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BRIEF COMMUNICATION

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Annual-perennial lifespan variation in *Chaenactis douglasii* suggests a drought escape strategy in warm-arid environments

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Abstract

Premise: Intraspecific variation in drought resistance traits, such as drought escape, appear to be frequent within wild, ruderal forb species. Understanding how these traits are arrayed across the landscape, particularly in association with climate, is critical to developing forbs for wildland restoration programs. Use of forbs is requisite for maintaining biological diversity and ecological services.

Methods: Using 6074 greenhouse-grown *Chaenactis douglasii* seedlings from 95 wild, seed-sourced populations across the western United States, we recorded bolting phenology and estimated genome size using flow cytometry. Mixed-effects regression models were used to assess whether climate of seed origin was predictive for bolting phenology and genome size.

Results: Variation in bolting, reflecting an annual vs. perennial lifespan in this species, was observed in 8.7% of the plants, with bolting plants disproportionately occurring in locations with warm, arid climates. Populations with increasing heat and aridity were positively correlated with observed bolting ($r = 0.61$, $p < 0.0001$). About one-third (22%) of the total (61%) lifespan variation was attributed to seed source climate and annual heat moisture index, a measure of aridity. Genome size had no significant effect on bolting. Projected climate modeling for mid-century (2041–2070) supports an increasing occurrence of annual lifespan.

Conclusions: Our analyses support a drought escape, bet-hedging strategy in *C. douglasii*. Populations exposed to greater aridity exhibited a higher proportion of individuals with an annual lifespan. Drought escape leading to an annual lifespan can affect how seeds are propagated and deployed for climate-informed restoration.

KEYWORDS

bet-hedging, bolting, climate change, dehydration escape, genome size, local adaptation, polyploidy, restoration

INTRODUCTION

Plant traits are typically attuned to local environments and a single fitness optimum. Highly varied environments can, however, confound trait optima, leading to bet-hedging strategies where alternate phenotypes persist in the population because of stochastic environments and varied fitness optima in different years (Starrfelt and Kokko, 2012). These plant species can alter their lifespan by varying the length or timing of vegetative and reproductive stages, becoming annuals, biennials, or perennials. Under certain years and conditions (e.g., drought years), alternate phenotypes may have fitness advantages (Kooyers et al., 2015; Welles and Funk, 2021). Three different drought adaptive strategies have been identified: escape, avoidance, and resistance (Kooyers, 2015). For example, drought escape, also known as dehydration escape (Volaire, 2018), is a reproductive strategy employed to avert drought by rapidly growing and reproducing prior to the onset of these events. Drought escape typically manifests as an adaptive strategy for populations in xeric environments (Franks, 2011; Paccard et al., 2014; Kooyers, 2015). For example, *Mimulus guttatus*

showed strong clines in flower phenology variation and other traits that relate to drought escape (Kooyers et al., 2015), and in *Brassica rapa*, offspring of plants under drought displayed earlier flower phenology than their predecessors (Franks et al., 2007).

Chaenactis douglasii Hook. & Arn. (Asteraceae) is a widespread native forb found in the semi-arid, intermountain regions of the United States and Canada (USDA NRCS National Plant Data Team, 2023). It is a ruderal species, predominantly found in disturbed and degraded soils and widely adapted, occurring from alpine (3000 m) to sagebrush steppe (100 m) ecosystems. This species plays an important role in advancing successional ecological development by capturing debris and building soil on barren soil types (Tilley et al., 2010; Gucker and Shaw, 2018). *Chaenactis douglasii* is described as a facultative biennial, a short-lived perennial, and rarely an annual (Cronquist et al., 1994). Indeed, *C. douglasii* var. *montana* M. E. Jones, a recognized synonym for *C. d.* var. *douglasii*, was more consistently a perennial at higher elevations (2100–3500 m; Cronquist et al., 1994). Similar to other forbs (Kooyers, 2015), the variation between annual and perennial life history could suggest that a drought escape adaptive strategy may be present in *C. douglasii*. Its broad occurrence and pollinator dependence have garnered interest for its use in revegetation seed mixes in post-fire restoration, reclaimed mines, and other disturbed sites (Gucker and Shaw, 2018). However, understanding how adaptive traits are arrayed on the landscape and influenced by the environment is necessary before developing restoration seed supply. This knowledge can increase plant establishment success, resiliency, and mitigate effects from climate change (Kilkenny, 2015; Richardson and Chaney, 2018).

Limited research has been directed toward adaptive genetic traits in wild forb species. Traits affecting drought resistance strategies are of particular interest for forbs occupying the arid regions of western North America. Genome size variation (i.e., within ploidy or whole genome duplication) is a common feature among angiosperms. Because this genome size variation can influence a variety of adaptive traits (e.g., Bilinski et al., 2018; Richardson et al., 2021; Blonder et al., 2023) that can foster species range expansion (Oh et al., 2022), it is important to evaluate potential trait–genome size interactions. Moreover, genome size information is needed to develop seed increase strategies in cultivation to avoid seed production losses from introducing reproductive incompatibilities caused by mixed ploidy (Kramer et al., 2018). Given the need to augment the use of forbs in restoration to maintain biological diversity as well as pollinator- and wildlife-friendly habitats (Dumroese et al., 2015a), an understanding of the genetic and environmental factors shaping intraspecific adaptive variation is critical to guide their appropriate use in restoration.

In the case of *C. douglasii*, taxonomic descriptions have noted annual, biennial, and perennial lifespan variation (e.g., Cronquist et al., 1994) and extensive, range-wide chromosome counts have found this species persists as a diploid ($2n = 12$), triploid, tetraploid, and hexaploid (Mooring, 1980). Data derived from greenhouse-grown collections of *C. douglasii* were used to analyze the relationships between lifespan variation, seed-origin climate, and genome size (1°C value). Here we ask three questions. (1) Does first-year flowering defining an annual lifespan—hereafter referred to as "bolting"—vary among *C. douglasii* populations? (2) Is landscape-level bolting variation explained by genome size variation or seed-origin climate? (3) Could climate change affect the predicted prevalence of lifespan variation? We hypothesize that populations with increasing propensity for summer drought are associated with an increasing frequency of an annual lifespan, and genome size is correlated with lifespan variation.

MATERIALS AND METHODS

In 2018, seeds were collected from about 50 individuals within each of 95 wild-sourced populations. Seed collection was informed by historic herbarium records and the expertise of botanists familiar with *C. douglasii*. Populations were selected to maximize representation of both the number of populations and the breadth of environmental conditions, while maintaining a minimum distance of 10 miles and/or at least a 300 m elevation difference between populations. Within-population sampling was done haphazardly within a 0.5 hectare polygon. Seeds of each population were bulked, cleaned, and stored at 4°C until mid-March 2019, when seeds were sown into containers (3.8 cm diameter, 21 cm depth, 164 mL volume, 528 seedlings m^{-2}; SC10 Ray Leach Cone-tainer, Stuewe and Sons, Tangent, Oregon, USA) and filled with a 1:1 (v:v) sphagnum peat moss:vermiculite substrate (Forestry #1, SunGro Horticulture, Agawam, Massachusetts, USA). Because germination was unknown, we sowed approximately six seeds per container to ensure the presence of at least one germinant. Containers with multiple germinants were thinned to one seedling after true leaves emerged, achieving a total of 6624 plants among 95 populations. Seedlings were grown in a semicontrolled greenhouse at the U.S. Department of Agriculture, Forest Service in Moscow, Idaho, USA (46.72N, −117.00W). Temperatures and photoperiod were ambient, except when heating was used to keep early spring minimum temperatures >5°C. Seedlings were fertigated with a complete, soluble fertilizer (Peters Professional 20N-20P_2O_5–20K_2O General Purpose Fertilizer, Allentown, Pennsylvania, USA) at 100 ppm nitrogen each time container water content reached 60–70% (scientist technique; Dumroese et al., 2015b).

Flow cytometry was conducted on 266 samples, equating to two to five plants per population, to estimate genome size. Fresh leaf tissue, about 0.5 cm^2, was added to approximately 0.25 cm^2 of internal standard, *Acer negundo* L., 1 C value = 0.54 pg (Loureiro et al., 2007). Genome size of the *A. negundo* standard was confirmed with the universal standard *Pisum sativum* L. cv. "Ctirad". The tissues were finely chopped with a razor blade in 330 mL of buffer (Nuclei Extraction Buffer, Sysmex, Kobe, Japan), and the

solutions were filtered with a 30 mm mesh sieve. The filtered solution was eluted with 1.3 mL of staining buffer (Cystain UV Precise P, Sysmex, Kobe, Japan). Stained samples were incubated at room temperature for about 1 min prior to analysis on the flow cytometer (Cyflow Space, Sysmex, Kobe, Japan) under ultraviolet (UV) fluorescence (375 nm). Genome size (1C-value) was calculated relative to the internal standard (Doležel et al., 2007). Periodically, samples were replicated to evaluate consistency in flow cytometry results. We chose not to use putative cytotypes in the analyses given the overlap among samples that would likely lead to erroneous cytotype assignment (Appendix S1).

Bolting tallies were conducted every four to five days from July 15 to September 27, 2019. We defined bolting as present (1) for each individual plant when a flowering stem and flower development were observed, or absent (0) if no reproductive structure appeared. We assumed that nonbolting plants would flower in subsequent years. By the end of September, bolted plants were either dead or dying (a product of *C. douglasii*'s semelparous reproductive strategy). For assessing bolting and climate variable correlation, bolting frequency was calculated for each population by dividing the number of bolted plants by the total number of plants.

Two mixed-effects models, a generalized linear mixed model (GLMM) and a linear mixed model (LMM), were developed for investigating predictor variables of bolting and genome size variation, respectively. Climate data for each population location were obtained from ClimateNA v6.40b (available at http://tinyurl.com/ClimateNA) based on methodology described in Wang et al. (2016), utilizing contemporary and projected climate data sets. Midcentury climate data (2041–2070) were acquired using an ensemble of eight general circulation models under the SSP5 climate scenario (see below). Climate variables were used as predictors. A total of 15 relevant climate variables representing the "normals" of a 30-year period (1991–2020); annual and seasonal estimates of air temperature, precipitation, and their interactions (Appendix S2); and genome size were used to assess associations with bolting. Two criteria, a Pearson's correlation coefficient of $|r| > 0.4$, and low collinearity ($|r| < 0.7$), were used in choosing climate variables as predictors in a general linear mixed-effects model (GLMM).

For the GLMM analysis, bolting was treated as binary data for each individual plant (i.e., present or absent). The GLMM was developed using glmmTMB v 1.1.8 (Brooks et al., 2017). Climate variables and cytotype were assigned as fixed effects, while site seed sources (i.e., populations) were assigned as random effects. A binomial distribution model was used in the GLMM. Initially, models were fitted with a zero inflation (Tweedie) distribution model, but this was not significant and was removed. The best fitting GLMM was determined with the analysis of variance (ANOVA) function using the Akaike information criterion (AIC), using the tab_model function in sjPlot v 2.8.15 (Lüdecke, 2023), and utilizing ggplot2 v 3.4.4 to construct graphical data representations (Wickham, 2016). Predicted fixed effects values were generated with the PREDICT function and transformed to predict bolting using the RESPONSE function within the glmmTMB package. Analyses were conducted using the R statistical framework v 4.3 (R Core Team, 2023). The data and scripts can be found at GitHub (see Data Availability section below). To predict the trajectory of bolting with climate change, annual heat moisture (AHM) values were extracted from the SSP585 pathway of Coupled Model Intercomparison Project 6 (CMIP6) in ClimateNA (see above). The SSP585 pathway represents the socio-economic conditions producing the highest greenhouse gas emissions.

RESULTS

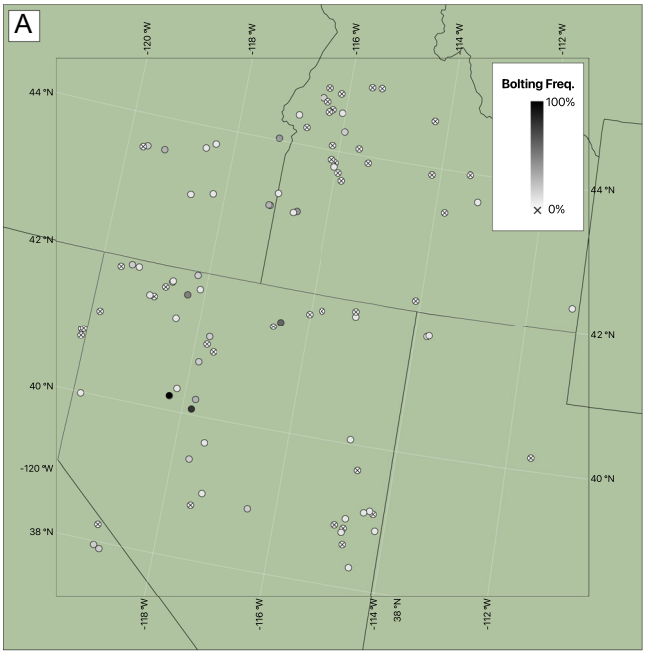
Bolting variation

A total of 526 plants (8.7% of 6,074 total) displayed an annual lifespan (i.e., bolting, senescence, and death) between July 15 and September 27, with at least one bolting plant present in 55% of the populations. Bolting varied widely among populations, ranging from 0–90.9% (Figure 1A). Overall, the data was skewed toward nonbolting plants with a bolting median of 1.1%. Bolting and climate variables that reflect summer temperature and aridity were positively correlated (Appendix S3). From the model selection, the AHM index yielded the lowest AIC value. Genome size, as a fixed effects predictor of bolting variation, was not significant and was removed from the final model (data not shown). The final GLMM found that AHM was significant in predicting bolting (Table 1, $p < 0.001$). Overall, fixed and random effects explained 61% (conditional R^2) of variation in bolting (Table 1). Fixed effects (i.e., climate) explained 22.5% of the variation (marginal R^2). Random effects (populations) explained 38.5% (conditional R^2 – marginal R^2). To evaluate the model, observed bolting frequencies of each population were plotted with the predicted, fixed effect values. The relationship was significant ($p < 0.0001$, $r = 0.61$, Figure 2).

To assess the potential effect of climate change on bolting variation, contemporary AHM values (1991–2010) were exchanged with midcentury (2041–2070) values from the SSP5 climate scenario. A 112% increase in the average AHM of the 95 populations was accompanied by a 65% increase in average bolting frequency between contemporary and midcentury climates. Given these data are based on frequencies, the plotted predicted values showed a sigmoidal pattern based on AHM as a climate predictor (Figure 3; Appendix S4). The greatest increase in bolting was predicted for populations exhibiting AHM values between 60 and 80 based on the fitted line (Figure 3).

Genome size

From 266 samples, 1 C values ranged from 1.86–8.76 pg, with two populations possessing samples that differed more than 1.5 pg in size. Thus, at least two out of 95 populations



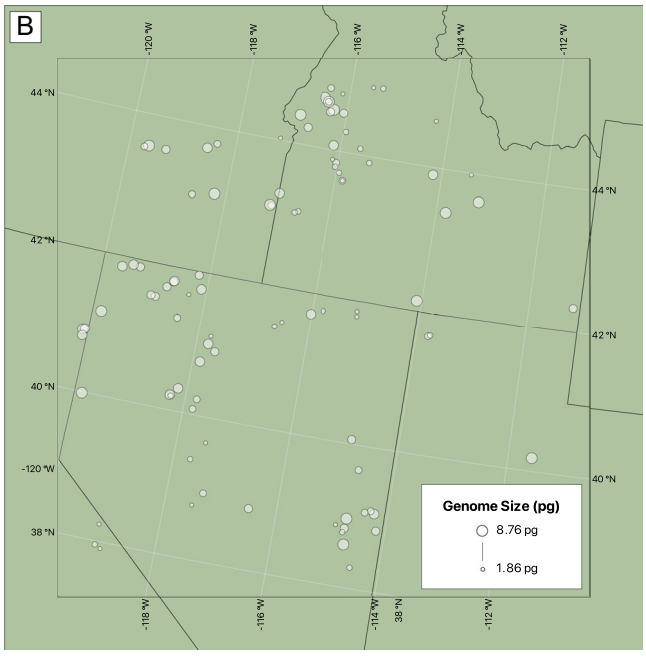


FIGURE 1 (A) Circles show the location of seed source populations of *Chaenactis douglasii* collected in the Intermountain region of the western United States. The black-white gradient within circles represents the bolting frequency within each population, with black representing a population entirely consisting of early-bolting plants. Circles containing a cross represent no observed bolting. (B) Circles and circle size show the location of seed source and genome size, respectively.

(2%) likely contain mixed ploidy (Figure 1B). Using the same data set of climate predictors as above, an LMM was fitted to the 1 C values. Two variables, summer frost-free days (NFFD_sm) and annual variation in NFFD (vNFFD), were found to be optimal based on AIC values. Random effects were assigned to populations. Fixed effects

TABLE 1 General linear mixed- model output of presence and absence of bolting in 95 *Chaenactis douglasii* plants ($n = 95$). Fixed effects were assigned to the annual heat moisture index (AHM). *P*-values and 95% confidence intervals (CI) are reported for fixed effects. Random effects are the assignment of individuals to seed source populations. σ^2 = mean random effect variance, $\tau_{00\ Population}$ = variation between populations, ICC = intraclass correlation coefficient (random effect variance/total variance).

	Bolting Presence/Absence		
Predictors	**Odds Ratios**	***CI***	***p***
(Intercept)	0.00	0.00–0.00	**<0.001**
AHM	1.09	1.06–1.13	**<0.001**
Random Effects			
σ^2	3.29		
$\tau_{00\ Population}$	3.25		
ICC	0.50		
$n_{\ Population}$	95		
Observations	6074		

climate predictors provided marginal support in explaining the genome size ($p < 0.09$; Appendix S5). The fixed effects explained 15.4% (marginal R^2) of the variation. Random effects (populations) explained most (78%) of the genome size variation (conditional R^2 – marginal R^2), and high intraclass correlation coefficient (random effect variance/total variance) (ICC) values further support our populations–genome size finding (Appendix S5).

DISCUSSION

Climatic effects on drought escape

Drought escape has been observed as an adaptive reproductive strategy in several forbs (Meyre et al., 2001; Franks et al., 2007; Kooyers et al., 2015; Welles and Funk, 2021). Similarly, our results show a significant, positive correspondence between drought escape via bolting and local seed source climate. Specifically, populations derived from more xeric regions with volatile hot and dry climates (i.e., high AHM and high AHM variation) showed increasing prevalence of expressing an annual lifespan (Table 1; Appendix S6). We hypothesize that expressing an annual vs. perennial lifespan may have fitness trade-offs that vary from year to year, depending on temperature and precipitation. Wet-cool climates may provide the resources for continued, multiyear vegetative growth, supporting increased second-year seed yield. However, in areas where these favorable climate conditions are more episodic, a perennial strategy may incur a cost because of a shortened growing season caused by drought. Thus, under more-frequent warm-dry conditions punctuated by occasional severe dry spells, a drought escape strategy may likely have higher fitness, reflected in the

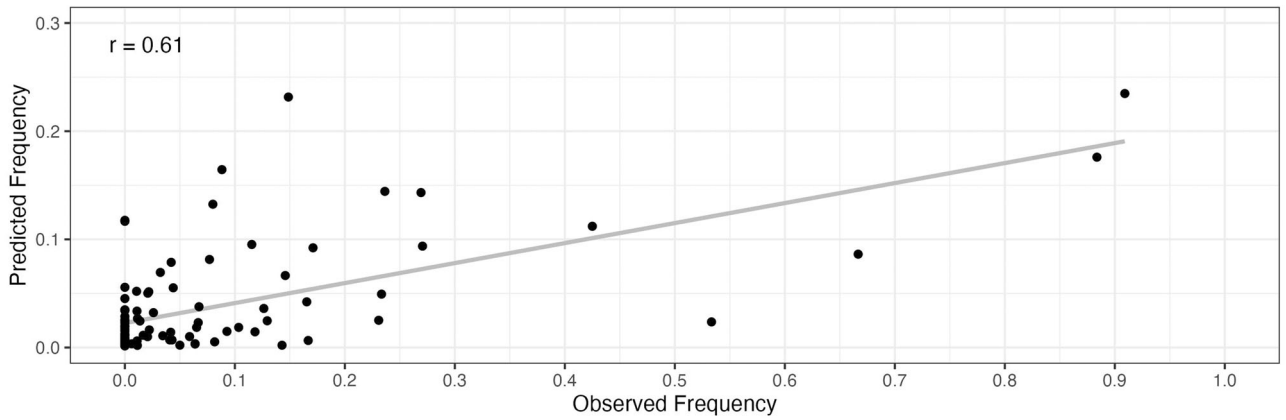


FIGURE 2 The observed frequency of bolting calculated for each of 95 *Chaenactis douglasii* populations plotted against the predicted fixed effects values (i.e., predicted frequency) from a generalized linear mixed model based on annual heat moisture index as a climate predictor. A linear regression is represented by the gray line. Pearson correlation coefficient (r) = 0.61, $p < 0.0001$.

increasing number of plants within populations exhibiting a drought escape response (Figure 3). Evolution does not necessarily prioritize immediate fitness advantages—the persistence of trait variation within a population may be evidence of bet-hedging that allows a particular trait variant, developed through exposure to environmental fluctuations over a long time period, to increase the mean fitness of a population (Seger and Brockmann, 1987; Starrfelt and Kokko, 2012). Indeed, xeric ecotypes of several plants that experience episodic droughts have been observed to grow and advance through life stages in a suboptimally conservative manner—a bet-hedging adaptation to ensure continuing survival in an unstable environment (Simons and Johnston, 2003; Sherrard and Maherali, 2006). Within *C. douglasii*, a perennial lifespan is the dominant phenotype across most populations; overall, 92% of plants in our experiment did not bolt the first year. However, most populations (55%) possessed at least one bolting plant, suggesting the drought escape bet-hedging strategy can exhibit significantly higher fitness in dry years, encouraging the typically suboptimal phenotype to persist within these populations. Further empirical data is needed to determine whether the expression of this trait reflects a geometric mean (i.e., bet-hedging) rather than an arithmetic mean (Simons, 2011).

Potential roles of climate variability

In addition to temperature increase, climate change is predicted to increase the variability in temperature and precipitation due to the amplification of heat waves and atmospheric convective processes, respectively (Rind et al., 1989; Lenderink and van Meijgaard, 2008; van der Wiel and Bintanja, 2021). Indeed, AHM variation drawn from a 30-year window (1989–2018) was the single-most predictive variable for bolting variation, explaining 24.8% of the variation within a GLMM (Appendices S3, S6). It is not yet possible, however, to accurately project yearly AHM variation in future climate. In contrast to the factors that drive mean climate shifts, the multifaceted and sometimes confounding factors that contribute towards climate variability and the contribution of climate variability towards extreme climatic events are either difficult to predict or poorly understood in many regions (Lewis and King, 2017). Thus, our modeled midcentury prediction may underestimate the increase in bolting from a more pronounced bet-hedging strategy because of future increases in AHM variation (Figure 3). As studied in other plant traits (Childs et al., 2010; Gremer and Venable, 2014), bet-hedging is a response to reduce risk due to environmental variability. In the case of *C. douglasii*, as the climate grows more volatile by the mid-century, the positive association between increased annual climate variability and bolting suggests an increase in the occurrence of annual plants compared to a model of solely AHM (Table 1; Appendix S6).

Bolting frequency plasticity

Bolting phenology within a plant's life cycle is a complex trait affected by genetic factors, environment, and genetic–environment interactions. Bolting phenology affected by environment (i.e., plasticity) is influenced by water availability and rosette size prior to bolting (Couvet et al., 1990; Mckay et al., 2003; Dong et al., 2013). Moreover, extreme heat prior to bolting can play a significant role in the delay of flowering in some facultative biennial species (Harbaugh et al., 1992; Giménez et al., 2013). We expect that the greenhouse conditions in our experiment would affect plasticity differently than field environments. Although we could not examine phenotypic plasticity caused by environment or genetic-environment interactions because our plants were grown in a single greenhouse environment, we expect, based on common garden studies from a variety of species (e.g., Richardson et al., 2017; Cooper et al., 2019), that plasticity would play a significant role in *C. douglasii* bolting, and could account for much of the unexplained

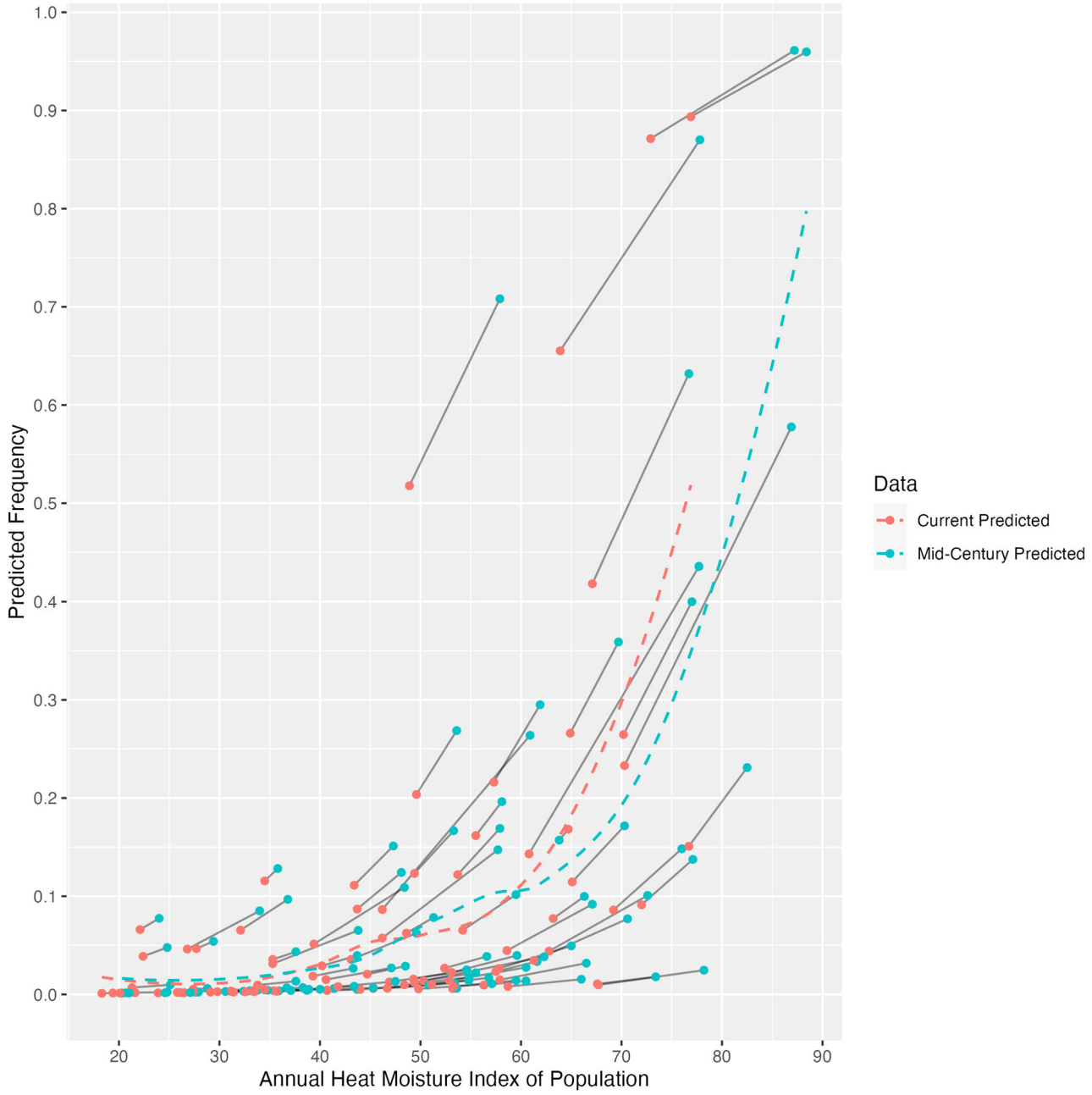


FIGURE 3 Predicted change in bolting frequency of *Chaenactis douglasii* resulting from projected midcentury increase in annual heat moisture due to climate change, SSP585 CMIP6 scenario. Red points represent contemporary-derived data (1991–2020), with blue representing data from midcentury projections (2041–2070) with lines connecting the same population at these time periods. Dashed lines indicate a smoothed local loess regression fit for each respective set of data.

variation in our GLMM and better define variation assigned to random effects.

Genome size variation and patterns

Because of partially indistinguishable histogram peaks of genome size among samples, we did not infer cytotypes for our analyses (Appendix S1). However, the variation in genome size and distribution supports a pattern of diploids, triploids, tetraploids, and hexaploids, as reported in chromosome counts by Mooring (1980). The ambiguity we observed in identifying cytotypes, especially between putative triploid and tetraploid histogram peaks, could result from extranumerary chromosomes, which Mooring (1980) reported in about 9% of samples. Mooring (1980) also reported diploids and tetraploids as the most prevalent, which we also observed in the greatest abundance (Appendix S1). Our data concur with Mooring (1980), who noted that about 1% of the analyzed populations had mixed cytotypes.

For our study, we estimate 2% of the populations contained mixed ploidy—a conservative estimate based on a population sample genome size difference of more than 1.5 pg (over half the size of the suspected diploid peak at 2.7 pg; Appendix S1).

In many plant species, adaptation to new environments is influenced by genome duplication. For example, specific cytotypes have been shown to have higher fitness in distinct edaphic conditions in *Achillea borealis* L. (Ramsey, 2011). Other studies have found patterns suggesting climate, such as aridity, as a catalyst for cytotype differentiation (Manzaneda et al., 2012) or disturbance history (Mráz et al., 2012). Our analysis suggests climate and climate variability may play a minor role in genome size landscape distribution (Figure 1B; Appendix S5). The marginal predictive power of the fixed effects in our model (i.e., NFFD and vNFFD) suggests that other environmental variables or demography processes (Wos et al., 2019) could partially explain the landscape patterns of genome size variation among *C. douglasii* populations. Variables that describe temperature variance such as vNFFD and temperature differential (TD), winter-summer TD (i.e., continentality) had stronger correlations with genome size than other climate variables (data not shown). It is also plausible that other factors influencing genome size differentiation are operating on a scale smaller than our geographic resolution (~1 km^2). For example, geographic and edaphic attributes like slope and soil particle size can affect the cytotype distribution of aspen (*Populus tremuloides*) and fescue (Festuca amethystina), respectively, and at subkilometer spatial scales (Blonder et al., 2020; Zielińska et al., 2024).

Implications for restoration

The genetic compatibility within or among seed collections (Kramer et al., 2018), and environmental compatibility at the restoration site (Erickson and Halford, 2020) are crucial to a species restoration program. To ensure the successful development of a *C. douglasii* restoration program, our results support the use of aridity (i.e., AHM) in defining seed transfer limits and the screening of seed collections for genome size prior to seed increase. These two steps will be important in considering where to acquire seeds to meet restoration needs, developing practices in cultivation to ensure genetic compatibility (i.e., like ploidy), and deploying seeds in areas appropriate to their drought escape strategy.

CONCLUSIONS

Here, we highlight an interaction between lifespan variation, a putative drought escape strategy in *C. douglasii*, and climate. A significant association between aridity and bolting suggests that variability in lifespan is a bet-hedging strategy. Annuals may have a fitness advantage in warm-dry years. Moreover, our climate-based model supports an increasing prevalence of annual vs. perennial lifespan as these populations experience increasing aridity and climatic variability during midcentury climates, however genome size did not show correspondence to lifespan variation. Developing a restoration program with this trait in mind will be crucial for the short- and long-term use of *C. douglasii* in restoration.

AUTHOR CONTRIBUTIONS

C.A. and B.R. collected data, performed the analyses, and wrote the manuscript. B.R., S.B., and F.K. designed the experiment. S.B., F.K., and K.D. coordinated seed collections and greenhouse propagation. All authors revised the manuscript.

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DATA AVAILABILITY STATEMENT

Data and R scripts can be found at https://github.com/Cameron-Amos/CHDO_Bolting.git.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. A histogram of sample counts and 1 C values (pg) for *Chaenactis douglasii*. Colors indicate putative cytotypes based on the genome size.

Appendix S2. Definitions of relevant climate variables (Pearson's *R* correlation coefficient to bolting frequency is > 0.4 or < −0.4), as well as climate variable definitions used in the calculations of relevant climate variables. More information can be found at the ClimateNA website: https://adaptwest.databasin.org/pages/adaptwest-climatena/

Appendix S3. Heat map matrix depicting the Pearson's correlation coefficient between significant predictor variables (correlated with bolting frequency at $r > 0.4$ or $r < -0.4$). A white dot represents a colinear relationship ($r > 0.7$ or $r < -0.7$). Climate acronyms can be found in Appendix S2.

Appendix S4. Predicted bolting frequency difference between current and mid-century for *Chaenactis douglasii* populations and the percentage change, derived from contemporary and midcentury annual heat moisture index values at each population location as a fixed effect predictor.

Appendix S5. Linear mixed model results of genome size variation in *Chaenactis douglasii*. Fixed effects, climate predictors are variation in the number of frost-free days (NFFD var) and summer (June, July, and August) NFFD (NFFD sm). Confidence intervals (*CI*) and *p*-values (*p*) are reported for fixed effects. Random effects are the assignment of individuals to seed source populations. σ^2 = mean random effect variance, $\tau_{00\ \text{Population}}$ = variation between populations, ICC = intraclass correlation coefficient (random effect variance/total variance).

Appendix S6. General linear mixed model results of presence and absence of bolting in *Chaenactis douglasii*, using yearly variation in annual heat moisture from 1991–2020 as a predictor variable.

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