here, 2018 was drier than 2017, as all points fall below the dashed 1:1 line). Block-level consistency in rank soil moisture within the garden permits approximation of unobserved values from off-site mean VWC values.

This indicates that, in addition to interannual variation in garden-level mean soil moisture, individual blocks are consistently drier or wetter than one another at a microgeographic scale, likely due to differences in soil quality and/or heterospecific perennial plant cover. Thus, we approximated block-level variation in 2016 and 2019 as described in section 2.2.3. Here we provide additional diagnostic plots generated during this process (Figures K2 – K4).

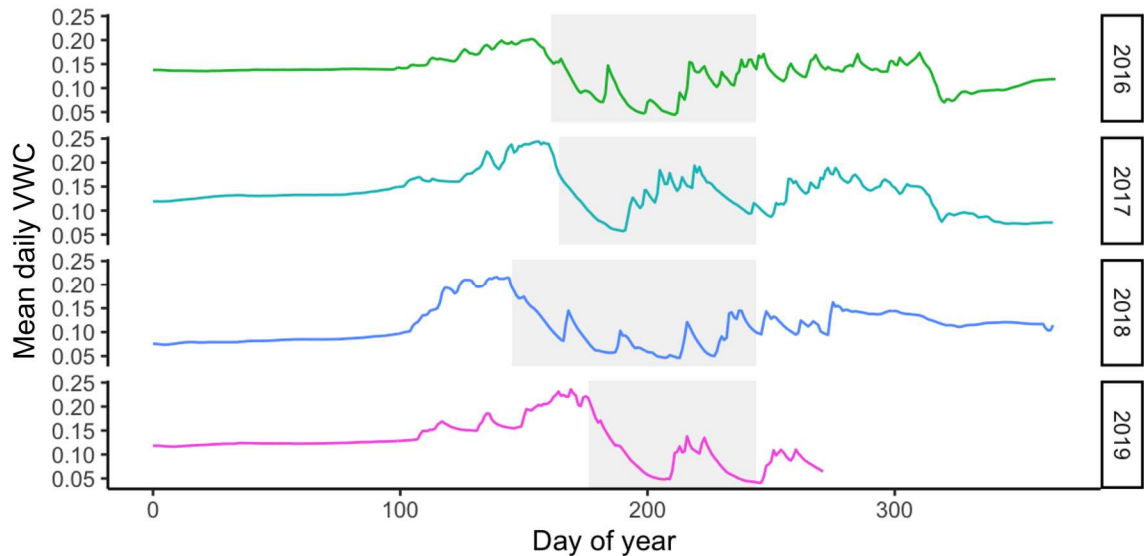
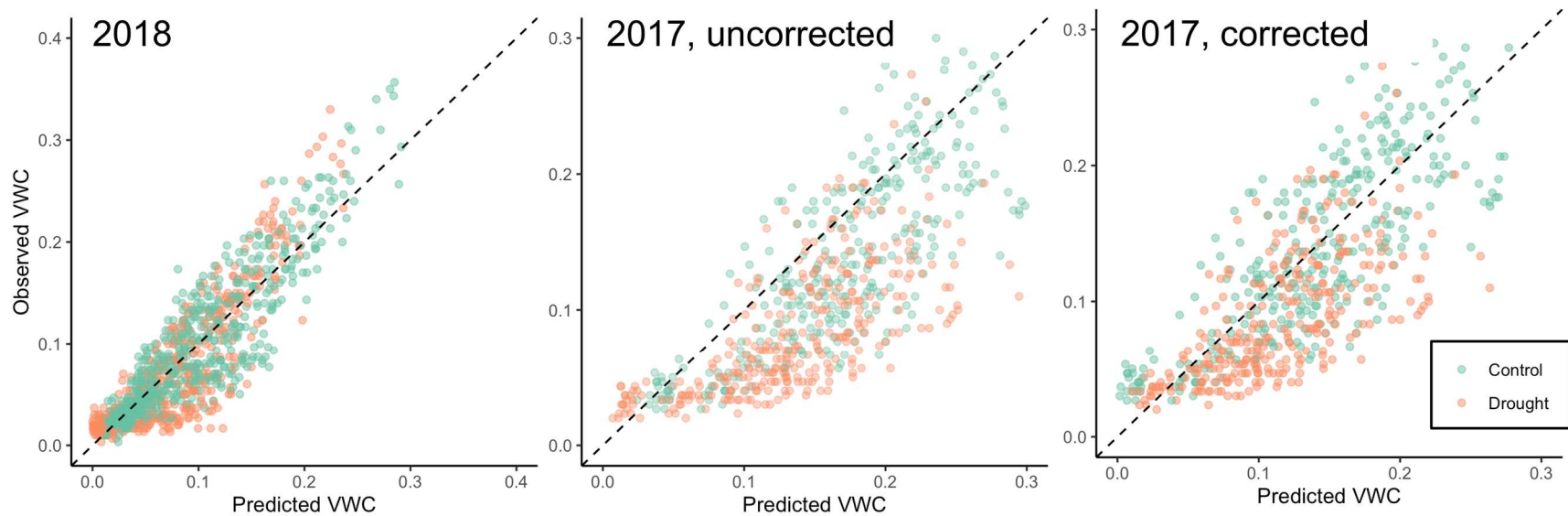


Figure K2. Off-site daily mean VWC across the four growing seasons. Gray boxes indicate the time periods considered to be the growing season for the purposes of this experiment: date of snowmelt through September 1. Within each growing season, the daily mean off-site VWC was used as the predictor in our linear models.



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Figure K3. VWC testing with daily predicted vs. observed values. We built the predictive VWC model using observed in-garden data from 2018 (left); thus, concordance between our own measurements and the model predictions was high. Because we also observed VWC directly in 2017, we assessed the accuracy of its predictions using this additional dataset. We found that raw predictions tended to overpredict VWC, by approximately the magnitude of the difference in mean growing season VWC between 2018 and 2017. Thus, by subtracting the mean difference between 2017 and 2018 from raw predictions, we re-centered the model's output around the 1:1 line (dashed). This method was preferable to fitting the predictive model from the full set of 2017 and 2018 data and using model estimates of the year effects, because, with no input data for 2016 and 2019, we would not be able to approximate model-estimated year effects. Using observed mean differences in VWC to correct for interannual variation provides a flexible approach that does not require observed data in all years.

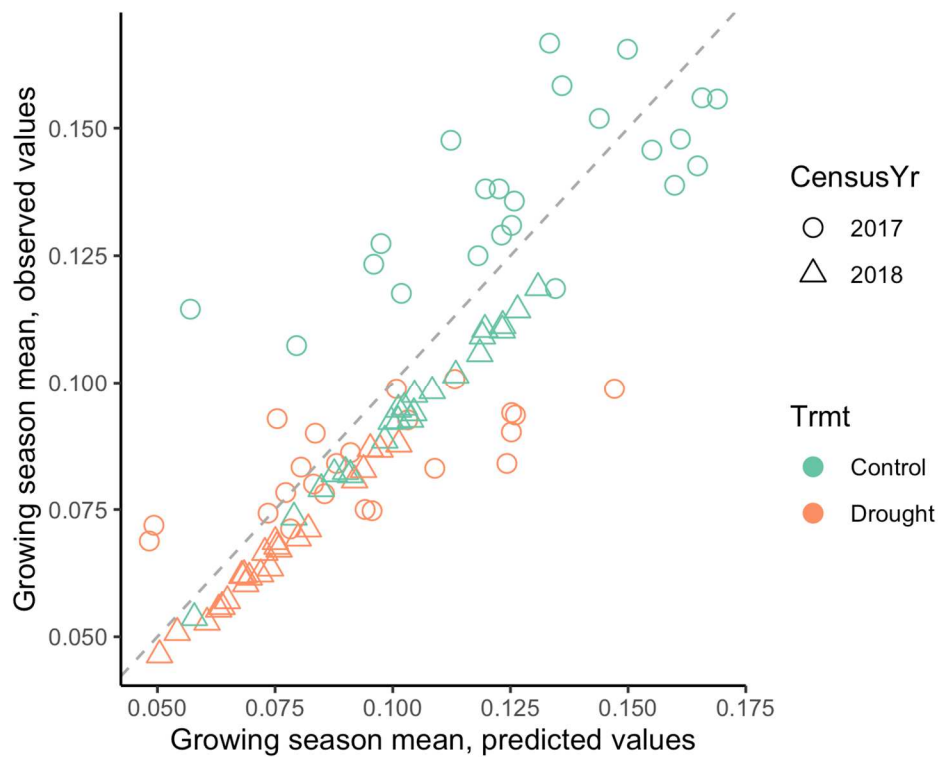


Figure K4. High concordance among predicted and observed block-level mean VWC. We fit our predictive models using daily VWC estimates to maximize power, but ultimately used growing season block-level means as the driver included in our IPM. We chose this resolution because we only censused plants once per growing season, so finer-scale temporal variation in soil moisture would not be matched with fine scale variation in vital rates within each growing season. Further, while predicted daily values were reliable, there was considerable variance around true values in years that were not included in the predictive model (see Figure K3, and justification in caption). Conversely, growing season means were highly concordant among predicted and observed values, in growing seasons for which we have both datasets.

Appendix L. Detailed modeling methods

State-specific IPM

Motivation

While constructing vital rate regressions, we observed that models typically predicted little to no growth among years, but very high variance in growth. Low mean predictions with high variance caused rampant “eviction” of individuals out of the model’s size space, *i.e.*, the prediction of individuals with a size_{t+1} outside of the pre-defined boundary points of the matrix, which are informed by the observed size ranges of the study organism. Eviction was driven by distinct growth patterns co-occurring when growth was modeled with a single function across all life stages (Figure L1).

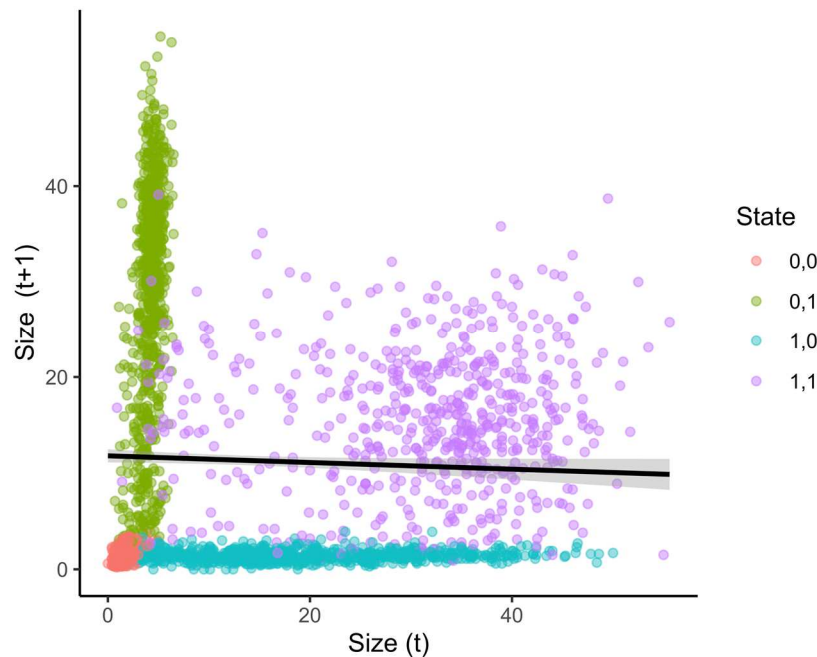


Figure L1. A single growth function across all states yields poor fit and wide variance in residuals. Color of points represents bolting state at times t , $t+1$.

Namely, growth almost always occurred among individuals that had not bolted previously (0,0 and 0,1 states), while shrinkage was common among individuals that had previously bolted (1,0 and 1,1 states). Further, the slopes of those lines, and variance of residuals around regression models, varied substantially among states. As such, we modified the standard IPM framework to accommodate continuous size-based vital rates within discrete state spaces (Figure L2).

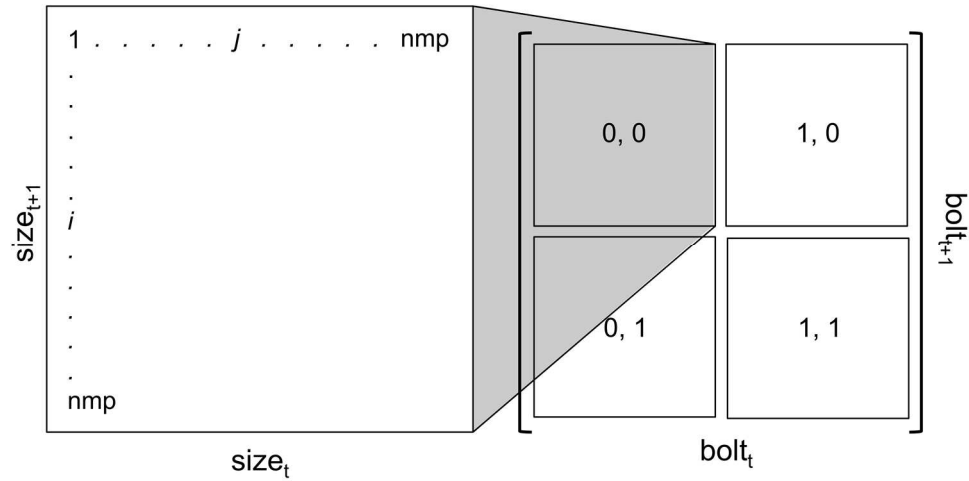


Figure L2. Schematic representation of hierarchical state-specific IPM, with standard size-based kernels nested within state-space quadrants of the full matrix. *nmp* refers to the number of meshpoints, or discretized intervals from continuous regressions, across which the IPM is evaluated.

Matrix design

Standard IPM kernels have the dimensionality $nmp \times nmp$, when *nmp* is equal to the number or kernel meshpoints, or fine-scale intervals across which continuous variation is discretized and then evaluated. To accommodate discrete state transitions, we expanded our model's dimensionality to be $2nmp \times 2nmp$, generating four quadrants

within each expanded matrix, which we assigned to correspond with discrete bolting states (Figure L2).

State-sensitive vital rates

To accommodate this design, we expanded the suite of vital rate regressions included in the IPM. Specifically, we modeled the probability of bolting for each genotype among subsets of data for individuals which had ($B1(z, h, w)$) and had not ($B0(z, h, w)$) bolted previously, across each size class z . We used these probabilities and their inverses ($1 - B1(z, h, w)$ and $1 - B0(z, h, w)$, respectively) to populate our expanded bolting matrices (Figure L3). Similarly, we modeled growth with functions for each of the four states separately, using subsets of our data depending on each individual's bolting history. We used these state-specific regressions to predict $size_{t+1}(z')$ across all sizes (z) within each state space (Figure L3). We used model selection, to remove superfluous predictors from global state-specific vital rate models, as described in the main text and Appendix M.

Because survival and reproduction did not vary considerably depending on state, we applied the same survival and reproduction functions across our P and F kernels, respectively. However, because new recruits cannot bolt until they have experienced vernalization (reproduction cued by cold weather), we restricted the population contributions of reproduction to the upper half of the F kernel, adding recruits into states for which $bolt_{t+1} = 0$ (Figure L3).

$$\begin{aligned}
 P &= \begin{bmatrix} S(z, h, w) & S(z, h, w) \\ S(z, h, w) & S(z, h, w) \end{bmatrix} \times \begin{bmatrix} 1-B0(z, h, w) & 1-B1(z, h, w) \\ B0(z, h, w) & B1(z, h, w) \end{bmatrix} \times \begin{bmatrix} G00(z', z, h, w) & G10(z', z, h, w) \\ G01(z', z, h, w) & G11(z', z, h, w) \end{bmatrix} \\
 F &= \begin{bmatrix} Fec(z', z, h, w) & Fec(z', z, h, w) \\ 0 & 0 \end{bmatrix} \times \begin{bmatrix} B0(z, h, w) & B1(z, h, w) \\ 1-B0(z, h, w) & 1-B1(z, h, w) \end{bmatrix} \\
 K &= P + F
 \end{aligned}$$

Figure L3. Schematic showing construction of state-specific IPM kernels. Growth (green matrix) was state-specific, depending on discrete bolting transitions. Thus, we calculated the probability of bolting (blue matrix) in year $t+1$ given bolting history in year t , and scaled growth, survival (orange matrix), and reproduction (red matrix) according to the probability of occupying a given state space. We then calculated the full IPM kernel as the sum of F and P .

Evaluation of the IPM

We evaluated our IPMs using standard approaches, but modified to accommodate state space. Specifically, prior to summing the P and F matrices, we scaled them based on the probability of individuals occupying each state-space, by multiplying the survival/growth and fecundity matrices by the bolting matrix (Figure L3). We then summed F and P to generate the full IPM kernel, K , from which we calculated λ .

We estimated λ across 400 distinct environmental combinations at a 20×20 resolution in herbivory and soil moisture space. We binned driver variation in $1/20^{\text{th}}$ increments of the full range for soil moisture, as we observed a substantial number of blocks in our garden experiencing the full range of driver values (Figure L3-A). However, because the distribution of herbivore damage was skewed toward small values (Figure L3-B), we divided this driver vector into bins on the log scale. This allowed us to estimate λ across the full range of observed herbivory levels, but to obtain higher resolution estimates of λ variation in the range of herbivory values that are most commonly observed in nature.

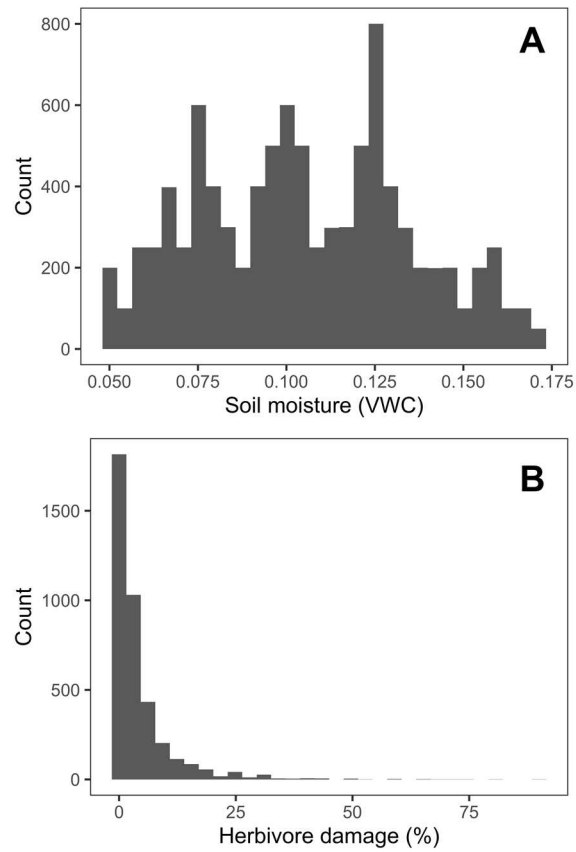


Figure L4. Distributions of driver values in field experiment. A: Soil moisture was generally normally distributed, with multiple modes showing variation induced by rainout shelter treatments. B: Herbivory was skewed toward low values, although observed damage levels ranged up to 90% total leaf area removal.

Appendix M. Details on vital rate regressions and model selection

We used an AICc-based model-selection approach to determine which driver values to retain from global models. AICc is similar to AIC but with greater penalties for over-parameterized models, especially when sample sizes are small (Burnham & Anderson 2002). We fit vital rate regressions, and thus performed model selection, separately for each vital rate and for each genotype. As such, different vital rate regressions for different genotypes often retained different combinations of drivers, in addition to allowing the magnitude and direction of effects to vary among those that were retained. While often times several models had similar AICc scores (ΔAICc between models < 2), we utilized parameter estimates from the model with the lowest AICc, which was generally the most parsimonious (fewer parameters retained than the model with the second-lowest AICc). Parameter estimates for driver values were generally similar across several of the best-supported models (Tables M1 – M18). In all tables, 'NA' indicates models which did not include the effect named in each column.

Table M1. Model selection comparisons: $S(z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
-1.498	NA	20.589	0.017	NA	NA	NA	NA	3.000	-1099.687	2205.387	0.000	0.288
-1.465	-0.009	20.513	0.018	NA	NA	NA	NA	4.000	-1099.077	2206.175	0.788	0.194
-1.620	NA	21.752	0.030	NA	NA	-0.117	NA	4.000	-1099.396	2206.813	1.426	0.141
-1.580	-0.009	21.610	0.030	NA	NA	-0.111	NA	5.000	-1098.820	2207.671	2.284	0.092
-1.527	0.006	21.054	0.018	-0.134	NA	NA	NA	5.000	-1098.939	2207.909	2.522	0.082
-1.449	-0.013	20.460	0.017	NA	0.000	NA	NA	5.000	-1098.996	2208.022	2.635	0.077
-1.627	0.005	22.015	0.030	-0.118	NA	-0.104	NA	6.000	-1098.716	2209.475	4.088	0.037
-1.564	-0.013	21.553	0.029	NA	0.000	-0.110	NA	6.000	-1098.741	2209.524	4.137	0.036
-1.510	0.002	20.996	0.017	-0.132	0.000	NA	NA	6.000	-1098.860	2209.764	4.377	0.032
-1.611	0.001	21.965	0.028	-0.118	0.000	-0.104	NA	7.000	-1098.636	2211.329	5.942	0.015
-1.586	-0.007	21.733	0.025	-0.044	0.001	-0.074	-0.007	8.000	-1098.595	2213.263	7.876	0.006
-1.428	NA	21.655	NA	NA	NA	NA	NA	2.000	-1108.248	2220.502	15.115	0.000
-1.414	-0.004	21.643	NA	NA	NA	NA	NA	3.000	-1108.152	2222.316	16.929	0.000
-1.475	0.012	22.183	NA	-0.136	NA	NA	NA	4.000	-1108.019	2224.058	18.672	0.000
0.663	-0.011	NA	0.025	NA	NA	NA	NA	3.000	-1164.825	2335.662	130.275	0.000
0.633	NA	NA	0.024	NA	NA	NA	NA	2.000	-1165.880	2335.766	130.379	0.000
0.692	-0.021	NA	0.022	NA	0.001	NA	NA	4.000	-1164.164	2336.349	130.962	0.000
0.888	NA	NA	NA	NA	NA	NA	NA	1.000	-1184.114	2370.231	164.844	0.000
0.901	-0.004	NA	NA	NA	NA	NA	NA	2.000	-1183.997	2372.000	166.613	0.000

Table M2. Model selection comparisons: $S(z, h, w)$, MM genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
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-1.327	-0.001	21.392	-0.004	-0.535	0.005	NA	NA	6.000	-1202.100	2416.239	0.000	0.285
-1.176	-0.053	19.954	-0.003	NA	0.005	NA	NA	5.000	-1203.238	2416.504	0.265	0.249
-1.398	-0.051	22.080	0.015	NA	0.005	-0.172	NA	6.000	-1202.558	2417.154	0.915	0.180
-1.509	-0.003	23.144	0.012	-0.499	0.005	-0.150	NA	7.000	-1201.593	2417.238	0.999	0.173
-1.597	0.038	23.985	0.023	-0.916	0.001	-0.250	0.038	8.000	-1201.013	2418.092	1.853	0.113
-1.395	NA	20.805	0.013	NA	NA	NA	NA	3.000	-1217.433	2440.876	24.637	0.000
-1.641	NA	23.207	0.034	NA	NA	-0.196	NA	4.000	-1216.555	2441.128	24.889	0.000
-1.404	0.003	20.845	0.012	NA	NA	NA	NA	4.000	-1217.388	2442.795	26.556	0.000
-1.660	0.004	23.317	0.034	NA	NA	-0.201	NA	5.000	-1216.474	2442.976	26.737	0.000
-1.436	0.013	21.137	0.013	-0.098	NA	NA	NA	5.000	-1217.343	2444.713	28.473	0.000
-1.662	0.005	23.334	0.034	-0.009	NA	-0.200	NA	6.000	-1216.474	2444.986	28.747	0.000
-1.308	NA	21.468	NA	NA	NA	NA	NA	2.000	-1222.134	2448.273	32.034	0.000
-1.341	0.008	21.539	NA	NA	NA	NA	NA	3.000	-1221.723	2449.457	33.218	0.000
-1.337	0.007	21.501	NA	0.013	NA	NA	NA	4.000	-1221.722	2451.463	35.224	0.000
0.878	-0.076	NA	0.000	NA	0.006	NA	NA	4.000	-1269.509	2547.036	130.797	0.000
0.698	NA	NA	0.019	NA	NA	NA	NA	2.000	-1291.444	2586.894	170.655	0.000
0.708	-0.005	NA	0.019	NA	NA	NA	NA	3.000	-1291.260	2588.531	172.292	0.000
0.929	NA	NA	NA	NA	NA	NA	NA	1.000	-1303.010	2608.022	191.783	0.000
0.918	0.004	NA	NA	NA	NA	NA	NA	2.000	-1302.913	2609.832	193.593	0.000

Table M3. Model selection comparisons: $B0(z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
0.957	NA	-50.613	-2.429	NA	NA	33.062	NA	4.000	-89.538	187.182	0.000	0.430
0.960	-0.006	-50.586	-2.411	NA	NA	32.956	NA	5.000	-89.522	189.204	2.022	0.157
-4.179	NA	NA	0.982	NA	NA	NA	NA	2.000	-93.270	190.571	3.389	0.079

-5.226	NA	11.851	0.907	NA	NA	NA	NA	3.000	-92.287	190.638	3.457	0.076
1.103	-0.044	-50.984	-2.494	NA	0.019	33.230	NA	6.000	-89.453	191.131	3.949	0.060
0.972	-0.025	-50.727	-2.392	0.218	NA	32.744	NA	6.000	-89.515	191.254	4.073	0.056
-4.166	-0.012	NA	0.996	NA	NA	NA	NA	3.000	-93.182	192.427	5.245	0.031
-5.193	-0.007	11.577	0.917	NA	NA	NA	NA	4.000	-92.255	192.616	5.434	0.028
1.130	-0.088	-51.073	-2.463	0.422	0.023	32.744	NA	7.000	-89.426	193.152	5.970	0.022
-4.078	-0.049	NA	0.954	NA	0.017	NA	NA	4.000	-93.132	194.370	7.188	0.012
-5.007	-0.074	9.681	0.907	0.762	NA	NA	NA	5.000	-92.165	194.490	7.309	0.011
-5.113	-0.037	11.527	0.880	NA	0.014	NA	NA	5.000	-92.216	194.592	7.411	0.011
0.911	0.017	-48.565	-2.362	-0.774	-0.024	31.554	0.545	8.000	-89.415	195.217	8.035	0.008
-4.188	NA	16.254	NA	NA	NA	NA	NA	2.000	-95.769	195.570	8.389	0.006
-4.772	-0.158	8.801	0.822	1.085	0.028	NA	NA	6.000	-92.044	196.312	9.130	0.004
-2.573	NA	NA	NA	NA	NA	NA	NA	1.000	-97.488	196.986	9.805	0.003
-4.194	0.001	16.282	NA	NA	NA	NA	NA	3.000	-95.769	197.601	10.420	0.002
-2.561	-0.004	NA	NA	NA	NA	NA	NA	2.000	-97.477	198.987	11.805	0.001
-3.921	-0.108	13.376	NA	1.191	NA	NA	NA	4.000	-95.601	199.307	12.126	0.001

Table M4. Model selection comparisons: $B0(z, h, w)$, MM genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
-6.308	NA	NA	1.894	NA	NA	NA	NA	2.000	-61.442	126.917	0.000	0.351
-6.430	0.020	NA	1.913	NA	NA	NA	NA	3.000	-61.173	128.412	1.495	0.166
-5.722	NA	-6.642	1.914	NA	NA	NA	NA	3.000	-61.240	128.545	1.628	0.156
-5.880	0.019	-6.252	1.934	NA	NA	NA	NA	4.000	-60.992	130.096	3.178	0.072
-3.943	NA	-26.674	1.146	NA	NA	8.608	NA	4.000	-61.057	130.225	3.307	0.067
-6.357	0.002	NA	1.873	NA	0.010	NA	NA	4.000	-61.153	130.416	3.499	0.061

-4.278	0.018	-24.185	1.245	NA	NA	7.700	NA	5.000	-60.847	131.861	4.943	0.030
-6.109	0.054	-3.951	1.947	-0.435	NA	NA	NA	5.000	-60.900	131.967	5.050	0.028
-5.754	-0.006	-6.566	1.880	NA	0.014	NA	NA	5.000	-60.956	132.079	5.161	0.027
-5.997	0.019	-2.489	1.811	-0.869	0.041	NA	NA	6.000	-60.705	133.644	6.727	0.012
-4.564	0.050	-21.142	1.291	-0.404	NA	7.307	NA	6.000	-60.771	133.775	6.858	0.011
-3.993	-0.014	-26.041	1.123	NA	0.018	8.325	NA	6.000	-60.789	133.813	6.895	0.011
-4.132	0.009	-22.793	0.999	-0.926	0.048	8.786	NA	7.000	-60.522	135.357	8.440	0.005
-3.690	-0.095	-27.995	0.754	0.397	0.103	11.605	-0.675	8.000	-60.497	137.396	10.479	0.002
-2.905	NA	NA	NA	NA	NA	NA	NA	1.000	-74.704	151.419	24.501	0.000
-2.956	0.012	NA	NA	NA	NA	NA	NA	2.000	-74.572	153.178	26.261	0.000
-2.402	NA	-5.427	NA	NA	NA	NA	NA	2.000	-74.579	153.191	26.274	0.000
-2.510	0.011	-4.747	NA	NA	NA	NA	NA	3.000	-74.478	155.023	28.105	0.000
-2.508	0.010	-4.765	NA	0.004	NA	NA	NA	4.000	-74.478	157.067	30.150	0.000

180 Table M5. Model selection comparisons: $B1(z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
-5.960	0.037	28.412	0.146	NA	NA	-0.535	NA	5.000	-305.931	621.967	0.000	0.273
-5.603	NA	26.377	0.140	NA	NA	-0.487	NA	4.000	-307.697	623.463	1.496	0.129
-4.224	0.033	14.144	0.081	NA	NA	NA	NA	4.000	-307.736	623.542	1.575	0.124
-5.843	-0.003	27.377	0.145	0.403	NA	-0.533	NA	6.000	-305.834	623.815	1.847	0.109
-5.987	0.044	28.464	0.147	NA	0.000	-0.537	NA	6.000	-305.905	623.957	1.990	0.101
-4.048	NA	13.486	0.080	NA	NA	NA	NA	3.000	-309.217	624.476	2.509	0.078
-4.110	-0.009	13.112	0.080	0.436	NA	NA	NA	5.000	-307.629	625.362	3.395	0.050
-4.240	0.039	14.140	0.082	NA	0.000	NA	NA	5.000	-307.720	625.544	3.577	0.046
-5.845	-0.006	26.969	0.148	0.609	-0.001	-0.537	NA	7.000	-305.724	625.644	3.677	0.044

-5.356	-0.209	22.260	0.126	2.594	0.008	-0.340	-0.081	8.000	-305.222	626.697	4.730	0.026
-4.101	-0.012	12.659	0.083	0.616	-0.001	NA	NA	6.000	-307.542	627.231	5.264	0.020
-2.605	NA	NA	0.086	NA	NA	NA	NA	2.000	-317.080	638.181	16.214	0.000
-2.682	0.024	NA	0.086	NA	NA	NA	NA	3.000	-316.239	638.519	16.552	0.000
-2.701	0.031	NA	0.087	NA	0.000	NA	NA	4.000	-316.216	640.502	18.535	0.000
-2.411	-0.096	16.357	NA	1.305	NA	NA	NA	4.000	-368.840	745.749	123.782	0.000
-2.734	0.035	19.453	NA	NA	NA	NA	NA	3.000	-369.966	745.974	124.007	0.000
-2.573	NA	18.880	NA	NA	NA	NA	NA	2.000	-371.662	747.346	125.378	0.000
-0.352	NA	NA	NA	NA	NA	NA	NA	1.000	-392.478	786.963	164.995	0.000
-0.411	0.022	NA	NA	NA	NA	NA	NA	2.000	-391.736	787.493	165.525	0.000

Table M6. Model selection comparisons: $B1(z, h, w)$, MM genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
-5.621	0.041	25.929	0.175	NA	NA	-0.635	NA	5.000	-405.312	820.705	0.000	0.241
-5.432	-0.021	25.461	0.167	NA	0.003	-0.623	NA	6.000	-404.614	821.341	0.636	0.176
-5.411	NA	24.937	0.170	NA	NA	-0.597	NA	4.000	-406.797	821.647	0.942	0.151
-5.623	0.042	25.943	0.175	-0.005	NA	-0.635	NA	6.000	-405.312	822.737	2.032	0.087
-5.628	0.043	27.546	0.168	-0.800	0.004	-0.644	NA	7.000	-404.307	822.765	2.060	0.086
-3.840	0.036	10.240	0.102	NA	NA	NA	NA	4.000	-407.594	823.242	2.537	0.068
-3.746	NA	10.184	0.101	NA	NA	NA	NA	3.000	-408.815	823.663	2.958	0.055
-3.686	-0.025	10.057	0.095	NA	0.003	NA	NA	5.000	-406.800	823.681	2.976	0.054
-5.538	-0.002	26.653	0.164	-0.357	0.006	-0.604	-0.020	8.000	-404.281	824.757	4.052	0.032
-3.798	0.018	9.863	0.101	0.176	NA	NA	NA	5.000	-407.568	825.216	4.511	0.025
-3.787	0.023	11.264	0.094	-0.628	0.004	NA	NA	6.000	-406.616	825.345	4.639	0.024
-2.744	-0.035	NA	0.102	NA	0.004	NA	NA	4.000	-412.814	833.682	12.977	0.000

-2.898	0.034	NA	0.110	NA	NA	NA	NA	3.000	-413.845	833.722	13.017	0.000
-2.812	NA	NA	0.109	NA	NA	NA	NA	2.000	-415.010	834.035	13.330	0.000
-2.261	NA	19.062	NA	NA	NA	NA	NA	2.000	-488.189	980.394	159.689	0.000
-2.331	0.027	19.158	NA	NA	NA	NA	NA	3.000	-487.215	980.462	159.757	0.000
-2.160	-0.051	17.473	NA	0.780	NA	NA	NA	4.000	-486.585	981.223	160.518	0.000
-0.134	NA	NA	NA	NA	NA	NA	NA	1.000	-518.192	1038.390	217.685	0.000
-0.185	0.023	NA	NA	NA	NA	NA	NA	2.000	-517.425	1038.865	218.160	0.000

Table M7. Model selection comparisons: $G00(z', z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
1.007	-0.051	-2.088	0.383	0.466	NA	NA	NA	6.000	-285.167	582.567	0.000	0.304
1.056	-0.060	-2.180	0.358	0.474	0.004	NA	NA	7.000	-284.643	583.598	1.031	0.182
0.768	-0.057	0.149	0.539	0.536	NA	-1.440	NA	7.000	-284.787	583.886	1.319	0.157
0.743	-0.070	0.852	0.564	0.571	0.005	-1.969	NA	8.000	-283.973	584.348	1.781	0.125
0.789	-0.011	NA	0.402	NA	NA	NA	NA	4.000	-288.624	585.358	2.792	0.075
0.649	-0.040	1.812	0.608	0.267	-0.006	-2.421	0.109	9.000	-283.844	586.191	3.625	0.050
0.823	-0.018	NA	0.380	NA	0.004	NA	NA	5.000	-288.240	586.646	4.080	0.040
0.843	-0.011	-0.637	0.406	NA	NA	NA	NA	5.000	-288.494	587.154	4.587	0.031
0.884	-0.018	-0.697	0.384	NA	0.004	NA	NA	6.000	-288.084	588.402	5.835	0.016
0.916	-0.011	-1.309	0.363	NA	NA	0.394	NA	6.000	-288.460	589.154	6.587	0.011
0.905	-0.018	-0.895	0.372	NA	0.004	0.117	NA	7.000	-288.081	590.475	7.908	0.006
0.782	NA	NA	0.383	NA	NA	NA	NA	3.000	-293.335	592.736	10.169	0.002
0.810	NA	-0.336	0.385	NA	NA	NA	NA	4.000	-293.299	594.709	12.142	0.001
0.854	NA	-0.744	0.359	NA	NA	0.240	NA	5.000	-293.287	596.741	14.174	0.000
1.453	-0.076	-0.796	NA	0.813	NA	NA	NA	5.000	-322.715	655.597	73.030	0.000

1.177	NA	2.079	NA	NA	NA	NA	NA	3.000	-332.161	670.388	87.821	0.000
1.202	-0.005	1.997	NA	NA	NA	NA	NA	4.000	-331.233	670.576	88.009	0.000
1.377	NA	NA	NA	NA	NA	NA	NA	2.000	-333.308	670.650	88.083	0.000
1.396	-0.006	NA	NA	NA	NA	NA	NA	3.000	-332.296	670.658	88.091	0.000

Table M8. Model selection comparisons: $G00(z', z, h, w)$, MM genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
0.972	-0.008	-2.208	0.425	NA	NA	NA	NA	5.000	-292.086	594.343	0.000	0.223
0.787	-0.008	NA	0.408	NA	NA	NA	NA	4.000	-293.435	594.984	0.641	0.162
1.020	-0.014	-2.343	0.401	NA	0.003	NA	NA	6.000	-291.543	595.326	0.983	0.136
0.818	-0.012	NA	0.387	NA	0.003	NA	NA	5.000	-293.052	596.275	1.932	0.085
0.884	-0.008	-1.392	0.479	NA	NA	-0.483	NA	6.000	-292.040	596.320	1.977	0.083
0.984	-0.011	-2.308	0.423	0.024	NA	NA	NA	6.000	-292.069	596.377	2.034	0.081
0.995	-0.008	-1.995	0.398	-0.098	0.005	NA	NA	7.000	-291.364	597.049	2.706	0.058
0.886	-0.014	-1.082	0.483	NA	0.004	-0.753	NA	7.000	-291.433	597.187	2.844	0.054
0.892	-0.011	-1.463	0.479	0.027	NA	-0.508	NA	7.000	-292.018	598.357	4.014	0.030
0.770	NA	NA	0.398	NA	NA	NA	NA	3.000	-296.410	598.888	4.545	0.023
0.855	-0.008	-0.678	0.483	-0.100	0.005	-0.782	NA	8.000	-291.246	598.905	4.562	0.023
0.926	NA	-1.872	0.413	NA	NA	NA	NA	4.000	-295.448	599.009	4.666	0.022
0.716	0.015	0.699	0.584	-0.310	-0.013	-1.743	0.159	9.000	-290.723	599.965	5.622	0.013
0.732	NA	-0.072	0.533	NA	NA	-1.075	NA	5.000	-295.218	600.608	6.265	0.010
1.436	-0.030	-0.401	NA	0.289	NA	NA	NA	5.000	-332.232	674.634	80.291	0.000
1.405	-0.005	NA	NA	NA	NA	NA	NA	3.000	-334.568	675.205	80.862	0.000
1.385	NA	NA	NA	NA	NA	NA	NA	2.000	-335.591	675.215	80.872	0.000
1.279	NA	1.114	NA	NA	NA	NA	NA	3.000	-335.302	676.671	82.328	0.000

1.313	-0.005	0.968	NA	NA	NA	NA	NA	4.000	-334.350	676.814	82.471	0.000
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Table M9. Model selection comparisons: $G01(z', z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$h \times z$	df	logLik	AICc	delta	weight
-14.492	-0.534	169.528	10.750	NA	NA	-35.138	NA	6.000	-1342.986	2698.212	0.000	0.135
-4.579	0.242	88.232	6.142	-5.395	NA	NA	NA	6.000	-1343.094	2698.427	0.216	0.122
-1.791	-0.152	45.645	6.931	NA	-0.095	NA	NA	6.000	-1343.181	2698.603	0.391	0.111
2.957	-0.088	NA	7.287	NA	-0.107	NA	NA	5.000	-1344.233	2698.636	0.424	0.110
-0.051	-0.529	52.320	6.263	NA	NA	NA	NA	5.000	-1344.329	2698.829	0.617	0.100
-5.490	0.467	77.860	6.732	-4.699	-0.081	NA	NA	7.000	-1342.262	2698.846	0.634	0.099
-15.064	0.062	173.518	9.747	-4.169	NA	-28.016	NA	7.000	-1342.300	2698.920	0.708	0.095
-12.799	-0.253	140.366	10.319	NA	-0.070	-27.880	NA	7.000	-1342.409	2699.139	0.927	0.085
5.773	-0.513	NA	6.573	NA	NA	NA	NA	4.000	-1345.727	2699.568	1.356	0.069
-13.470	0.280	146.463	9.419	-3.887	-0.064	-21.829	NA	8.000	-1341.814	2700.042	1.831	0.054
-16.410	1.079	168.446	10.256	-9.545	-0.267	-28.008	1.430	9.000	-1341.729	2701.977	3.765	0.021
1.409	NA	NA	6.583	NA	NA	NA	NA	3.000	-1384.229	2774.527	76.315	0.000
1.092	NA	2.779	6.566	NA	NA	NA	NA	4.000	-1384.226	2776.566	78.354	0.000
-8.547	NA	80.746	9.566	NA	NA	-23.472	NA	5.000	-1383.747	2777.664	79.452	0.000
0.457	0.935	233.699	NA	-10.490	NA	NA	NA	5.000	-1394.488	2799.146	100.934	0.000
9.625	-0.565	168.217	NA	NA	NA	NA	NA	4.000	-1398.032	2804.177	105.965	0.000
32.395	-0.515	NA	NA	NA	NA	NA	NA	3.000	-1409.710	2825.488	127.276	0.000
11.352	NA	121.155	NA	NA	NA	NA	NA	3.000	-1432.372	2870.811	172.599	0.000
28.058	NA	NA	NA	NA	NA	NA	NA	2.000	-1437.591	2879.217	181.005	0.000

Table M10. Model selection comparisons: $G01(z', z, h, w)$, MM genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
7.907	0.513	NA	5.440	NA	-0.207	NA	NA	5.000	-1354.206	2718.578	0.000	0.179
4.248	0.476	38.659	5.051	NA	-0.198	NA	NA	6.000	-1353.247	2718.726	0.148	0.166
-9.812	0.482	151.792	8.856	NA	-0.200	-29.836	NA	7.000	-1352.263	2718.836	0.258	0.157
-30.715	3.426	319.920	12.981	-23.389	-0.771	-63.353	4.589	9.000	-1350.372	2719.246	0.668	0.128
0.797	1.007	71.612	4.779	-4.729	-0.168	NA	NA	7.000	-1352.550	2719.411	0.834	0.118
-13.216	1.011	184.347	8.576	-4.712	-0.170	-29.762	NA	8.000	-1351.567	2719.535	0.957	0.111
3.141	0.623	93.221	3.533	-7.204	NA	NA	NA	6.000	-1354.580	2721.391	2.814	0.044
-10.424	0.622	202.903	7.201	-7.221	NA	-28.878	NA	7.000	-1353.664	2721.638	3.061	0.039
9.647	-0.384	44.336	3.615	NA	NA	NA	NA	5.000	-1356.338	2722.842	4.264	0.021
-3.837	-0.388	153.378	7.264	NA	NA	-28.741	NA	6.000	-1355.440	2723.112	4.535	0.019
14.154	-0.386	NA	3.989	NA	NA	NA	NA	4.000	-1357.586	2723.282	4.705	0.017
8.401	0.856	163.854	NA	-8.723	NA	NA	NA	5.000	-1370.137	2750.440	31.863	0.000
16.464	-0.364	106.345	NA	NA	NA	NA	NA	4.000	-1372.515	2753.140	34.563	0.000
7.094	NA	48.179	3.406	NA	NA	NA	NA	4.000	-1373.761	2755.631	37.054	0.000
11.977	NA	NA	3.811	NA	NA	NA	NA	3.000	-1375.102	2756.269	37.692	0.000
-3.274	NA	131.877	6.204	NA	NA	-22.052	NA	5.000	-1373.279	2756.723	38.145	0.000
31.085	-0.364	NA	NA	NA	NA	NA	NA	3.000	-1380.075	2766.216	47.638	0.000
13.666	NA	106.590	NA	NA	NA	NA	NA	3.000	-1386.975	2780.015	61.438	0.000
28.318	NA	NA	NA	NA	NA	NA	NA	2.000	-1394.007	2792.047	73.470	0.000

Table M11. Model selection comparisons: $G10(z', z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
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1.085	NA	3.198	NA	NA	NA	NA	NA	3.000	-277.072	560.215	0.000	0.293
1.063	NA	3.012	0.002	NA	NA	NA	NA	4.000	-276.679	561.477	1.262	0.156
1.095	-0.002	3.157	NA	NA	NA	NA	NA	4.000	-277.020	562.159	1.944	0.111
1.187	-0.041	2.261	NA	0.397	NA	NA	NA	5.000	-276.198	562.576	2.361	0.090
0.907	NA	4.389	0.011	NA	NA	-0.075	NA	5.000	-276.277	562.733	2.518	0.083
1.074	-0.003	2.961	0.002	NA	NA	NA	NA	5.000	-276.610	563.399	3.184	0.060
1.164	-0.040	2.118	0.002	0.380	NA	NA	NA	6.000	-275.855	563.963	3.748	0.045
0.919	-0.003	4.332	0.011	NA	NA	-0.074	NA	6.000	-276.212	564.676	4.461	0.032
0.990	-0.044	3.661	0.012	0.422	NA	-0.089	NA	7.000	-275.295	564.928	4.713	0.028
1.053	0.006	2.994	0.003	NA	0.000	NA	NA	6.000	-276.456	565.163	4.948	0.025
1.147	-0.033	2.002	0.004	0.458	-0.001	NA	NA	7.000	-275.436	565.210	4.995	0.024
0.972	-0.037	3.546	0.014	0.500	-0.001	-0.089	NA	8.000	-274.875	566.185	5.970	0.015
0.902	0.005	4.337	0.012	NA	0.000	-0.073	NA	7.000	-276.073	566.484	6.269	0.013
1.364	NA	NA	0.004	NA	NA	NA	NA	3.000	-280.892	567.856	7.641	0.006
1.068	-0.079	2.701	0.009	0.877	0.001	-0.047	-0.017	9.000	-274.668	567.881	7.666	0.006
1.435	NA	NA	NA	NA	NA	NA	NA	2.000	-282.000	568.035	7.820	0.006
1.377	-0.006	NA	0.004	NA	NA	NA	NA	4.000	-280.614	569.348	9.133	0.003
1.448	-0.005	NA	NA	NA	NA	NA	NA	3.000	-281.755	569.582	9.367	0.003
1.365	0.000	NA	0.004	NA	0.000	NA	NA	5.000	-280.539	571.259	11.044	0.001

Table M12. Model selection comparisons: $G10(z', z, h, w)$, MM genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
1.317	NA	NA	0.005	NA	NA	NA	NA	3.000	-314.341	634.742	0.000	0.196
1.411	NA	NA	NA	NA	NA	NA	NA	2.000	-315.908	635.846	1.104	0.113
1.266	NA	0.596	0.004	NA	NA	NA	NA	4.000	-314.144	636.389	1.647	0.086

1.308	0.003	NA	0.005	NA	NA	NA	NA	4.000	-314.224	636.549	1.807	0.079
1.304	NA	1.030	NA	NA	NA	NA	NA	3.000	-315.264	636.588	1.846	0.078
1.333	-0.009	NA	0.003	NA	0.001	NA	NA	5.000	-313.255	636.663	1.921	0.075
1.073	NA	2.425	0.015	NA	NA	-0.095	NA	5.000	-313.656	637.465	2.723	0.050
1.406	0.002	NA	NA	NA	NA	NA	NA	3.000	-315.852	637.765	3.024	0.043
1.330	-0.029	-0.165	0.004	0.355	NA	NA	NA	6.000	-312.829	637.873	3.131	0.041
1.372	-0.030	0.252	NA	0.361	NA	NA	NA	5.000	-313.961	638.075	3.333	0.037
1.256	0.003	0.609	0.005	NA	NA	NA	NA	5.000	-314.018	638.189	3.447	0.035
1.286	-0.009	0.541	0.003	NA	0.001	NA	NA	6.000	-313.093	638.400	3.658	0.031
1.297	0.003	1.049	NA	NA	NA	NA	NA	4.000	-315.186	638.474	3.733	0.030
1.141	-0.027	1.618	0.014	0.340	NA	-0.091	NA	7.000	-312.386	639.058	4.316	0.023
1.051	0.004	2.543	0.016	NA	NA	-0.100	NA	6.000	-313.479	639.172	4.430	0.021
1.288	-0.099	0.033	0.004	1.179	0.005	0.008	-0.052	9.000	-310.454	639.370	4.628	0.019
1.101	-0.008	2.275	0.013	NA	0.001	-0.089	NA	7.000	-312.663	639.612	4.870	0.017
1.324	-0.026	0.006	0.004	0.262	0.000	NA	NA	7.000	-312.723	639.732	4.990	0.016
1.141	-0.024	1.727	0.014	0.259	0.000	-0.088	NA	8.000	-312.302	640.973	6.231	0.009

Table M13. Model selection comparisons: $G11(z', z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
4.301	1.034	78.187	0.004	-14.488	0.026	NA	NA	7.000	-809.162	1632.809	0.000	0.407
10.910	-1.119	13.367	-0.244	7.406	0.110	2.264	-0.786	9.000	-807.491	1633.768	0.958	0.252
5.558	1.027	67.937	-0.035	-14.432	0.026	0.311	NA	8.000	-809.138	1634.902	2.092	0.143
6.982	NA	40.205	0.076	NA	NA	NA	NA	4.000	-814.376	1636.924	4.114	0.052
9.258	NA	41.983	NA	NA	NA	NA	NA	3.000	-816.200	1638.502	5.693	0.024
8.853	NA	24.878	0.018	NA	NA	0.474	NA	5.000	-814.321	1638.899	6.090	0.019

Table M14. Model selection comparisons: $G11(z', z, h, w)$, MM genotype.

6.886	0.016	40.484	0.076	NA	NA	NA	NA	5.000	-814.357	1638.972	6.163	0.019
5.350	0.540	54.052	0.081	-5.261	NA	NA	NA	6.000	-813.406	1639.174	6.365	0.017
7.463	-0.133	40.605	0.046	NA	0.009	NA	NA	6.000	-813.485	1639.333	6.523	0.016
11.863	NA	NA	0.081	NA	NA	NA	NA	3.000	-817.210	1640.523	7.713	0.009
9.218	0.008	42.120	NA	NA	NA	NA	NA	4.000	-816.196	1640.562	7.753	0.008
8.674	0.013	25.930	0.021	NA	NA	0.448	NA	6.000	-814.308	1640.979	8.169	0.007
8.020	0.460	53.959	NA	-4.555	NA	NA	NA	5.000	-815.489	1641.235	8.425	0.006
6.654	0.532	43.427	0.042	-5.206	NA	0.323	NA	7.000	-813.381	1641.246	8.436	0.006
9.555	-0.138	23.623	-0.019	NA	0.009	0.523	NA	7.000	-813.418	1641.321	8.512	0.006
14.535	NA	NA	NA	NA	NA	NA	NA	2.000	-819.250	1642.551	9.741	0.003
11.866	-0.001	NA	0.081	NA	NA	NA	NA	4.000	-817.210	1642.592	9.782	0.003
12.452	-0.148	NA	0.051	NA	0.009	NA	NA	5.000	-816.376	1643.009	10.200	0.002
14.565	-0.010	NA	NA	NA	NA	NA	NA	3.000	-819.242	1644.586	11.777	0.001

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
16.447	-1.272	-4.625	NA	10.748	NA	NA	NA	5.000	-1182.843	2375.860	0.000	0.201
16.016	-4.397	0.927	0.012	35.281	0.114	-0.143	-0.887	9.000	-1179.337	2377.203	1.343	0.103
9.740	-1.252	50.279	0.235	10.626	NA	-1.897	NA	7.000	-1181.566	2377.459	1.599	0.090
15.947	NA	NA	NA	NA	NA	NA	NA	2.000	-1186.728	2377.490	1.630	0.089
13.752	NA	18.221	NA	NA	NA	NA	NA	3.000	-1185.713	2377.496	1.636	0.089
16.203	-1.264	-5.106	0.010	10.680	NA	NA	NA	6.000	-1182.807	2377.858	1.998	0.074
10.254	-1.512	52.791	0.214	9.805	0.011	-1.940	NA	8.000	-1181.149	2378.721	2.861	0.048
6.948	NA	72.679	0.241	NA	NA	-1.902	NA	5.000	-1184.401	2378.977	3.117	0.042
15.210	NA	NA	0.025	NA	NA	NA	NA	3.000	-1186.512	2379.094	3.234	0.040

16.818	-1.506	-3.939	-0.014	9.917	0.010	NA	NA	7.000	-1182.448	2379.223	3.364	0.037
13.402	NA	17.267	0.015	NA	NA	NA	NA	4.000	-1185.630	2379.376	3.516	0.035
15.851	0.040	NA	NA	NA	NA	NA	NA	3.000	-1186.655	2379.379	3.519	0.035
13.658	0.040	18.213	NA	NA	NA	NA	NA	4.000	-1185.641	2379.397	3.538	0.034
6.799	0.044	73.184	0.242	NA	NA	-1.919	NA	6.000	-1184.313	2380.870	5.011	0.016
15.131	0.038	NA	0.024	NA	NA	NA	NA	4.000	-1186.446	2381.009	5.149	0.015
7.879	-0.480	74.264	0.211	NA	0.016	-1.979	NA	7.000	-1183.407	2381.142	5.282	0.014
13.321	0.039	17.286	0.015	NA	NA	NA	NA	5.000	-1185.562	2381.299	5.439	0.013
16.335	-0.482	NA	-0.014	NA	0.016	NA	NA	5.000	-1185.563	2381.301	5.441	0.013
14.547	-0.462	16.638	-0.021	NA	0.016	NA	NA	6.000	-1184.741	2381.727	5.867	0.011

Table M15. Model selection comparisons: $Pr(z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
-2.425	NA	NA	0.294	NA	NA	NA	NA	2.000	-184.892	373.798	0.000	0.375
-2.716	NA	2.465	0.293	NA	NA	NA	NA	3.000	-184.715	375.459	1.661	0.163
-2.425	0.000	NA	0.294	NA	NA	NA	NA	3.000	-184.892	375.812	2.015	0.137
-3.440	NA	8.394	0.369	NA	NA	-0.612	NA	4.000	-184.470	376.989	3.191	0.076
-2.552	0.023	NA	0.307	NA	-0.002	NA	NA	4.000	-184.626	377.300	3.502	0.065
-2.721	-0.002	2.621	0.293	NA	NA	NA	NA	4.000	-184.704	377.456	3.658	0.060
-3.476	-0.003	8.851	0.372	NA	NA	-0.635	NA	5.000	-184.444	378.961	5.163	0.028
-2.792	0.019	2.234	0.305	NA	-0.002	NA	NA	5.000	-184.493	379.059	5.261	0.027
-2.645	-0.023	1.973	0.294	0.142	NA	NA	NA	5.000	-184.681	379.436	5.638	0.022
-3.435	0.014	7.716	0.371	NA	-0.002	-0.552	NA	6.000	-184.305	380.711	6.914	0.012
-3.398	-0.028	8.193	0.375	0.175	NA	-0.648	NA	6.000	-184.411	380.925	7.127	0.011
-2.806	0.023	2.329	0.305	-0.022	-0.002	NA	NA	6.000	-184.492	381.087	7.289	0.010

-5.110	0.416	20.159	0.534	-2.859	-0.037	-1.783	0.260	8.000	-182.471	381.118	7.321	0.010
-3.418	0.007	7.607	0.372	0.043	-0.002	-0.559	NA	7.000	-184.303	382.742	8.944	0.004
-1.664	0.272	26.209	NA	-2.038	NA	NA	NA	4.000	-350.559	709.166	335.368	0.000
-0.473	NA	16.730	NA	NA	NA	NA	NA	2.000	-357.386	718.786	344.988	0.000
-0.459	-0.010	17.069	NA	NA	NA	NA	NA	3.000	-357.068	720.165	346.367	0.000
1.628	NA	NA	NA	NA	NA	NA	NA	1.000	-370.008	742.020	368.222	0.000
1.642	-0.003	NA	NA	NA	NA	NA	NA	2.000	-369.987	743.988	370.190	0.000

Table M16. Model selection comparisons: $Pr(z, h, w)$, MM genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
-4.078	-0.251	18.706	0.601	1.573	NA	-2.765	NA	6.000	-268.129	548.333	0.000	0.510
-4.052	-0.258	18.873	0.597	1.537	0.001	-2.764	NA	7.000	-268.089	550.279	1.946	0.193
-4.538	-0.039	22.169	0.567	NA	NA	-2.518	NA	5.000	-270.473	551.000	2.667	0.135
-3.880	-0.314	17.612	0.583	1.950	0.005	-2.667	-0.030	8.000	-268.065	552.259	3.926	0.072
-4.457	-0.073	22.626	0.562	NA	0.002	-2.561	NA	6.000	-270.151	552.377	4.044	0.068
-4.596	NA	21.613	0.563	NA	NA	-2.522	NA	4.000	-273.296	554.627	6.294	0.022
-0.449	-0.169	-10.150	0.241	0.970	NA	NA	NA	5.000	-276.884	563.821	15.488	0.000
-0.943	-0.042	-6.289	0.241	NA	NA	NA	NA	4.000	-277.895	563.826	15.493	0.000
-1.624	-0.045	NA	0.237	NA	NA	NA	NA	3.000	-279.389	564.799	16.465	0.000
-0.878	-0.057	-6.311	0.237	NA	0.001	NA	NA	5.000	-277.835	565.723	17.390	0.000
-0.436	-0.172	-10.112	0.240	0.962	0.000	NA	NA	6.000	-276.879	565.833	17.500	0.000
-1.569	-0.058	NA	0.233	NA	0.001	NA	NA	4.000	-279.341	566.718	18.385	0.000
-0.971	NA	-7.106	0.236	NA	NA	NA	NA	3.000	-281.000	568.020	19.687	0.000
-1.750	NA	NA	0.231	NA	NA	NA	NA	2.000	-282.951	569.912	21.579	0.000
0.674	NA	9.562	NA	NA	NA	NA	NA	2.000	-442.944	889.899	341.566	0.000

0.698	-0.018	10.050	NA	NA	NA	NA	NA	3.000	-442.114	890.250	341.917	0.000
1.089	-0.114	6.959	NA	0.743	NA	NA	NA	4.000	-441.284	890.604	342.271	0.000
1.853	NA	NA	NA	NA	NA	NA	NA	1.000	-447.923	897.850	349.517	0.000
1.912	-0.012	NA	NA	NA	NA	NA	NA	2.000	-447.500	899.011	350.678	0.000

Table M17. Model selection comparisons: $R(z, h, w)$, BB genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
-45.659	-0.971	233.350	7.619	NA	0.080	-18.094	NA	7.000	-3874.306	7762.776	0.000	0.276
-12.211	-0.916	NA	5.078	NA	0.077	NA	NA	5.000	-3876.768	7763.624	0.848	0.181
2.557	-0.817	-132.583	5.173	NA	0.075	NA	NA	6.000	-3875.937	7763.996	1.220	0.150
-37.432	-2.751	169.731	7.643	13.051	0.080	-18.180	NA	8.000	-3873.984	7764.178	1.403	0.137
10.809	-2.554	-196.358	5.186	12.735	0.075	NA	NA	7.000	-3875.631	7765.426	2.650	0.073
-55.596	1.047	302.514	8.530	-13.912	-0.104	-24.615	1.310	9.000	-3873.622	7765.507	2.731	0.070
-46.420	0.827	167.250	7.686	NA	NA	-15.664	NA	6.000	-3877.583	7767.289	4.514	0.029
-4.257	0.856	-148.554	5.546	NA	NA	NA	NA	5.000	-3878.806	7767.698	4.923	0.024
-21.141	0.811	NA	5.454	NA	NA	NA	NA	4.000	-3879.845	7767.748	4.972	0.023
-38.328	-0.922	104.566	7.710	12.838	NA	-15.745	NA	7.000	-3877.275	7768.713	5.937	0.014
3.881	-0.857	-211.544	5.559	12.574	NA	NA	NA	6.000	-3878.510	7769.143	6.367	0.011
-46.649	NA	215.130	7.796	NA	NA	-16.948	NA	5.000	-3880.416	7770.920	8.144	0.005
-15.462	NA	NA	5.402	NA	NA	NA	NA	3.000	-3882.583	7771.201	8.426	0.004
-0.906	NA	-125.722	5.478	NA	NA	NA	NA	4.000	-3881.839	7771.736	8.961	0.003
48.746	NA	596.586	NA	NA	NA	NA	NA	3.000	-4102.823	8211.681	448.905	0.000
49.479	-0.229	600.299	NA	NA	NA	NA	NA	4.000	-4102.707	8213.471	450.696	0.000
37.869	2.182	686.455	NA	-17.674	NA	NA	NA	5.000	-4102.399	8214.886	452.110	0.000
127.328	NA	NA	NA	NA	NA	NA	NA	2.000	-4112.324	8228.666	465.890	0.000

127.919	-0.111	NA	NA	NA	NA	NA	NA	3.000	-4112.298	8230.630	467.854	0.000
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Table M18. Model selection comparisons: $R(z, h, w)$, MM genotype.

Int.	h	w	z	$h \times w$	$h \times z$	$w \times z$	$w \times h \times z$	df	logLik	AICc	delta	weight
-39.965	-1.094	314.501	6.797	NA	0.102	-19.790	NA	7.000	-5326.713	10667.543	0.000	0.519
-38.674	-1.495	303.680	6.809	3.131	0.101	-19.837	NA	8.000	-5326.688	10669.524	1.982	0.193
3.013	-1.029	NA	4.083	NA	0.099	NA	NA	5.000	-5330.447	10670.957	3.414	0.094
11.781	-0.955	-88.125	4.176	NA	0.097	NA	NA	6.000	-5329.689	10671.465	3.922	0.073
-41.221	-0.740	322.444	6.933	-2.342	0.065	-20.740	0.260	9.000	-5326.677	10671.540	3.998	0.070
12.803	-1.244	-96.640	4.180	2.263	0.097	NA	NA	7.000	-5329.676	10673.467	5.924	0.027
-44.826	1.339	276.512	7.029	NA	NA	-18.349	NA	6.000	-5331.477	10675.040	7.497	0.012
-41.289	0.218	248.757	7.055	8.295	NA	-18.508	NA	7.000	-5331.296	10676.707	9.165	0.005
-6.241	1.326	NA	4.486	NA	NA	NA	NA	4.000	-5334.921	10677.883	10.340	0.003
3.517	1.365	-96.435	4.581	NA	NA	NA	NA	5.000	-5334.019	10678.101	10.558	0.003
6.986	0.383	-123.609	4.586	7.273	NA	NA	NA	6.000	-5333.881	10679.848	12.306	0.001
-46.871	NA	337.057	7.235	NA	NA	-19.790	NA	5.000	-5341.330	10692.722	25.179	0.000
-1.318	NA	NA	4.531	NA	NA	NA	NA	3.000	-5344.631	10695.287	27.744	0.000
5.306	NA	-64.494	4.595	NA	NA	NA	NA	4.000	-5344.232	10696.505	28.963	0.000
41.098	1.472	484.835	NA	NA	NA	NA	NA	4.000	-5544.044	11096.129	428.587	0.000
37.995	2.341	508.368	NA	-6.436	NA	NA	NA	5.000	-5543.973	11098.009	430.466	0.000
43.151	NA	521.202	NA	NA	NA	NA	NA	3.000	-5551.784	11109.592	442.050	0.000
101.635	1.712	NA	NA	NA	NA	NA	NA	3.000	-5560.704	11127.433	459.890	0.000
109.399	NA	NA	NA	NA	NA	NA	NA	2.000	-5570.919	11145.851	478.309	0.000

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Biography

Lauren N. Carley received a B.A. in Biology from Reed College in 2012.

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