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Original Article

Life history and chemical defense interact to drive patterns of adaptation in an annual monkeyflower

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Abstract

Although chemical defenses and herbivore pressure are widely established as key targets and agents of selection, their roles in local adaptation and determining potential evolutionary responses to changing climates are often neglected. Here, we explore fitness differences between 11 rangewide *M. guttatus* populations in a field common garden experiment and assess the agents and targets of selection driving relative fitness patterns. We use piecewise structural equation models to disentangle associations between chemical defenses, (phenylpropanoid glycosides; PPGs), and life history traits with herbivory and fitness. While the historical environment of populations is not predictive of fitness differences between populations, >90% of variation in fitness can be predicted by the flowering time and foliar PPG defense arsenal of a population. Piecewise structural equation models indicate that life history traits, particularly earlier flowering time, are strongly and directly linked to fitness. However, herbivory, particularly fruit predation, is also an important agent of selection that creates indirect links between fitness and both chemical defenses and life history traits. Our results emphasize the multivariate nature of the agents and targets of selections in producing adaptation and suggest that future responses to selection must navigate a complex fitness landscape.

Keywords: *Mimulus guttatus* (common monkeyflower), *Erythranthe guttata*, flowering time, herbivory, frugivory, common garden, piecewise structural equation modeling

A classic debate in evolutionary biology is the extent to which organisms are well adapted to their environment (Hendry & Gonzalez, 2008). Many evolutionary biologists use the relative prevalence of local adaptation observed through reciprocal transplant experiments to justify that species are well adapted to their environment (e.g., Hereford, 2009; Leimu & Fischer, 2008). Alternatively, maladaptation could be prevalent because of demographic constraints, lags in natural selection, limited genetic architectures, and the dynamic and multivariate nature of environments (Barton & Partridge, 2000; Hendry & Gonzalez, 2008; Lenormand, 2002). Examples of such maladaptation and adaptation lags to changing climatic conditions have been increasing documented (Anderson & Wadgymar, 2020; Kooyers et al., 2019; Wilczek et al., 2014). These examples highlight that multiple different abiotic and biotic selection pressures are simultaneously acting within populations (CaraDonna et al., 2014; Kooyers & Olsen, 2013; Wadgymar et al., 2018) and that genetic architecture of ecologically important traits may limit the efficiency of natural selection (Etterson & Shaw, 2001). Deciphering how such selection pressures interact with phenotypic variation to produce variation in fitness among populations is the key for determining how well organisms fit their environment as well as future responses to selection.

Plant defenses, by definition, reduce the negative impact of herbivores on a plant's reproductive success and thus should be key ecological phenotypes (Erb, 2018; Holeski, 2021). Plant defenses include both resistance and tolerance to herbivory (Strauss & Agrawal, 1999); here we focus on resistance traits and use "resistance" and "defense" interchangeably. Evidence that various plant defenses are selected in response to variation in herbivore pressure is widespread (Ahern & Whitney, 2014; Baldwin, 1998; Erb, 2018; Kerwin et al., 2015; Muola et al., 2010; Prasad et al., 2012). This includes substantial evidence for links between defense traits and herbivory (e.g., Dirzo & Harper, 1982; Rasmann & Agrawal, 2009), herbivory and fitness (e.g., Agrawal et al., 2012; Marquis, 1984), and defense traits directly to fitness (e.g., Lankau, 2007; Ochoa-López et al., 2020; Rausher & Simms, 1989; Shonle & Bergelson, 2000). This literature indicates that resistance traits are effective in deterring herbivory to different tissue types, including foliar tissue, floral tissue, fruits, or seeds (Cogni & Futuyma, 2009; McCall & Irwin, 2006; Whitehead & Bowers, 2014) and that variation in resistance traits between tissues also may potentially impact multiple aspects of plant fitness, potentially increasing survival, fecundity, or the probability of success of the next generation (Adler et al., 2001). Despite this immense literature, the relative role and impact that plant defenses play in promoting relative patterns of adaptation among populations is not well understood (Erb 2018) and it has been argued that defenses play a less valuable role in adaptation than traits that directly correspond to abiotic selection factors (Hargreaves et al., 2020).

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The relative role of plant defenses in adaptation is complicated by the multivariate nature of resistance traits, herbivory, and fitness as well as correlations between resistance and life history traits. Plants may produce multiple structurally similar compounds derived from a single biosynthetic pathway (Fraenkel, 1959; Keefover-Ring et al., 2014; Raguso et al., 2015), each of which may have unique properties in defense (Agrawal & Fishbein, 2006). Alternatively, either the concentration of an entire class of secondary metabolites may impact herbivores similarly (Erb & Robert, 2016; Kos et al., 2012; Rotter et al., 2018) or differences in the specific combinations of secondary metabolites may interactively impact herbivores (hereafter termed "arsenal"; Coley et al., 2018). Substantial genetic correlations often exist between different secondary metabolites (Coley et al., 2018) or between defenses and either growth rate, the rate of reproduction, or other ecologically important phenotypes (Strauss et al., 2002; Züst & Agrawal, 2017; Züst et al., 2015). Such correlations make it difficult to disentangle which compounds are actually under selection (Cope et al., 2021; Kliebenstein et al., 2001; Kooyers et al., 2020). Thus, determining the relative role that secondary metabolites play in defense and adaptation requires careful experimental designs that quantify variation within potential resistance traits, different types of herbivory, and variation in fitness within a natural populations (Erb, 2018).

Mimulus guttatus (*syn. Erythranthe guttata*) has been widely studied as an ecological genetic model for life history adaptation (Hall & Willis, 2006; Kooyers et al., 2015, 2019; Lowry & Willis, 2010; Nelson et al., 2018; Troth et al., 2018). However, herbivore pressure and chemical defenses are also important agents and targets of selection in this system (Kooyers et al., 2017; Lowry et al., 2019b; Rotter et al., 2018). The dominant bioactive secondary compounds implicated in defense in *M. guttatus* are phenylpropanoid glycosides (PPGs) (Holeski et al., 2013; Keefover-Ring et al., 2014). Greenhouse common garden and feeding trial studies with multiple plant species, including *M. guttatus*, have demonstrated that PPGs can deter feeding by generalist herbivores and stimulate feeding by specialist herbivores (Holeski et al., 2013, 2014; Molgaard, 1986; Rotter et al., 2018). In annual *M. guttatus,* PPG arsenals consist of seven different PPGs. These arsenals are constitutively expressed, highly heritable, and make up a substantial proportion of dry leaf tissue (avg. ~8% dry weight; Holeski et al., 2013; Kooyers et al., 2017, 2020). Both total foliar PPG concentrations (hereafter "total PPGs") and the relative composition of PPG arsenals vary clinally across gradients in latitude, elevation, and herbivore pressure; patterns that are indicative of selection acting on both traits (Kooyers et al., 2017; Rotter et al., 2019).

Our understanding of the adaptive significance of PPGs is complicated by genetic correlations with other traits that also vary clinally across the same ecological gradients. Total PPG levels are strongly correlated with both growth rate and reproductive timing with individuals that constitutively produce higher concentrations of PPGs growing more slowly and flowering later (Kooyers et al., 2017, 2020). These correlations are relevant for understanding patterns of natural selection on defense because multiple studies have found evidence for selection on growth rate, reproductive speed, and phenology that correspond to the timing and duration of the growing season of the annual *M. guttatus* population (Kooyers et al., 2015, 2019; Nelson et al., 2018; Troth et al., 2018). For instance, in a low-elevation Oregon population with one of the highest total PPGs levels in the species' range, strong directional selection was found for earlier flowering time, more rapid growth, and (surprisingly) *greater* foliar herbivory (Kooyers et al., 2019).

Here, we investigate how variation in life history and defense generate variation in herbivory and fitness among different populations of *M. guttatus*. We conduct a field-based common garden experiment including plants from 11 rangewide populations of *M. guttatus* to ask: (1) Does the native population have the greatest fitness? and (2) Is variation among populations in fitness better associated with abiotic factors or a particular combination of traits? Concurrently, in the same garden, we parse the roles of different selection pressures and traits potentially involved in creating variation in fitness among populations by using the rangewide populations as well as F_4 lines within structural equation models and follow-up linear models. Specific, we ask: (3) Is herbivory an important agent of selection? and (4) How do tradeoffs between life history and defense traits create variation in fitness among populations? Our results demonstrate that total PPG concentration and arsenal are associated with some measures of herbivory, and that the vast majority of variation in fitness among populations can be predicted when both life history and defense arsenal are included within models.

Methods

Experimental design and line development

We conducted common garden experiments in a seepy meadow site near Lookout Point Dam in Lowell, Oregon (LPD, lat. 43.91667, long. 122.75603; 277 m asl) as well as a parallel common garden in a geographically proximate greenhouse to measure phytochemical defenses. Our experiments took place in 2019, which was close to the historical averages for temperature and precipitation during the growing season (Supplementary Figure S1). We constructed two types of lines to plant in each common garden. First, to examine range-wide relative differences in phenotypes and fitness, we created outbred lines from 11 populations occurring throughout the range of *M. guttatus* by crossing maternal lines within each population that had previously been grown in a common growth chamber environment (Figure 1; Supplementary Appendix 1). We created between 5 and 9 (ave. 7.4) outbred lines per population (hereafter "outcrossed population lines"). In a few cases where populations were underrepresented, we supplemented the outbred lines with selfed lines that were grown in the same common environment as the outbred lines (13 selfed lines). Cross type had no effect on fitness in downstream analyses.

Second, in order to break apart phenotypic correlations to the maximal extent possible and assess relationships between phenotypic variation and fitness, we constructed a panel of F_4 lines by crossing between F_3 lines that had been selfed from a previous F_2 mapping population (BEL × IM; Kooyers et al., 2020). This mapping population was originally derived from a cross between a population in the foothills of the Sierra Nevada and a second population from the Cascades in Oregon; these populations represent extreme divergence for total PPGs, PPG arsenal, and growth rate. We preferentially crossed F_3 individuals that were at the extremes of trait distributions as measured in the F_2 generation (Kooyers et al., 2020). This resulted in the creation of 45 different lines that adequately recreate the chemical defense variation of the

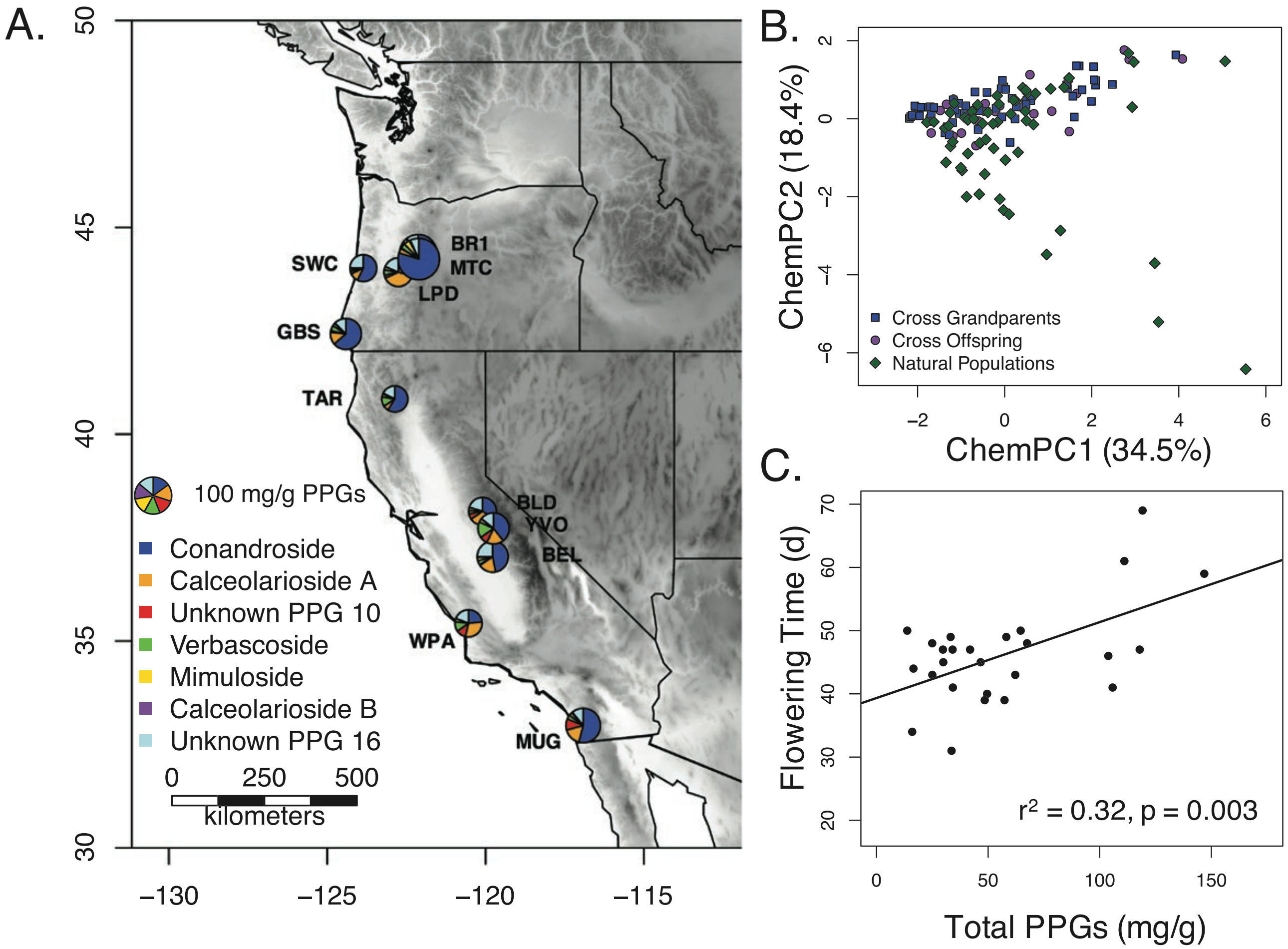

Figure 1. Summary of key defense phenotypes and locations for each population. (A) Map depicts the locations and average concentrations of constitutively produced phenylpropanoid glycoside (PPGs) for each population. Sizes of pie charts represent average total PPG concentration and sizes of slices represent the relative composition of each PPG in a population. Raster represents elevation above sea level with darker values corresponding to higher elevations. Three-letter population abbreviations are given adjacent to each site. (B) Principal component analysis of PPG arsenals summarized by ChemPC1 and ChemPC2 from the greenhouse experiment. Percentages reflect the variance explained by each PC axis. Point color and shape indicate whether lines are outbred population lines, F_4 lines, or the grandparents of the F_4 lines (F_2 lines). Note that grandparents were not grown in the same greenhouse garden, rather values come from Kooyers et al. (2020). (C) Correlation between total PPGs and flowering time within F_4 lines. All PPG concentrations refer to mg/g dry weight.

entire mapping population (hereafter referred to as "F_4 lines"). Seeds from both types of lines were cold-stratified in 2.5" pots containing Fafard 3B potting soil for seven days before moving to the University of Oregon greenhouse. Germination was recorded daily. Seedlings remained in the greenhouse for 14 days receiving ambient light at ~20 °C before being transplanted into either field or greenhouse conditions.

Greenhouse experiment

We left a single individual per line under greenhouse conditions to determine constitutive levels of each PPG and assess phenotypic correlations between PPGs and other traits (*N* = 121 plants). Lines were randomized across flats and flats were rotated weekly. Plants were surveyed for flowering every other day. Flowering time was calculated as the number of days from germination to the opening of the first flower. At first flower, we counted the number of leaves and measured plant height from ground to apical meristem. To assess levels of each PPG, we flash froze leaf tissue (second, third, and fourth leaf pairs) 77 days after flats were moved to the greenhouse. A number of samples were lost due to thawing during shipping leading to a reduced sample size for PPG analysis (*N* = 87). Each sample was lyophilized, ground, and weighed prior to phytochemical analysis. PPG analysis followed previously described procedures (Holeski et al., 2013, 2014). We quantified the PPG content of each sample via high-performance liquid chromatography (HPLC, Agilent 1260 HPLC with a diode array detector and Poroshell 120 EC-C18 analytical column [4.6 mm inner diameter, 250 mm length, 2.7 µm particle size]; Agilent Technologies, Santa Clara, CA) maintained at 30 °C. We calculated total PPG concentration as the sum of all PPG concentrations for each individual.

Field common garden experiment

At the LPD site, seedlings were transplanted into a randomized block design to minimize microsite variation. As germination allowed, a single individual from each line was randomized in each block and we planted 12 blocks across the site (*N* = 1241 plants, ave. 10.0 seedlings/line). Plants were surveyed for flowering as in the greenhouse experiment and the same phenotypes were taken at flowering. Both foliar and floral herbivory were observed at flowering. Foliar herbivory was estimated as a proportion of tissue removed on all true leaves by a single observer (A. Scharnagl; Johnson et al., 2016). This measure is likely an underestimate because we miss plants that were completely eaten as seedlings (N = 16 or ~1.3%). Floral herbivory was estimated as a proportion of tissue removed for all flowers by the same observer. This estimate of floral herbivory is an underestimate of herbivory as the corolla would likely have had additional herbivory after the time of observation (generally 1–2 days longer before falling off). We note that sample numbers for foliar and floral herbivory differ because corollas occasionally fell off plant before trait data could be processed.

We also recorded a number of fitness measures. We surveyed each plant for survival every other day and recorded every new flower. At the end of the field season, we totaled up the number of flowers for each plant. We collected ripe fruits at multiple times across the growing season. However, we noticed that many of our fruits experienced predation, as has been consistent from other studies at this site (Kooyers et al., 2019). Thus, we approximated fruit predation as the proportion of fruits lost to herbivory relative to floral abundance. This only approximates true fruit predation as fruits may not have been completely consumed and thus some viable seeds could fall to the ground. Seeds from each fruit collected were manually counted. Fitness was approximated in two different ways. First, we consider "floral abundance" as one measure of absolute fitness with plants not surviving to flowering or not flowering assigned zero fitness. Second, we consider seed set as absolute fitness with plants producing no seeds assigned zero fitness. We consider both measures imperfect but valid as total flowers is likely a better proxy for male reproduction, but total seed set is likely a better proxy for female reproduction.

Statistical analysis

All statistical analyses were done in R version 4.0.5. We assessed the abundance of individual PPGs and correlations between PPGs and other important traits using data from all lines in our greenhouse garden. Patterns of variation in PPG arsenals were assessed in both types of lines as well as the grandparents of the phenotypically selected lines using a principal component analysis. We used a z-score transformation to center each individual PPG and then conducted a PCA using the pcaMethods package (ver. 1.60.0; Stacklies et al., 2007) with imputation of missing data using *svdimpute* function (0.004% of total data missing). We examine phenotypic correlations between individual PPGs as well as between total PPGs, flowering time, and number of leaves at flowering using Pearson correlations using the *Hmisc* package v4.2.0 (Harrell, 2015).

Assessing variation among populations in fitness

We assessed whether there was a signal of adaptation using ANOVAs and post-hoc comparisons between populations in a general linear model framework. We used two models, one with floral abundance as a response variable and a second with total number of seeds as a response variable. Both fitness measures were log-transformed. In both models, population was used as an independent variable. Linear models were implemented using the *lm()* function. Significance of associations between response and independent variables within models was assessed via ANOVA using the Wald χ^2 test implemented in the *car* package v3.0-7 (Fox et al., 2013). We then conducted Dunnett's Tests via the *DunnettTest()* function in the DescTools v0.99.31 package (Signorell, 2022) to compare each non-native population to the native population (LPD) using a 95% family-wise confidence level. A signature of adaptation would consist of the LPD population having significantly higher fitness than the other populations—i.e., a local-foreign effect. We note that this is not a signal for local adaptation, as this would require a second site and a reciprocal home-away effect in each population (Kawecki & Ebert, 2004).

To assess what factors were most important for adaptation, we modeled average population fitness as a function of geographic distance, environmental differences, and trait variation. Population averages for phenotypic and fitness variables were calculated as average values of all line means. We used univariate linear models with either log-transformed population averages for floral abundance or number of seeds as response variables and several different independent variables including geographic distance from LPD, latitude, elevation, mean annual temperature, date when the frost-free period begins, as well as the key phenotypic variables found in the analyses above (flowering time, number of leaves, total PPGs, ChemPC2, and individual PPG concentrations). Environmental variables were extracted from ClimateWNA (Wang et al., 2016). Statistical significance of associations was assessed as above with a Bonferroni correction to account for multiple testing. Because multiple traits may be responsible for variation in fitness and we are specifically interested in the impacts of defense traits on adaptation, we examined whether fitness was better explained by adding either total PPGs or any individual PPG concentrations as variables to the only significant univariate model (flowering time). We compared nested models with and without defense traits using the *anova()* function.

Assessing the importance of herbivory

To better understand the importance of herbivores within our field populations, we examined levels of herbivory as well as associations between herbivory and fitness. We summarized levels of foliar and floral herbivory and fruit predation on all individuals grown in the field common garden by examining means, standard deviations, and histograms for each herbivory type. To determine relationships among different types of herbivory, we ran pairwise Spearman correlations, which were used because of zero-inflation in the herbivory data. We examined the relationships at the individual scale between each type of herbivory (foliar, floral, and fruit predation) and absolute fitness (either based on floral abundance or number of seeds) using univariate linear models. All univariate linear models were conducted using *lm()* function with a fitness measure as a response variable and a herbivory measure as an independent variable. Fitness measures were log-transformed. Because there were clear non-linear patterns in the data, we also ran quadratic models where a squared herbivory term was included in each model. We chose which model to report based on model comparison via the *anova()* function, only using the quadratic model when it had a statistically better fit than the linear model. Significance of associations was assessed via ANOVA as above.

Assessing associations between traits, herbivory, and fitness

To holistically examine relationships between herbivory, chemical defense traits, life history traits, and fitness, we used a piecewise structural equation modeling approach

(PSEM) that included data from both outbred population lines and F_4 lines. We use PSEM to compare five hypothesis-driven models, with the aim of understanding how life history traits (including vegetative growth and reproductive time), resistance traits (total PPGs and PPG arsenal), and herbivory (foliar, floral, and fruit predation) directly and indirectly affect both measures of absolute fitness (floral abundance and seed set). Our full model (Figure 2) represents the hypothesis that life history traits, resistance traits, and herbivory all directly affect fitness, with resistance traits also indirectly affecting fitness through herbivory. The remaining four models are reduced models that are nested within the full model. Two "life history and defense" models (Figure 2B and C) represent different versions of the hypothesis that herbivory is unimportant, such that only life history traits and resistance traits affect fitness. The second model, but not the third, allows defense traits to affect herbivory. A "life history and herbivory" model (Figure 2D) represents the hypothesis that defense traits are unimportant, such that only life history traits and herbivory affect fitness. Finally, a "life history" model (Figure 2E) represents the hypothesis that only life history traits affect fitness.

Within each of the five categories above, there are multiple variables. In general, when we state that we allow one category to affect another, it means we draw all possible links between variables; for example, in Figure 2A, we allow each defense trait to predict each type of herbivory. However, we exclude the link between fruit predation and floral abundance, which would not be sensible as fruit predation occurs after flowers have senesced. We made composite variables for "vegetative growth" and "foliar herbivory" (Grace & Bollen, 2008). Vegetative growth was a composite of plant height at flowering and number of leaves, and foliar herbivory was a composite of the linear and quadratic effects of foliar herbivory.

All structural equation modeling was performed using package *piecewiseSEM* (Lefcheck, 2016). To meet model assumptions, each response variable was modeled using Poisson generalized linear mixed effect models (GLMMs), fit via penalized quasi-likelihood (*glmmPQL()* in package *MASS*). As above, population was included as a random effect with F_4 lines assigned as an additional population. We selected the best model via two criteria. First, each proposed PSEM has one model-wide *p*-value, which either rejects or fails to reject the hypothesized causal structure (meaning no important paths are omitted). Thus, a high model-wide PSEM *p*-value indicates that most or all the omitted paths are not statistically significant. For simplicity, we call a model "accepted" if PSEM fails to reject the hypothesized causal structure. Second, we compared bias-corrected Akaike information criterion (AICc) values across the five PSEMs (Akaike, 1973; Sugiura, 1978) as relying only on *p*-values may select for overly complicated causal structures. Finally, we chose the most promising model and refined the causal structure by adding significant paths that were not part of our initial hypotheses and removing non-significant paths. Thus, the final model may not fit neatly within any of the original hypotheses. This post-hoc refining is a recommended practice for structural equation modeling (Grace et al., 2012).

The above PSEM models suggests there are several interesting relationships between chemical defense and life history traits with herbivory. To assess these relationships in greater depth and connect these patterns to variation in relative fitness between populations, we modeled relationships between traits and herbivory using linear mixed models implemented using *lmer()* function in the *lme4* package v1.1-21 (Bates et al., 2014). Models included only line means of the outbred population lines. Each herbivory measurement (foliar, floral, and fruit predation) was treated as a univariate response

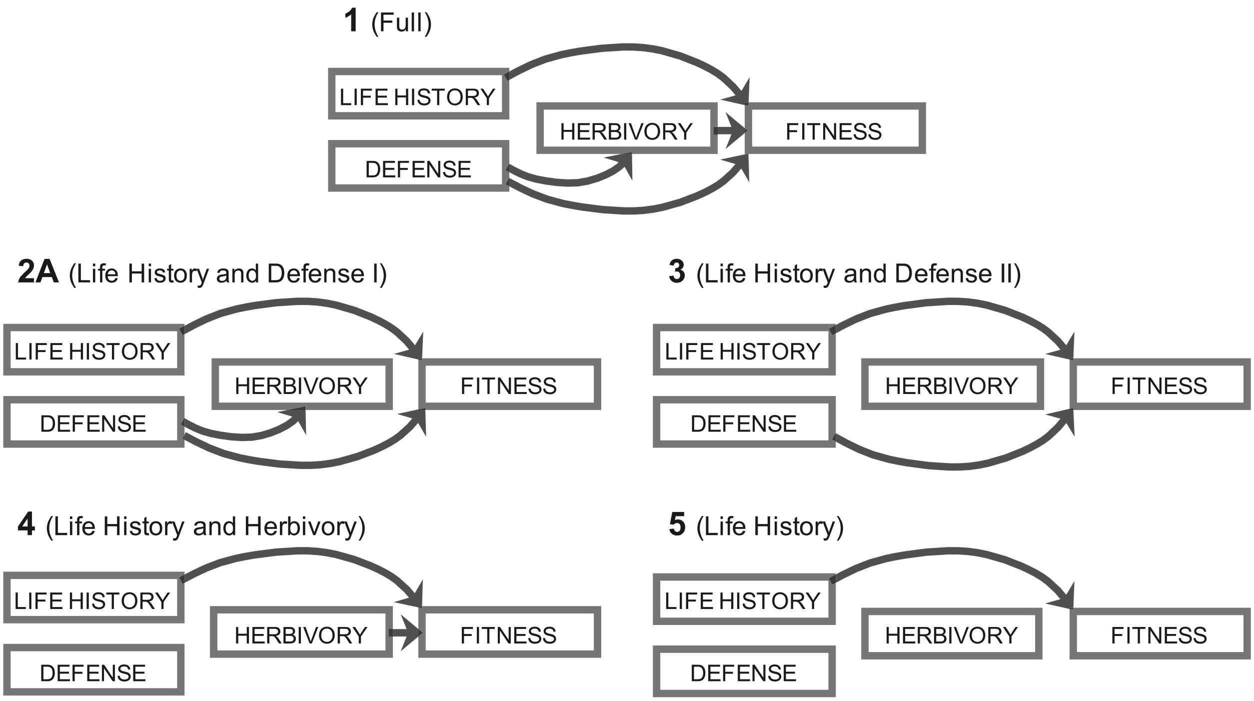

Figure 2. Conceptual diagrams for our full piecewise structural equation model (A) and four reduced models that are nested within the full model (B–E). There are multiple variables within each box of the conceptual diagrams (e.g., foliar herbivory, floral herbivory, and fruit predation within herbivory). The piecewise structural equation models are specified with these conceptual diagrams as a starting point, as described in the main text.

variable in separate models. In independent models, we assessed the association between each measure of herbivory and 10 different traits: total PPGs, chemical defense arsenal (ChemPC2), reproductive timing (flowering time), allocation to growth (number of leaves at flowering) as well as the concentration of each individual PPG. Models included population as a random term. Foliar herbivory was logit-transformed prior to modeling (Lim et al., 2015). However, similar preliminary models for both floral herbivory and fruit predation were strongly zero-inflated and produced poor model fits. Thus, we constructed hurdle models for each of these variables that subsequently examined whether each of the four traits were associated with any herbivory (Yes/No) via a generalized linear model with a binomial distribution and a logit link, followed by assessing whether the quantitative level of herbivory was associated with a particular trait using logit transformed data within a linear mixed model. We note that PPG concentrations were assessed only in foliar tissue and these concentrations are not necessarily correlated with PPG concentrations in flowers, fruits, or seeds. Significant associations between response and independent variables were assessed with ANOVA as above.

Results

Analysis of PPG concentrations in our greenhouse common garden indicated that populations vary dramatically in total PPG concentration as well as PPG arsenal, and that F_4 lines incorporated much of this variation (Figure 1B). Principal component analysis of PPG arsenals in our greenhouse garden demonstrated that levels of individual PPGs were highly correlated with one another and with flowering time (Figure 1C; Supplementary Appendix 2). The first principal component axis (34.5% of variation) loaded positively for each PPG, but higher levels of ChemPC1 were most closely associated with greater levels of conandroside, calceolarioside B, verbascoside, and mimuloside (Supplementary Appendix 3). Since conandroside concentration was the largest contributor to total PPGs, ChemPC1 was strongly correlated with total PPGs ($r^2 = 0.86$, $p < .001$) and for this reason we did not use it in models below to represent differences in defense arsenals. The second principal component axis (18.4% of variation) represents a tradeoff among concentrations of PPGs. Higher values of ChemPC2 represents lower levels of calceolarioside A, verbascoside, unknown PPG 10, and unknown PPG 16 and higher levels of conandroside. We use ChemPC2 to represent divergent arsenals of PPGs in all analyses below ($r^2 = 0.27$ with total PPGs). The loadings on the ChemPC1 and ChemPC2 axes are very similar to the PCA in previous studies of annual *M. guttatus* populations (Kooyers et al., 2017, 2020).

Variation in fitness among populations

There was significant variation in fitness among populations both for floral abundance and for number of seeds (Male: $F_{10,70} = 6.52$, $p < .001$; Female: $F_{10,70} = 4.26$, $p < .001$; Figure 3). On average, the native population, LPD, produced more flowers and seeds than all but one other population (Supplementary Appendix 4). However, Dunnett's tests reveal that a limited number of these comparisons between populations were statistically significant (5/10 for floral abundance and 2/10 for number of seeds). Indeed, much of the variation in either measure of fitness occurred within populations and we had a relatively limited number of lines per population. The only population that produced more flowers and seed than LPD on average was a high elevation population in the Sierra Nevada (BLD), 680 km away from LPD. This population produced 0.68 more flowers and 26.2 more seeds than LPD on average and neither pairwise measure of fitness was statistically significant (Floral Abundance: diff = 0.28, $p = .23$; Number of Seeds: diff = 1.18, $p = .23$).

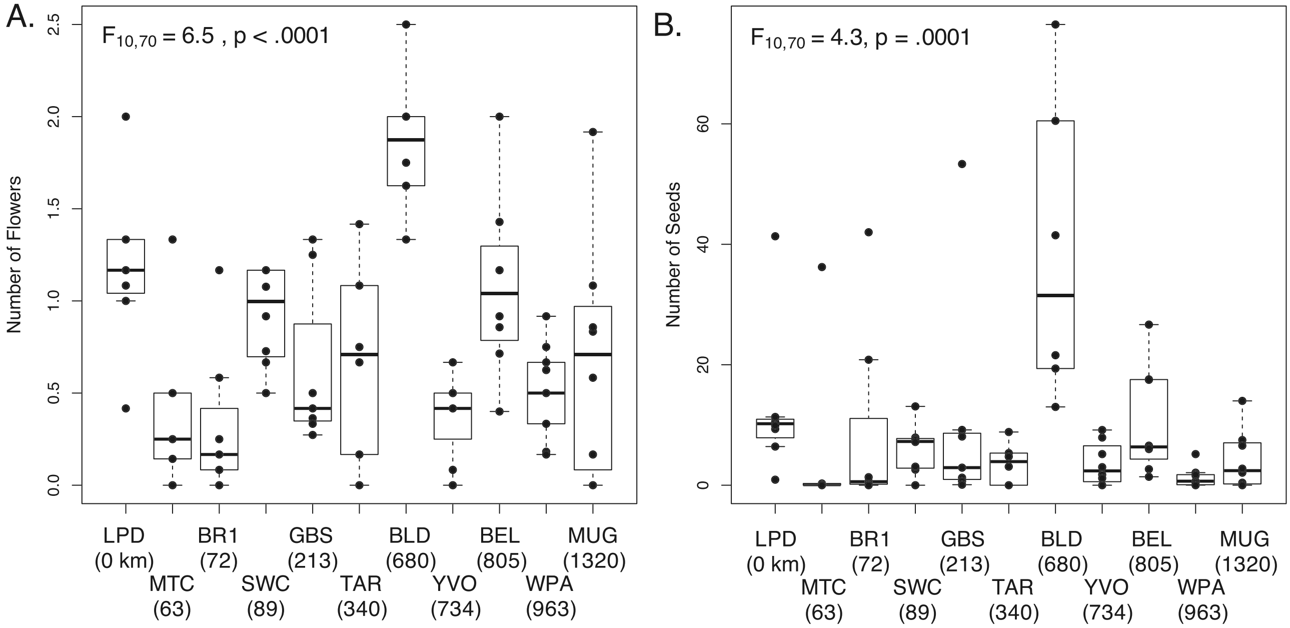

Figure 3. Variation within and among populations in floral abundance and seed production (A, B), respectively. The whiskers of the boxplot are the minima and maxima of the data without outliers, box lower and upper limits are quartiles and the heavy line is the sample median. Points represent line means. Values in parentheses below population names indicate the distance in kilometers the population occurs from the common garden site (LPD). Statistics come from ANOVAs examining variation among populations as described in the main text.

Neither geographic distance from the LPD site nor any environmental variable was associated with population averages for either fitness variable (Supplementary Appendix 5). The only significant association between population averages for traits and fitness was an association between flowering time and floral abundance ($F_{1,9}$ = 19.8, p = .002). However, the addition of defense arsenal (ChemPC2) to the models for either fitness variable improved model fit dramatically with models explaining either 96% or 84% of the variance in floral abundance or number of seeds respectively (Figure 4, Supplementary Appendix 6). Interestingly, lower values of ChemPC2 were associated with greater floral abundance while higher values of ChemPC2 were associated with producing more seeds. These relationships were driven by multiple PPGs including calceolarioside A and unknown PPG 10 (Supplementary Figure S2; Supplementary Appendix 6). Together, these results suggest that patterns of local adaptation or maladaptation are best explained by including both life history and defense traits.

Substantial herbivory impacts fitness

We measured foliar herbivory, floral herbivory, and fruit predation only on individuals that flowered. There was evidence of foliar herbivory on 49.7% of plants, evidence of floral herbivory on 23.3% of plants, and evidence of fruit predation on 46.4% of plants. Overall, 75.6% of plants had some type of herbivore damage. Damaged plants had an average of 4.9% (*SD* 11.3%) of leaf tissue missing, 4.5% (*SD* 12.8%) of floral (corolla) tissue missing, and lost an average of 36.8% of their fruits (*SD* 43.9%) to predation. However, no measure of herbivory was normally distributed (Figure 5A–C). The majority of plants had either no herbivory or < 5% foliar or floral herbivory with just a few plants exhibiting high levels of herbivory. Fruit predation nearly followed a binary distribution as plants often either had no fruits missing or lost all of their fruits. There were no significant associations between different types of herbivory—for example, plants with greater foliar herbivory were not more likely to have greater floral herbivory or fruit predation than those with lesser foliar herbivory (Supplementary Appendix 7).

Different types of herbivory had different relationships with fitness. While there was no relationship between floral herbivory and either floral abundance or number of seeds, there were clear linear and quadratic relationships between foliar herbivory and both fitness measures and between fruit predation and number of seeds (Figure 5; Supplementary Appendix 8). At low levels of foliar herbivory, there was a positive effect of foliar herbivory on both fitness measures (linear term: floral abundance: t = 4.7, p < .001; number of seeds: t = 3.3, p = .001), but this relationship became negative when foliar herbivory was high (quadratic term: floral abundance: t = −4.2, p < .001; number of seeds: t = −3.2, p = .002). Variation in fitness was high at low levels of herbivory. There was also a strong relationship between fruit predation and fitness (full quadratic model: female: $F_{2,103}$ = 18.4, p < .001, r^2 = 0.26). Plants with higher levels of fruit predation produced fewer seeds with higher levels of variance at lower levels of fruit predation. Additionally, there was a relationship between floral abundance and fruit predation, with plants that produced more flowers having fewer of them predated ($F_{2,103}$ = 10.6, p < .001, r^2 = 0.17). This suggests that fruit predation may have been intense for only a portion of the growing season.

Trait variation affects herbivory and fitness

We leveraged variation among range-wide populations of *M. guttatus* as well as within F_4 lines to examine how changes in chemical defenses (concentration and arsenal) as well as phenology and morphology affected herbivory and fitness. Our models reveal the importance of including both herbivory and life history traits when explaining fitness. Of the five tested PSEM models, only the “full model” and Life History and Defense IPSEMs were accepted, with each of the other reduced models judged to be missing important paths (Table 1).

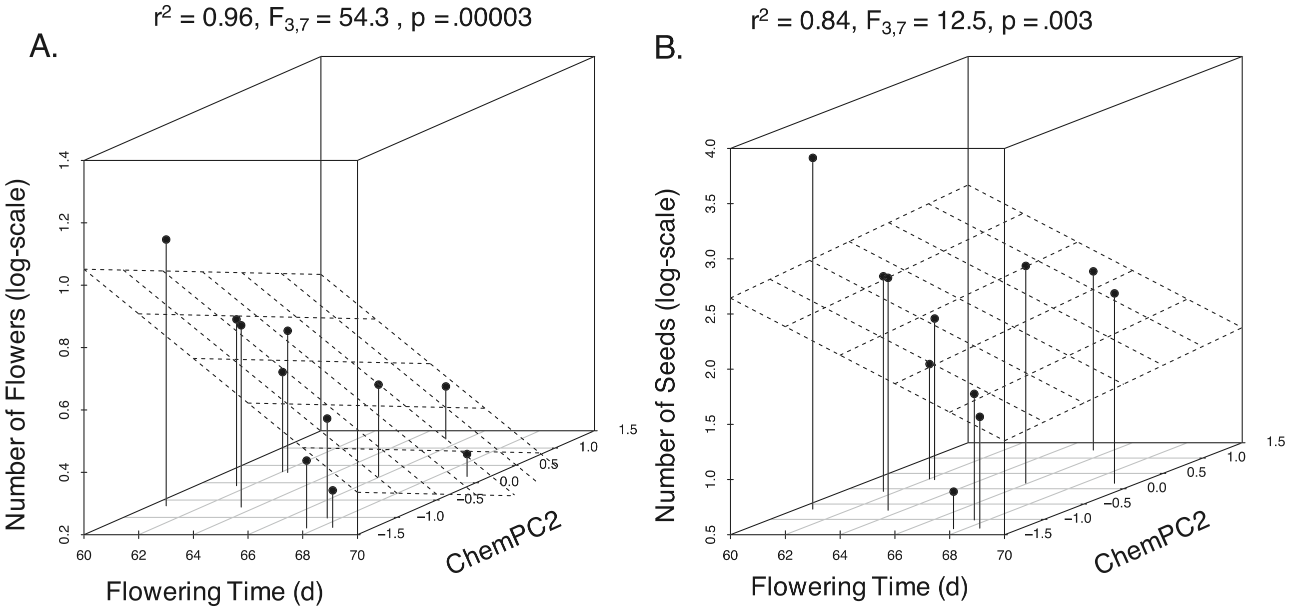

Figure 4. Relationships between population means for flowering time and ChemPC2 with floral abundance and seed production (A, B), respectively. *F*-statistics and *p*-values are the model fits derived from linear models described in the main text. Planes were created from these same models.

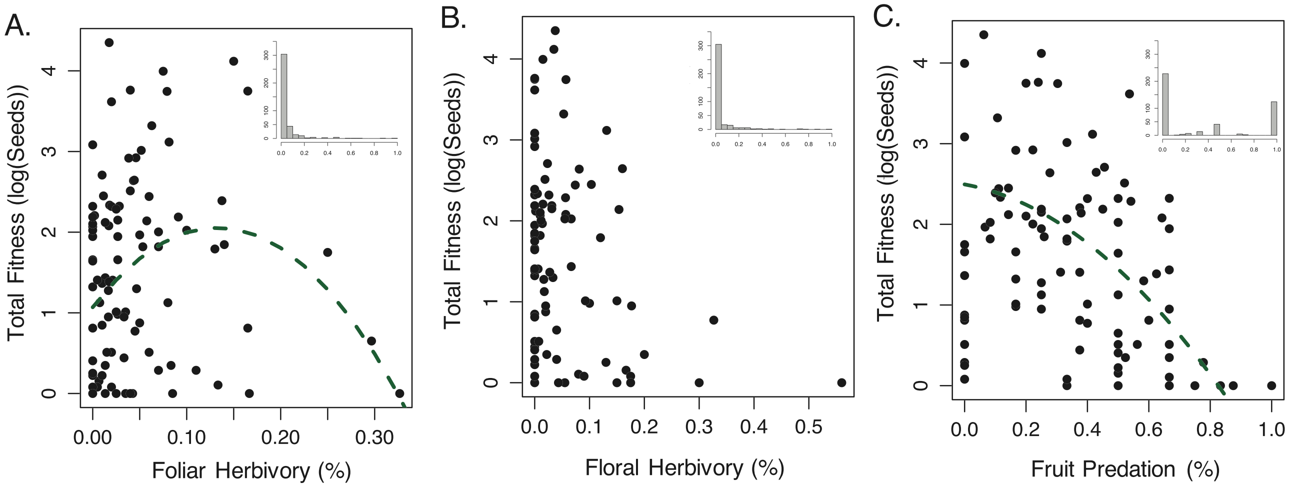

Figure 5. Relationship between in foliar, floral, and fruit predation and seed production (A–C). Inlayed graphs are histograms describing individual-level variation in foliar, floral and fruit predation. Best fit quadratic regression lines are only depicted if $p < .05$ and quadratic models better fit data than linear models via the model selection criterion described in the main text.

Table 1. *p*-values and AICc scores for the five hypothesized piecewise structural equation models, and the refined model.

Model	*p* value	AICc
Full Model	**.076**	**161.92**
Life history and Defense I	**.068**	**161.12**
Life history and Defense II	.002	114.23
Life history and Herbivory	.008	108.99
Life history	.007	106.22
Refined Model	**.481**	**124.38**

Note. For each model, the proposed causal structure is the null hypothesis. Bolded rows (those with $p > 0.05$) are therefore models with proposed causal structures that were accepted. AICc measures model quality by assessing how well a given model fits a given dataset, while accounting for model complexity; lower AICc values indicate higher model quality. We prefer the refined model as it has the lowest AICc score among models with accepted causal structures.
AIC = Akaike information criterion.

However, the full model was overly complicated: it contained many non-significant paths (Figure 6A) and its AICc value was much higher than any of the reduced models (Table 1). We refined the full model by removing non-significant paths and adding two new paths, first between flowering time and number of seeds, and then between flowering time and fruit predation (Figure 6B). The refined model was accepted and had a lower AICc than the full model (Table 1). The refined model reveals that life history traits and herbivory directly affect fitness, but defense traits affect fitness only indirectly through their effects on herbivory. More specifically, there was a highly significant link between total PPGs and foliar herbivory ($p = .009$), as well as a weaker link between total PPGs and floral herbivory ($p = .074$), and a non-significant relationship between PPG defense arsenal and fruit predation ($p = .108$).

To better dissect the relationships between traits, herbivory, and fitness that were viewed as important within the PSEM models, we modeled and visualized significant associations within the PSEM models using linear mixed models. We first examined predictors of different kinds of herbivory. Foliar herbivory was associated with variation in flowering time, number of leaves at flowering, and total PPGs (Figure 7, Supplementary Appendix 9). Lines that flowered later, had more leaves at flowering, and had higher total PPG levels had lower percentages of foliar herbivory (flowering time: $\chi^2 = 4.2$, $p = .04$; number of leaves: $\chi^2 = 3.8$, $p = .05$; total PPGs: $\chi^2 = 13.0$, $p < .001$). Notably, there was no association in PSEM between flowering time and foliar herbivory, and this association may stem from the correlation between Total PPGs and flowering time.

Relationships between either floral herbivory or fruit predation with defense traits are more complex. It is important to note that we measured phytochemical defenses only in foliar tissue, and these concentrations may or may not be related to phytochemical defenses in corolla or fruit tissue. We therefore have more confidence in the models that include foliar herbivory as a response and recommend more caution when interpreting other herbivory models. In the PSEM models, floral herbivory was positively associated with total PPGs. Our hurdle models reveal that the presence or absence of floral herbivory and the quantitative amount of herbivory were associated with total PPGs, but in opposite directions. Lines with no floral herbivory had greater total foliar PPGs than lines that had some floral herbivory ($\chi^2 = 3.8$, $p = .05$); however, among lines that had floral herbivory, lines with greater floral herbivory had higher levels of total PPGs ($\chi^2 = 5.6$, $p = .02$). The relationship among lines with some floral herbivory was largely driven by a single line that had the highest floral herbivory of any line (Figure 7EF). In PSEM models, fruit predation was associated with foliar defense arsenal. Hurdle models reveal that lines that had fruit predation had greater values of ChemPC2 ($\chi^2 = 4.3$, $p = .04$). We explored which foliar PPGs may have driven this relationship. The only individual foliar PPG related to the presence or absence of fruit predation was calceolarioside A, where lines with lower levels calceolarioside A in the foliar tissue are less likely to have fruit predation ($\chi^2 = 4.0$, $p = .04$). Additionally, lines with greater concentrations of unknown PPG 10 had higher quantitative levels of fruit predation ($\chi^2 = 6.4$, $p = .01$). Our refined PSEM model also indicates there is a relationship between flowering time and fruit predation. Indeed,

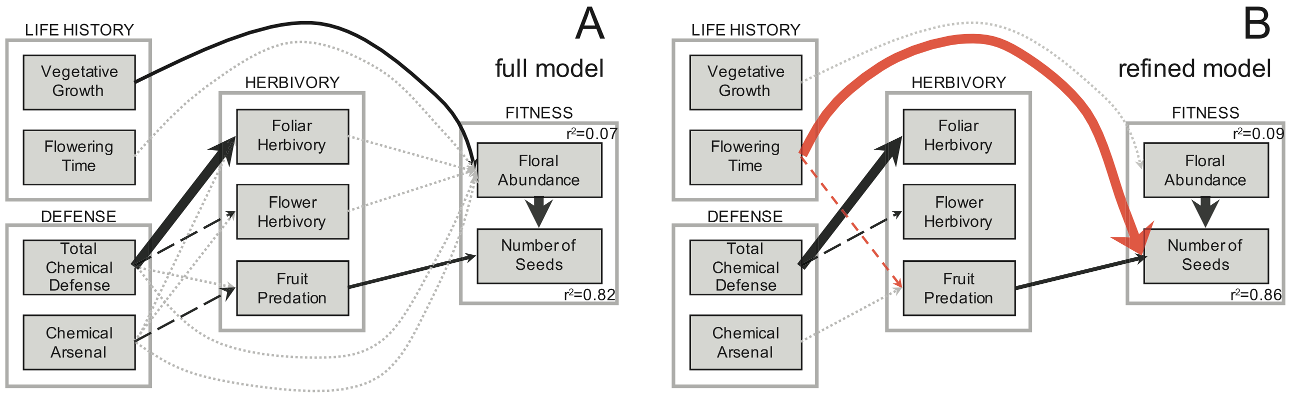

Figure 6. Results for two piecewise structural equation models: the full model (A), and a refined model (B). Arrow width and pattern indicate the sign of regression coefficients and the corresponding *p*-values: dotted lines, $p > .10$; dashed lines, $.05 < p < .10$; thin solid lines, $.01 < p < .05$; thick solid lines, $p < .01$. Black and red arrows indicate positive and negative regression coefficients, respectively. Paths with $p > .10$ are always gray to reflect uncertainty.

plants that flowered earlier had fewer fruits eaten ($\chi^2 = 7.3$, $p = .007$), possibly reflecting differences in intensity of fruit predation throughout the growing season.

PSEM models suggest that growth and reproductive traits directly impact fitness while chemical defense traits impact fitness only through herbivory. Our linear models suggest that growth and reproductive traits influence both floral abundance and number of seeds rather than just one or the other (Supplementary Figure S3, Supplementary Appendix 10). Lines that flowered earlier had greater floral abundance ($\chi^2 = 14.0$, $p < .001$) and number of seeds ($\chi^2 = 22.4$, $p < .001$). Lines with more leaves at flowering produced more seeds ($\chi^2 = 5.61$, $p = .02$), and produced marginally more flowers ($\chi^2 = 2.83$, $p = .09$). As in the PSEM, there were no direct relationships between either the levels or arsenals of foliar chemical defenses (i.e., total PPG concentration or ChemPC2, respectively) and either floral abundance or seed production. Thus, it appears that resistance traits affect fitness only indirectly through relationships with other variables such as herbivory.

Discussion

We demonstrate the relative roles of plant resistance traits and life history in creating variation in fitness among populations. The native population had higher fitness on average than nearly every population—except a single population from over 600 km away. This seemingly odd result can be explained in the context of trait variation. That is, differences in fitness between populations are nearly entirely explained by variation in flowering time and the phytochemical defense arsenal. Our non-linear models and PSEMs break down how these traits impact fitness as mediated by herbivory. Earlier flowering and rapid growth are favored at this site and directly impact fitness. However, there is also substantial herbivory. Defenses, including total PPG and PPG arsenal, are associated with herbivory, but are only indirectly correlated with fitness. Notably, the populations with the highest fitness also have a divergent PPG arsenal and high levels of fruit predation. These populations produce more seeds only because they produce more flowers. Below we discuss these results in the context of the two fields that they synthesize—plant–herbivore interactions and the evolutionary ecology of adaptation.

Role of abiotic factors and biotic interactions in generating adaptation

Over time, selection on traits in populations in divergent environments should create local adaptation and cause differentiation in phenotypes between populations based on the environmental characteristics of each population. While local adaptation is frequently found in reciprocal transplant experiments (Hereford, 2009; Leimu & Fischer, 2008), experiments that compare many populations within the same common garden are less common and have less frequently identified a home site advantage (Anderson & Wadgymar, 2020; Hereford & Winn, 2008). The home site in this study, LPD, had higher average fitness than all but one population. Interestingly the population with the highest average fitness was a population from >600 km south in the Sierra Nevada. This is similar to a past finding at the same Oregon common garden site in that a low-elevation Sierra Nevadan population had higher fitness than native populations (Kooyers et al., 2019). Here, we planted more populations that spanned a wider degree of environmental conditions than in previous work to identify factors predictive of variation in fitness. We find no relationship between the geographical or climatic distance between populations and the average fitness. In fact, populations that are geographically close to the site of the common garden do not have relatively high fitness. Of the three populations that occur within 100 km of LPD (SWC, MTC, BR1), all have lower average fitness with two of these sites (MTC and BR1) having the lowest fitness of any of the populations surveyed.

Rather than geographic distance or climate, our results suggest that the key determinant of relative fitness relationships between populations is the phenotypic composition of a population. We find that variation in fitness at a population scale is nearly entirely explained by the average flowering time and the foliar phytochemical defense arsenal of the population (Figure 4). Interestingly, the phytochemical arsenal that is most beneficial depends on which measure of fitness is assessed. Populations with lower foliar ChemPC2 values (high relative concentrations of calceolarioside A, unknown PPG 10, and verbascoside) produce more flowers, but fewer seeds. This difference becomes clearer when taking into consideration the relationship between fruit predation and ChemPC2, where populations with higher foliar ChemPC2 values have less fruit predation, but also produce less seeds per flower. In a broader geographical context, phytochemical arsenal varies dramatically across the range

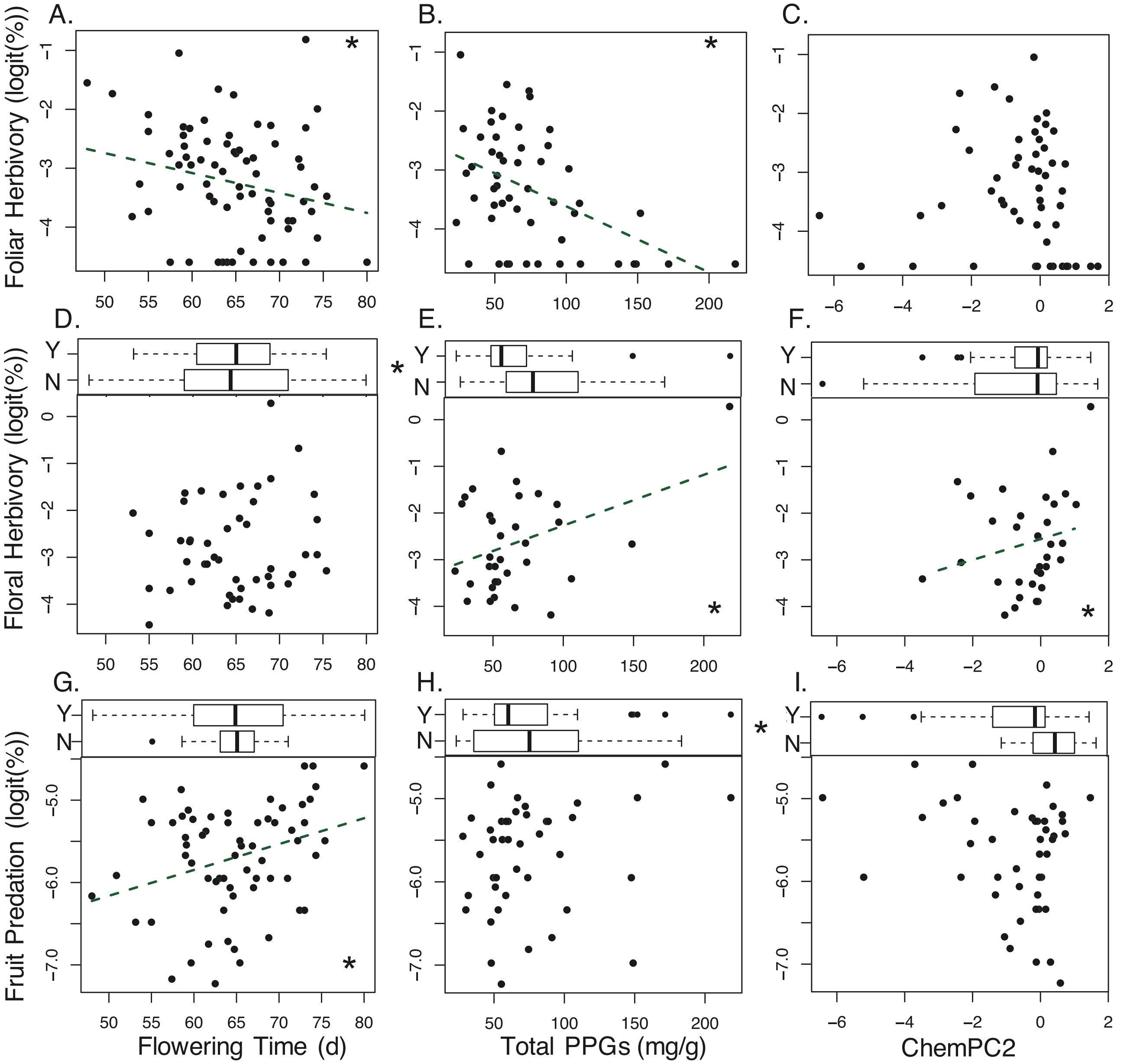

Figure 7. Relationships between foliar herbivory (A–C), floral herbivory (D–F), and fruit predation (G–I) with flowering time, total phenylpropanoid glycosides (PPGs), and PPG defense arsenal as summarized by ChemPC2. All points are line means from outbred population lines. Regressions lines are taken from models that include population as a random factor and are only depicted when statistically significant at $p < .05$. Boxplots examine floral and fruit predation as a discrete variable and asterisks indicate significant relationship with each phenotype. Scatterplots and regressions for floral and fruit predation do not include lines with zero damage. All PPG concentrations refer to mg/g dry weight.

of *M. guttatus*, going from the low to high ChemPC2 values from California to Oregon (Figure 1, Kooyers et al., 2017). The major exception to this latitudinal gradient is the population from our common garden site, LPD, which is more similar phytochemically to the low elevation California populations than the Oregon population (Figure 1). Thus, we hypothesize that a key target of selection in the LPD site is phytochemical defense arsenal which, along with flowering time, promotes adaptation of the native population.

Quantifying herbivory is important for understanding patterns of adaptation

The role of chemical defenses and herbivory in shaping fitness is less clear than the relationship between life history traits and fitness. Herbivore pressure is often found to be a key selective factor for perennial plant species, but there is less evidence for its role in shaping patterns of local adaptation in short-lived annual plant species (Cogni & Futuyma, 2009; Erb, 2018). Here, measuring herbivory on multiple tissues seems important as each type impacts a substantial number of plants (>23%) and most plants had some form of damage (75.6%). More importantly, both foliar herbivory and fruit predation had significant non-linear relationships with fitness (Figures 2,5 and 6). Interestingly, a negative relationship between foliar herbivory and fitness exists only when examining individual plants at the highest levels of foliar herbivory. This non-linear pattern suggests that plants tolerate low levels of foliar damage well and can partially or fully compensate for this tissue loss as has been observed in other species (Carmona & Fornoni, 2013). We note that death due to foliar herbivory during early development may be a strong effect that is not included here and is the most difficult to observe and quantify.

Fruit predation had a much stronger negative relationship with fitness and a significant portion of plants lost all potential reproductive output due to fruit predation (29%; Figure 5). Fruit predation in this system stems at least partially from Lepidoptera larvae and weevils (Rotter & Holeski, 2017) as well as potentially from vertebrate browsers and birds (Rotter, 2020). The importance of the link between fruit predation and fitness is reflected in our PSEM models (Figure 6), as those that include herbivory are of higher quality both in terms of p-value and AIC criterion (Table 1). However, fruit predation also represents a paradox as the PSEM suggests that *greater fruit predation* is associated with producing higher numbers of seeds—exactly the opposite of the conclusions at the individual level. We address this conundrum below in the context of trait interactions between foliar defense arsenal (ChemPC2) and flowering time.

Life history traits influence fitness both directly and indirectly

The relative importance of life history, chemical defenses, and herbivory as drivers of variation in fitness remains a knowledge gap. The most apparent result within our common garden is the importance of life history traits—earlier flowering and rapid growth are strongly selected (Figure 4, Supplementary Figure S3). These results are not unique, as past results from this system have demonstrated a strong connection between life history traits and fitness in the field (Hall & Willis, 2006; Kooyers et al., 2019; Troth et al., 2018). Growing seasons are starting much earlier than historical averages at the LPD site and create strong selection for earlier flowering (Kooyers et al., 2019). However, our PSEMs provide a more nuanced view of how these traits affect fitness. Not only does flowering time directly impact fitness, but it also may indirectly impact fitness by changing patterns of herbivory. Plants that reproduce later had higher levels of fruit predation. This is consistent with temporal variation in herbivore presence, where there may be fewer herbivores when early flowering lines produced seeds (e.g., Krimmel & Pearse, 2015). The combined effects of flowering time and herbivory were highly predictive of the number of seeds produced for a given plant (Figure 6B, $r^2 = 0.86$). Thus, any future selection pressure on flowering time (e.g., earlier growing seasons) will influence defense and herbivory as well as fitness through fruit predation.

Chemical defense arsenals indirectly influence fitness through fruit predation

It is logistically very difficult to characterize links between compound concentrations, herbivory, and plant fitness in nature (Erb, 2018). We found distinct associations between foliar PPG concentrations and/or arsenal with different types of herbivory, but no direct links between defense levels or arsenal and fitness. PSEMs including links between defense, herbivory, and fitness were clearly better than PSEMs where either (1) defense does not contribute to herbivory and/or fitness or (2) herbivory does not contribute to fitness. The refined model provides more nuance and suggests that defense influences fitness only indirectly, by changing patterns of herbivory. Phytochemical defense arsenal is more strongly linked to herbivory and fitness than is total PPG concentration, and is also a significant predictor of fitness differences among populations. Plants with foliar arsenals tending toward high values of ChemPC2 (lower relative concentrations of calceolarioside A, verbascoside, and unknown PPG 10) are less likely to incur fruit predation. This result could suggest that certain PPGs act as attractants while others act as deterrents for potential fruit predators as has been demonstrated in laboratory experiments (Holeski et al., 2013; Rotter et al., 2018).

We note a few important limitations to our above conclusions regarding relationships between PPGs, herbivory, and fitness. First, we measured PPG concentrations only in foliar tissue; this could impact our conclusions and interpretation if PPG concentrations and arsenals in the flowers and fruits are not positively correlated with foliar arsenals. Empirical evidence in other systems suggests that positive correlations across tissue types are not always the case for chemical defenses. Defense concentrations may be lower, higher, or uncorrelated across tissues and/or arsenals may differ (Alves et al., 2007; Brown et al., 2003; Miranda-Pérez et al., 2016; Tuller et al., 2018; Whitehead & Bowers, 2013; Whitehead et al., 2013). Preliminary studies suggest that at least some of the PPGs present in the foliar tissue of *M. guttatus* are also present in corollas and seeds (Holeski, personal observation). Defense arsenals in fruit tissue are unknown. However, we suggest these associations are important to include because inclusion of these paths in our refined PSEM model substantially improved the model fit and justified the follow up analysis. Second, we measure only constitutive production of PPGs in a greenhouse setting rather than any changes in PPG production due to induction by herbivores. Previous work has shown that there is little induction of PPGs in the annual populations of *M. guttatus* used here, but this study used only mechanical induction versus actual herbivores (Holeski et al., 2013; Kooyers et al., 2017). Finally, we note that this study only included a single year and there is likely temporal heterogeneity in selection pressures between years (e.g., Anderson & Wadgymar, 2020; Lowry et al., 2019a; Troth et al., 2018). However, similar selection pressures (life history and herbivore pressure) were found in 2014 at the same common garden site (Kooyers et al., 2019), suggesting that this selective regime is relatively frequent.

Synthesis between trait models and variation in fitness between populations

The associations between ChemPC2, flowering time, fruit predation, and fitness in our linear models can help test trait-based hypotheses explaining variation in relative fitness among populations. Variation in fitness among populations was best explained by defense arsenal and flowering time, but the favored defense arsenal differed between fitness metrics (Figure 4). Our LMM and PSEM clear up the relationships between defense, herbivory, and fitness, suggesting that lines with lower ChemPC2 values (as in the native population) are more likely to have fruit herbivory, and that greater fruit herbivory is associated with producing *more* seeds (Figures 6 and 7). Unlike in the population-scale models, lines with lower ChemPC2 do not necessarily produce more flowers; however, we have limited information to draw this comparison within models at a maternal-line scale as lines with low ChemPC2 all come from just a few populations. The fitness benefit of having the defense arsenal associated with lower ChemPC2 is yet undetermined. However, this hypothesis would explain why nearby populations do very poorly compared to the native populations—they flower later (BR1, MTC) and have very divergent PPG arsenals (SWC, BR1, and MTC)—but also have lower fruit herbivory. Alternatively, the California population with the highest fitness (BRD) is very phenotypically similar to LPD in terms of phytochemical arsenal and flowering time. In sum, these results suggest that the scale of adaptation may be very fine in this highly variable outcrossing species and reflects biotic conditions as well as abiotic conditions that are more often studied.

Conclusion

Our results suggest that growth rates, flowering times, and defense traits each play a substantial and interactive role in structuring variation in fitness within a natural population. We find that variation in traits (flowering time and foliar phytochemical defense arsenal) rather than historical environmental data or geographic distance is strongly predictive of population level differences in fitness. While life history is directly and strongly predictive of fitness within this population, there are also indirect relationships where different types of herbivory mediate the relationship between life history traits or foliar phytochemical defense arsenal and fitness. Notably, there are also tradeoffs to possessing the traits linked to high fitness—populations that have the highest fitness also have the highest fruit predation. More broadly, this work exemplifies the complex fitness landscape, complete with genetically-correlated traits with multiple functions, that responses to selection will need to navigate to respond to changing climates.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut/qpac048)

Data availability

All data from the greenhouse common garden and from the field common garden study are deposited on Dryad (https://doi.org/10.5061/dryad.p5hqbzkss).

Author contributions

N.K., A.S., and L.H. conceived the ideas and designed the methodology. A.S., L.H., and N.K. collected the data. N.K. and M.G. analyzed the data. N.K. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Conflict of interest: The authors declare no conflict of interest.

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