

Transgenerational and Within-Generation Plasticity in Response to Climate Change: Insights from a Manipulative Field Experiment across an Elevational Gradient

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ABSTRACT: Parental environmental effects—or transgenerational plasticity—can influence an individual's phenotype or fitness yet remain underexplored in the context of global change. Using the perennial self-pollinating plant *Boechera stricta*, we explored the effects of climate change on transgenerational and within-generation plasticity in dormancy, germination, growth, and survival. We first conducted a snow removal experiment in the field, in which we transplanted 16 families of known origin into three common gardens at different elevations and exposed half of the siblings to contemporary snow dynamics and half to early snow removal. We planted the offspring of these individuals in a factorial manipulation of temperature and water level in the growth chamber and reciprocally transplanted them across all parental environments in the field. The growth chamber experiment revealed that the effects of transgenerational plasticity persist in traits expressed after establishment, even when accounting for parental effects on seed mass. The field experiment showed that transgenerational and within-generation plasticity can interact and that plasticity varies clinally in populations distributed across elevations. These findings demonstrate that transgenerational plasticity can influence fitness-related traits and should be incorporated in studies of biological responses to climate change.

Keywords: seed dormancy, germination, maternal effects, parental effects, seed mass, reciprocal transplant.

Introduction

Contemporary changes in climate are jeopardizing the persistence of species and populations across the globe (Bellard et al. 2012; Garcia et al. 2014). Evolutionary ecologists have

evaluated the extent to which natural populations are capable of evolving, migrating, and responding plastically to contemporary changes in climate (Cotton 2003; Perry et al. 2005; Parmesan 2006; Aitken et al. 2008; Crozier et al. 2008; Lenoir et al. 2008; Anderson et al. 2012; Merilä and Hendry 2014). However, an organism's phenotype can also be influenced by the experiences or actions of its parents, most typically by its mother (Mousseau and Fox 1998; Pick et al. 2016). Plasticity that occurs across generations—or transgenerational plasticity—can arise when parents from contrasting environments provision their offspring with different proteins, nutrients, hormones, epigenetic factors, or antibodies (Wolf and Wade 2009). The nongenetic inheritance of gene products from parents can induce transgenerational plasticity in size, growth, phenology, viability, or immunity in offspring (Holeski et al. 2012; Herman et al. 2013). Parents can also influence the conditions that their offspring experience through altered reproductive timing or differences in the habitat into which offspring are dispersed (Galloway and Etterson 2007; Donohue 2009). Transgenerational plasticity (also known as parental effects; Uller 2008) can alter an individual's phenotype or environment, yet we know little about whether these shifts are adaptive in nature (but see Galloway and Etterson 2007), particularly in the context of rapid environmental change.

Plasticity can arise in response to the parental environment (transgenerational plasticity), the offspring environment (within generation plasticity), or both (Ezard et al. 2014; Walsh et al. 2015). The duration and magnitude of fluctuations in environmental conditions relative to an organism's generation time can select for the evolution of different forms of plasticity (Leimar and McNamara 2015). All else being equal, the evolution of transgenerational plasticity is more likely when conditions fluctuate across generations, while within-generation plasticity can arise when fluctuations occur within generations (Kuijper and Hoyle 2015). Furthermore, the extent or periodicity of environmental fluctuations can vary spatially (e.g., McCabe et al. 2008), which could result in differ-

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ences in the form and strength of plasticity across environmental gradients (e.g., Fernández-Pascual et al. 2013). Importantly, these forms of plasticity can evolve in populations that experience both short- and long-term environmental fluctuations (Ezard et al. 2014; Walsh et al. 2016). Transgenerational and within-generation plasticities can differ in scope and magnitude and can have reinforcing, opposing, or interactive effects on offspring phenotype (Walsh et al. 2015; Luquet and Tarel 2016; Auge et al. 2017; Vayda et al. 2018). Thus, the influence of climate change on transgenerational plasticity may depend on within-generation plastic responses and vary among populations or habitats.

The adaptive value of transgenerational plasticity depends, in part, on the correlation between the environments experienced by the parents and their offspring (Karban et al. 1999; Burgess and Marshall 2011; Kuijper and Hoyle 2015). Transgenerational plasticity can be adaptive when the environmental conditions that the parents experience are predictive of the environment their offspring will encounter (Donohue and Schmitt 1998; Galloway 2005). Indeed, as with within-generation plasticity, theoretical work has demonstrated that environmental variation must be predictable for transgenerational plasticity to evolve (Schlichting and Levin 1986; Scheiner 1993; Marshall and Uller 2007; Uller 2008). As the climate continues to change rapidly, previously adaptive transgenerational plasticity may become maladaptive if parental environments are no longer predictive of offspring environments (Visser 2008; Auge et al. 2017). This possibility could be more likely in regions where climatic fluctuations or weather extremes are common or when parental and offspring growth are separated in time, as is the case for species with persistent seed/egg banks or long generation times or where seeds are collected from natural populations for future restoration or relocation efforts (Ezard et al. 2014; Kuijper and Hoyle 2015; but see Auge et al. 2017).

To assess the fitness consequences of transgenerational plasticity under changing environments, parents and offspring should be reciprocally transplanted among treatments or locations that vary in conditions relevant to climate change (Uller et al. 2013; Donelson et al. 2018). For instance, manipulations of temperature, CO₂ concentration, and soil moisture in a wide variety of species have revealed that the performance of individuals depends on whether their mothers experience similar conditions (Sultan et al. 2009; Donelson et al. 2012; Miller et al. 2012; Salinas and Munch 2012; Shama et al. 2014). These findings suggest that transgenerational plasticity can mediate biological responses to environmental change or can temporarily buffer populations from climatic stressors. However, most studies of the potential influence of transgenerational plasticity on adaptive responses to climate change are conducted under controlled conditions, which can be poor surrogates for natural environments (Kellermann et al. 2015; Poorter et al. 2016). As such, empirical data from experiments

that manipulate variables relevant to climate change in a field setting are vital for discerning the influence of transgenerational plasticity on fitness (Donohue 2009; Uller et al. 2013; Donelson et al. 2018).

Transgenerational plasticity influences a wide range of fitness-related traits in plants, including seed size and dormancy dynamics, and has stronger effects on traits expressed early in the life cycle (Roach and Wulff 1987; Donohue and Schmitt 1998; Galloway 2005; Hereford and Moriuchi 2005; Galloway and Etterson 2007; Donohue 2009; Herman et al. 2012). Manipulative field studies have demonstrated that these traits are sensitive to many of the variables projected to shift with climate change, including increases in temperature and CO₂ concentrations and altered precipitation regimes (Arft et al. 1999; Dunne et al. 2003; Lloret et al. 2004; Sherry et al. 2007; Wipf and Rixen 2010; Wu et al. 2011; Anderson et al. 2012; Dieleman et al. 2012). Winter conditions are also important drivers of growth and development for plants in temperate or alpine environments (Aerts et al. 2006; Williams et al. 2015). For instance, the mountainous regions of western North America have experienced widespread declines in winter snowpack as a result of contemporary climate change (Pederson et al. 2011; Fyfe et al. 2017; Wadgyman et al. 2018b) and are projected to encounter rapid increases in winter temperatures and further decreases in snowpack in the coming decades (Miller et al. 2016). These altered winter dynamics can reduce fitness by suppressing the probability of flowering (Wadgyman et al. 2018b) and have exacerbated extinction threats for flora of montane regions (Engler et al. 2011; Dullinger et al. 2012). Furthermore, environmental fluctuations in precipitation are more pronounced at lower elevations in this area (Mote et al. 2005; Regonda et al. 2005), which suggests that the extent or form of plasticity may vary in low- versus high-elevation populations. An understanding of whether transgenerational plasticity facilitates or impedes adaptation to climate change in nature would improve predictions of the fate of species or populations inhabiting vulnerable areas.

Here, we examine the adaptive significance of transgenerational and within-generation plasticities in the perennial forb, *Boechera stricta* (Brassicaceae), in relation to climate change through complementary field and laboratory experiments using the same seed sources. In the Colorado Rocky Mountains, we manipulated snowpack and timing of snowmelt in a two-generation reciprocal transplant experiment in three common gardens to ask whether transgenerational and within-generation plasticities in life-history traits (1) arise in response to altered snow dynamics; (2) reinforce, oppose, or interact with each other; and (3) vary geographically in populations distributed across an elevational gradient. Fluctuations in annual and seasonal precipitation occur across years and decades in this region (Cayan et al. 1998; Ault and St. George 2010; Wadgyman et al. 2018b), particularly at low elevations (Mote et al. 2005; Regonda et al. 2005). This predictable en-

vironmental variation may have set the stage for the evolution of transgenerational and within-generation plasticities in plants whose growth and life-history transitions span multiple years (Schlichting and Levin 1986; Scheiner 1993; Uller 2008). We hypothesize that transgenerational plasticity is most apparent in early-expressed traits (Roach and Wulff 1987; Mousseau and Fox 1998; Donohue 2009; Burgess and Marshall 2011). Furthermore, we predict that gradients in the extent of environmental variability across elevations have resulted in clinal variation in both transgenerational and within-generation plasticities. Exploring the eco-evolutionary consequences of transgenerational plasticity may reveal a mechanism that contributes to unexplained or variable response to climate change.

Methods

Study Species

The perennial forb, *Boechera stricta* (Brassicaceae), is broadly distributed across its native range in the U.S. and Canadian Rocky Mountains (Song et al. 2006; Al-Shehbaz and Windham 2010; Rushworth et al. 2011). Populations of *B. stricta* are locally adapted to fine-scale environmental variation (Lee and Mitchell-Olds 2013; Anderson et al. 2015; Wadgyrmar et al. 2017), suggesting that this species is an excellent model for testing whether transgenerational plasticity plays a role in maintaining local adaptation with climate change. Our study sites near the Rocky Mountain Biological Laboratory (Gothic, Gunnison Co., Colorado; 38°57'N, 106°59'W) lie within the portion of the range where genetic diversity for *B. stricta* is highest (Kiefer et al. 2009). *Boechera stricta* is almost exclusively self-pollinating and has geographically restricted seed dispersal capabilities, resulting in highly inbred populations across the landscape (average $F_{IS} = 0.89$; Song et al. 2006). Consequently, seeds derived from the same maternal lineage are highly homozygous, enabling us to distinguish between the contributions of genetic effects, transgenerational plasticity, and within-generation plasticity on offspring performance.

Seed Sources

We collected seeds from 46 populations along an elevational gradient (2,694–3,690 m; for a schematic of the experimental design, see fig. 1). We grew plants in the greenhouse for a generation to standardize maternal effects and generate full-sibling maternal families. In the fall of 2013, we transplanted juvenile rosettes generated from these grandparental greenhouse-produced seeds into a snow removal experiment in three common gardens spanning the elevations from which the source populations were collected ($N = 4,079$ total juvenile transplants from 107 maternal families across the 46 source populations; garden elevations: 2,710, 2,890, and 3,430 m). These gardens span gradients of temperature, soil moisture, and

winter duration (Wadgyrmar et al. 2017). In each garden, experimental maternal plants were divided into two treatments: ambient snowpack (control) or early snow removal, wherein snow was shoveled to a 10-cm depth in half of the blocks once the snowpack naturally receded to 1 m (as in Anderson and Gezon 2015; Wadgyrmar et al. 2018b). Early snow removal treatments impose several of the winter conditions forecasted to arise with climate change in our study region, including reduced snowpack, earlier date of snowmelt, increased exposure to frost events, and decreased soil moisture (Anderson and Gezon 2015; Gezon et al. 2016; Wadgyrmar et al. 2018b).

To examine the effects of transgenerational plasticity on local adaptation and responses to climate change, we used maternal families for which individuals produced >200 seeds in each garden and treatment. Other maternal families would also have been suitable, but including a greater number of families would have resulted in unfeasibly large sample sizes. These field-produced seeds represent families of full-sibling homozygous individuals that vary in parental environments. In July–September 2014, we collected seeds from one maternal family from each of 16 populations that were well represented across the six parental environments (two treatments $\times$ three common gardens) and whose source elevations spanned a broad gradient (2,721–3,530 m). To illuminate the role of transgenerational plasticity on offspring fitness, we planted both greenhouse- and field-produced seeds in a growth chamber experiment in January 2016 and a field experiment in October 2015, both of which we describe below ($N = 16$ maternal families $\times$ seven parental environments [six field + one greenhouse] = 112 experimental lineages). The time lag between seed maturation and planting was sufficient to ensure the release of dormancy (after ripening).

Growth Chamber Experiment

We assessed the influence of transgenerational plasticity on offspring phenotypes under controlled conditions in two identical growth chambers (Convion CMP5000 Growth Chamber, Winnipeg, Canada). To test for differential provisioning among maternal plants of the same genotype that experienced different environments, all seeds were individually weighed before planting (Cubis Micro Balance MSE3.6P000DM, Sartorius, Göttingen; $N = \sim 23.5$ seeds $\times$ 16 genotypes $\times$ seven parental environments = 2,643 total seeds). Seeds were randomly assigned to temperature and precipitation treatments that resembled the two extreme parental environments: the warm and dry condition found at the low-elevation garden and the cool and moist conditions of the high-elevation garden. To disentangle the roles of temperature and precipitation on plasticity, we fully crossed the two temperature and two moisture treatments, resulting in four total offspring environments. Temperature treatments mimicked typical seasonal changes in conditions at each site, with lower simulated spring

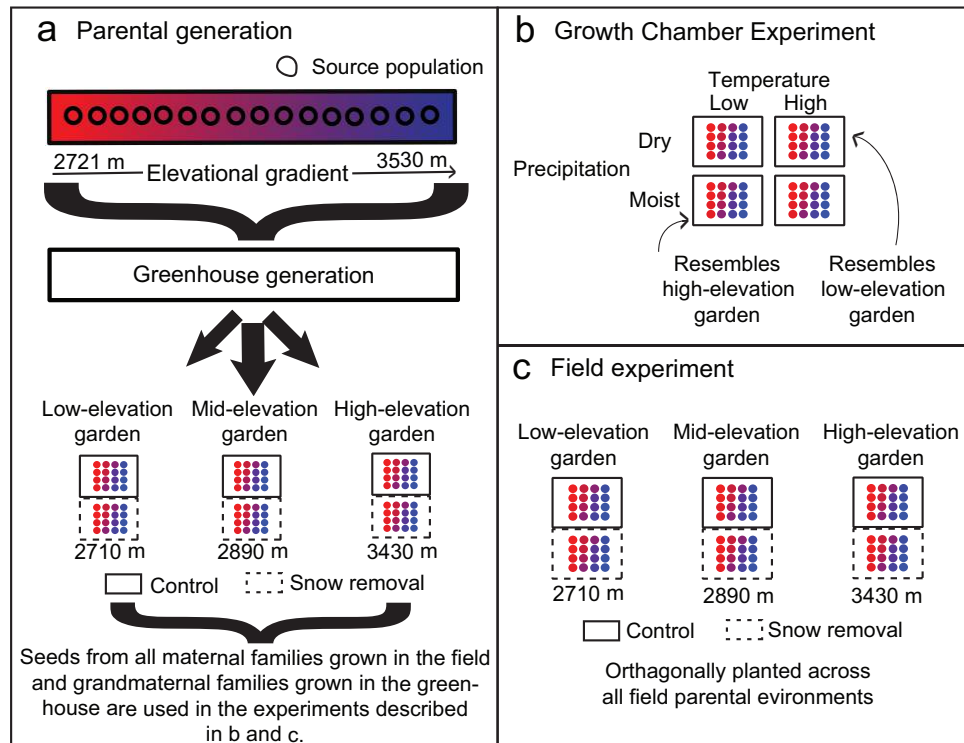


Figure 1: Schematic depicting the greenhouse and field experiments applied to grandparental and parental generations, respectively (a), and the growth chamber (b) and field conditions (c) to which their offspring were exposed. a, As a first step in this experiment, we collected seeds from individuals (hereafter, the grandparental generation) in natural *Boechera stricta* populations distributed across an elevational gradient in Colorado. We then reared these grandparental seeds in the greenhouse to homogenize maternal effects and generate full-sibling maternal families of this primarily self-pollinating plant. We exposed these parental seeds to control and snow removal treatments in three common gardens in the field. For illustration purposes only, we depict experimental gardens as divided spatially between control and snow removal treatments. In fact, snow removal and control blocks were spatially interspersed in the common gardens, such that microtopography is not conflated with treatment. b, c, After this maternal generation, we exposed offspring seeds to growth chamber (b) and field (c) experiments. In the field, we planted offspring seeds orthogonally into all parental field experiments. Seeds of the parental generation from greenhouse-reared plants (with homogenized maternal effects) were also planted as a control within each of the offspring environments in the growth chamber and snow removal experiments.

and fall temperatures and higher simulated summer temperatures. We maintained a constant 4°C difference between temperature levels between each of two growth chambers, with daytime temperatures consistently 3.5°C higher than nighttime temperatures in both treatment levels (low-temperature treatment ranges: 11.5–17°C daytime, 8–14°C nighttime; high-temperature treatment ranges: 15.5–21.5°C daytime, 12–18°C nighttime; fig. S1a; figs. S1–S6 are available online). Day lengths resembled seasonal progressions of photoperiod at our study site and were the same in both temperature treatments (day length range: 14.03–14.88 h; fig. S1b). Relative humidity was kept at a constant 55%, which approximates the average humidity from April through October 2014 in Crested Butte (<http://www.wunderground.com>).

We planted seeds on January 22–24, 2016, on moist filter paper within individual petri dishes to preserve unique identities. We kept seeds in the dark until germination and

monitored all seeds daily for germination. We exposed germinants to the light until the seed coat was shed and the radicle and cotyledons were extended, after which we planted them in 12-cm-deep Ray Leach Cone-tainers (Stuewe & Sons, Tangent, OR) and monitored survival and growth. We provided ample water to all seedlings until 42 days after planting, at which point we randomly assigned them to a precipitation treatment. We applied precipitation treatments at the block level, and low- and high-temperature growth chambers each contained three trays of both precipitation treatments. We watered plants assigned to the well-watered treatment every day and watered those in the dry treatment every other day. We rotated trays weekly within growth chambers to reduce positional effects and every 4 weeks between growth chambers to minimize chamber effects.

We evaluated the viability of seeds that did not germinate by extracting embryos from the seed coats and soaking them

in a 1% solution of 2,3,5-triphenyltetrazolium chloride, which will turn respiring tissue pink. After 48 hours, we assigned a dormant status to seeds if the radicle and at least one cotyledon had stained pink. We considered embryos that did not stain (or only partially stained) as nonviable. We assessed germination and dormancy for seeds derived from all three common gardens but collected postgermination data only on germinants from the low- and high-elevation gardens whose thermal and precipitation regimes were simulated in the growth chambers. After 10 weeks, plants were moved to a cold room for 6 weeks at 4°C to mimic overwintering and provide vernalization cues for flowering. Because of malfunctions with one of the growth chambers near the end of the experiment, we were unable to use any data collected after vernalization. Because of contamination by mold, 156 seeds were excluded from the experiment after seed weights were obtained ($N = 2,486$ seeds used to assess germination success and dormancy). Similarly, we had to exclude 18 seedlings from postgermination analyses because of mislabeling of tags ($N = 2,468$ seeds used in postgermination analyses).

Snow Manipulation Experiment in the Field

We tested the influence of transgenerational plasticity on offspring performance under field conditions by reciprocally planting greenhouse- and field-produced seeds back into each of the parental gardens and treatments. We randomly planted seeds within plastic grids containing 13 columns and 13 rows of uniquely labeled 1×1 -cm cells that allowed us to track the identity and fate of individual seeds ($N = \sim 15.6 \text{ seeds} \times 16 \text{ genotypes} \times \text{seven parental environments} \times \text{six offspring environments} = 10,494 \text{ seeds}$). We planted seeds into these grids in the fall of 2015 after natural seed dispersal to minimize contamination and to expose our experimental seeds to winter conditions and early spring snowmelt needed for germination. We confirmed the accuracy of seed placement by randomly assigning control unplanted cells, which we monitored for germinants. Half of the grids within each garden experienced ambient conditions, while the other half was subject to the early snow removal treatment. We monitored all seeds for the following traits in 2016 and 2017: emergence, number of true leaves during each census, and overwinter and summer survival. For reference, we present weather conditions during the parental and offspring generations in figure S2. All data are deposited in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.nf45q26> (Wadgyamar et al. 2018a).

Statistical Analyses

We assessed the influence of parental environment, population elevation of origin, and their interaction on seed mass with a linear mixed model using the nlme package (Pinheiro et al. 2014) in R (ver. 3.2.2; R Development Core Team, <https://www.R-project.org>). Elevation is an excellent

proxy for climate in this system because temperatures and aridity decline with increasing elevation (Dunne et al. 2003; see also fig. S3). For the growth chamber experiment, we modeled seed status (germinated, dormant, or nonviable) using a multinomial regression using the Laplace estimation method (multinomial distribution and logit link for nominal data, ProcGlimmix, SAS ver. 9.4). We included fixed effects for offspring temperature, elevation of origin, parental environment, and all two- and three-way interactions. Additionally, we included seed mass as a covariate to test for transgenerational effects apart from differential seed provisioning and random effects for maternal identity nested within genotype and block nested within offspring temperature treatment. Because water treatment levels were not imposed until after the seedling stage, we did not include this treatment as a fixed effect in this analysis. We considered dormant the reference group, which allowed us to simultaneously estimate intercepts and slopes for fixed effects in comparisons between nonviable versus dormant seeds and dormant seeds versus germinated seeds. We analyzed survival and leaf number (a proxy for growth rate) using generalized linear models with binary and Poisson distributions, respectively (Proc Glimmix), with the same fixed and random terms described above.

For the field snow removal experiment, we analyzed the timing of germination and survival into the second year using a mixed effects Cox proportional hazards model with random effects for maternal identity nested within genotype and for planting grid nested within offspring environment. This analysis was conducted using the coxme package in R (Therneau 2018), which is currently incapable of generating direct estimates of absolute risk (i.e., the final probability of germinating or surviving). Therefore, we base our conclusions off of the statistical results generated from a coxme model, but our figures and estimates of absolute risk are derived from simpler fixed effects Cox proportional hazards models without random effects, using the coxph package (Therneau and Lumley 2017) and survminer packages (Kassambara et al. 2017). Results from the coxme and coxph models were qualitatively and quantitatively similar (see tables S6, S9; tables S1–S12 are available online). We did not use seed mass as a covariate in analyses of the field experiment because we did not weigh those seeds before transplanting.

We compared the extent of within-generation and transgenerational plasticities for any trait where we detected a significant interaction between offspring- and parental-environmental effects by estimating the relative distance plasticity index (RDPI) for each genotype for both forms of plasticity (Valladares et al. 2006). This metric computes pairwise distances among individuals of each genotype grown under different environments (when examining within-generation plasticity) or whose parents were grown under different environments (when examining transgenerational plasticity). These distances are averaged across environments and within a genotype, yielding an

estimate of the degree of both forms of plasticity for each genotype. Index values are bounded between 0 (no plasticity) and 1 (high plasticity), which enables comparisons across genotypes. A perfectly reciprocal, cross-generational planting design would allow us to directly contrast RDPI values between the two forms of plasticity. Thus, we excluded data for seeds derived from the greenhouse parental environment when calculating these indices. We calculated the mean and variance for values of within-generation and transgenerational RDPI for each genotype and statistically evaluated them using linear models and two-way ANOVAs with mean or variance of RDPI as the response variable; elevation of origin, form of plasticity, and their interaction as explanatory variables; and an error covariate for the form of plasticity to homogenize residual variance. Residuals were normally distributed.

In analyses from both experiments, seed mass (when included as a covariate) and elevation were standardized to a mean of zero and a standard deviation of 1 before analyses. We tested whether the relationship between traits of interest and elevation of origin were nonlinear by including a quadratic term for elevation, but it was not significant in any case and was dropped from all analyses. In some cases, our models identified significant differences among parental or offspring environments or significant interactions that were not apparent when P values from contrasts were corrected for multiple testing. The detection of many significant effects before P value correction can be indicative of important biological processes at work (Moran 2003). As such, we report raw P values here for contrasts, but note that we would expect four out of 79 tests to erroneously assign significance by chance, assuming $\alpha = 0.05$.

Results

Snow Removal Treatment

Our snow removal experiments effectively accelerated the timing of snowmelt in all years and sites. During the parental spring season in 2014, snowmelt in our low-, mid-, and high-elevation gardens naturally occurred on ordinal day of years 123, 146, and 166, respectively, and occurred on days 112, 138, and 162 in our early snow removal treatment. We observed similar trends in both spring seasons that the offspring experienced (listed as ambient/removal; 2016 season: low elevation, 110/104; midelevation, 131/114; high elevation, 162/151; 2017 season: low elevation, 112/102; midelevation, 140/125; high elevation, 165/163).

Are Transgenerational and Within-Generation Plasticities Sensitive to Climate Change?

Seed Mass. Elevation of origin and parental environment additively influenced seed mass. Genotypes derived from high-elevation populations produced larger seeds than those from

low-elevation populations, with a 12.6-mg increase in seed mass for every 100 m gain in elevation ($F_{1,14} = 15.14$, $P = .001$; fig. 1a; tables S1, S2). In addition, the parental environment significantly affected seed mass ($F_{6,84} = 4.13$, $P = .001$; fig. 1b; tables S1–S3). Seeds produced by plants in the greenhouse were smaller relative to those produced by plants in the high-elevation snow removal environment and the midelevation ambient environment. Additionally, plants from the snow removal treatment in the high-elevation garden produced larger seeds than those from the ambient treatment.

Germination. The multinomial regression revealed that viability, dormancy, and germination probabilities for the growth chamber experiment were influenced by seed mass, source elevation, parental environment, and source elevation by parental environment (fig. 2c; table S4). The probability of germinating (vs. going dormant) differed substantially across parental environments according to the timing of snowmelt, with the highest proportion of dormant seeds produced by parents in the low-elevation removal environment and fewest dormant seeds produced by parents in the high-elevation ambient environment. Additionally, smaller seeds had a greater probability of being nonviable than being dormant and of remaining dormant than germinating. The odds of producing dormant (vs. nonviable) seeds increased by 38% for every 1 SD unit increase in seed mass (odds ratio = 0.38; 95% CI = 0.27–0.52; $t_{2,221} = -6.1$, $P < .001$; fig. 3a; table S4). Similarly, the odds of germinating (vs. going dormant) increased by 213% for every 1 SD unit increase in seed mass (odds ratio = 2.13; 95% CI = 1.61–2.82; $t_{2,221} = 5.27$, $P < .001$; fig. 3b; table S4). The probability of producing dormant (vs. nonviable) seeds varied significantly across source elevations in three of seven parental environments. The odds of producing dormant (vs. nonviable) seeds from parents that experienced the midelevation ambient and snow removal environments declined by 237% and increased by 43%, respectively, for every 1 SD unit increase in source elevation (ambient odds ratio = 2.37; 95% CI = 1.01–5.59; $t_{2,221} = 1.98$, $P = .048$; removal odds ratio = 0.43; 95% CI = 0.19–0.96; $t_{2,221} = -2.05$, $P = .040$; fig. 3c, 3d; table S4). For parents from the low-elevation removal environment, the odds of producing dormant (vs. nonviable) seeds declined by 317% for every 1 SD unit increase in source elevation (odds ratio = 3.17; 95% CI = 1.44–6.95; $t_{2,221} = 2.87$, $P = .004$; fig. 3e; table S4). Last, our analysis uncovered a 26% reduction in the odds of producing germinated (vs. dormant) seeds for every 1 SD unit increase in source elevation when parents experienced the midelevation snow removal environment (odds ratio = 0.26; 95% CI = 0.11–0.67; $t_{2,221} = -2.8$, $P = .005$; fig. 3f; table S4).

For the snow manipulation experiment in the field, germination success varied with two-way interactions between elevation of origin, parental environment, and offspring en-

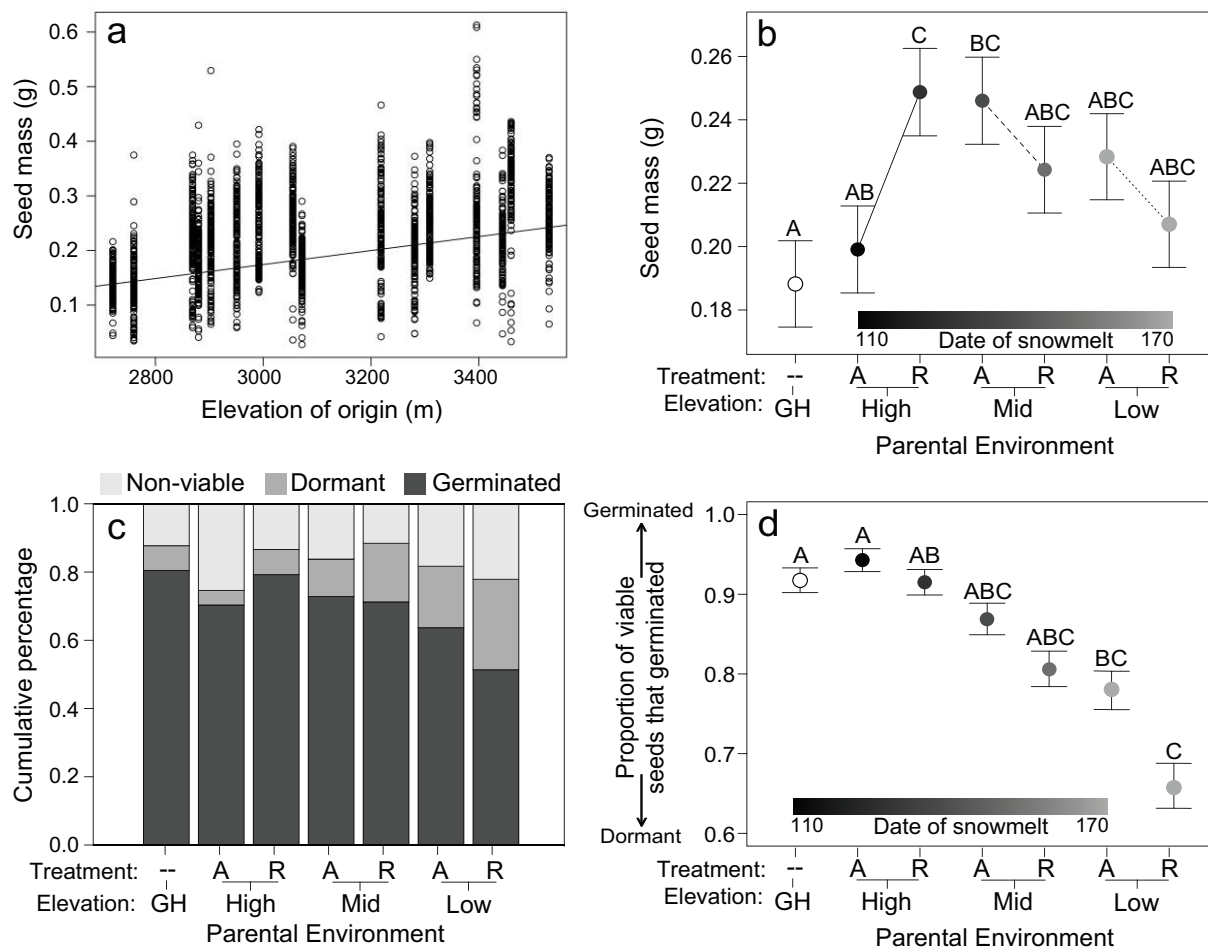


Figure 2: Effects of elevation of origin (a) and parental environment (b) on seed mass; cumulative proportion of seeds that germinated, were dormant, or were nonviable in each parental environment (c); and proportion of viable seeds that germinated as a function of parental environment for the growth chamber experiment (d). b displays least squares means $\pm$ SE for greenhouse-produced seeds (GH) and for the ambient (A) and early snow removal (R) treatments in the high-, mid-, and low-elevation gardens. Letters denote significant differences among parental environments.

environment (tables S6, S7). Germination success declined with source elevation for parents exposed to greenhouse conditions (hazard ratio = 0.42, $z = -2.25$, $P = .023$; fig. 4a) and midelevation ambient environments (hazard ratio = 1.96, $z = 2.51$, $P = .012$; fig. 4c; table S8). In contrast, germination success increased significantly with source elevation when parents experienced the high-elevation ambient environment (hazard ratio = 0.572, $z = -2.18$, $P = .029$; fig. 4b; table S8). Germination success also varied with source elevation in some offspring environments, with higher germination in low-elevation populations planted in the mid-elevation ambient environment (hazard ratio = 0.57, $z = -2.18$, $P = .029$; fig. 4d) and the low-elevation removal environment (hazard ratio = 0.58, $z = -2.39$, $P = .017$; fig. 4e; table S8).

Germination success was most strongly explained by the interactive effects of transgenerational and within-generation plasticities ($\chi^2_{30} = 56.7$, $P = .002$; tables S6–S8). Variation in within-generation plasticity depended on the environmental conditions experienced by the parents, with parental effects masking the offspring environmental effects in some cases (fig. 5, left). For example, the probability of germination differed between the high-elevation ambient and removal offspring environments when the parents experienced the greenhouse environment (fig. 5a) but did not differ significantly when parents experienced five of the six other environments. Similarly, variation in transgenerational plasticity among parental environments was contingent on the environment in which offspring were reared (fig. 5, right). For instance, germination probabilities for offspring that experienced the

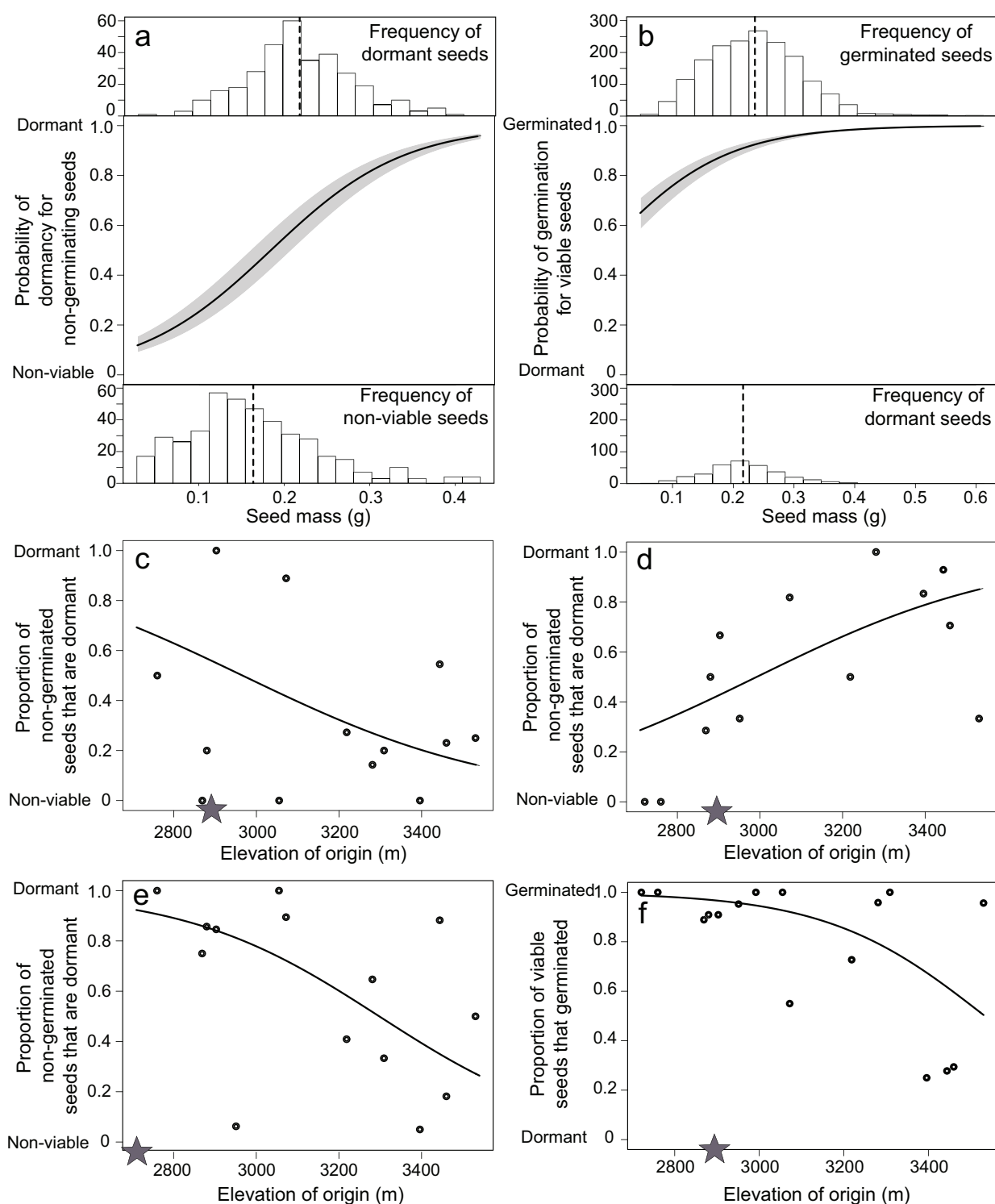
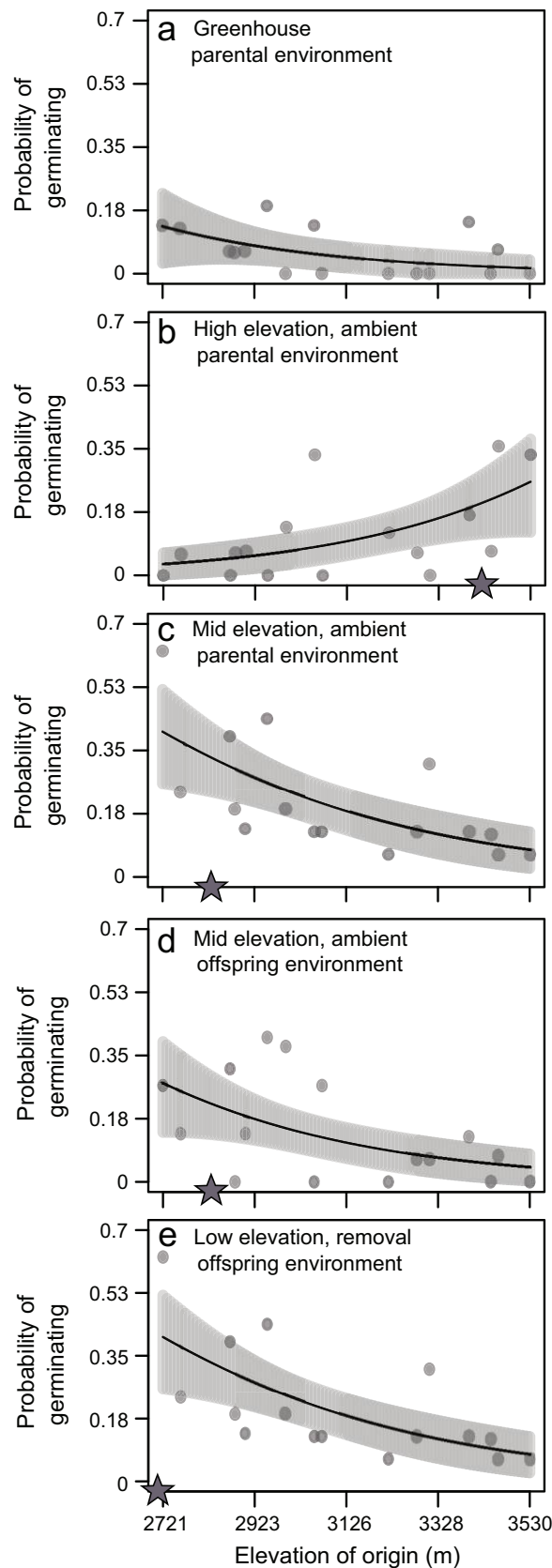


Figure 3: Multinomial regression revealed that seed mass influences the probability of being dormant versus nonviable (a) and germinating versus remaining dormant (b). Additionally, these analyses uncovered genetic clines in seed status in several parental environments: the probability of being dormant versus nonviable against source elevation in the midelevation ambient (c), midelevation removal (d), and low-elevation removal (e) parental environments, and the probability of germinating versus remaining dormant for the midelevation removal parental environment (f). These results are from the growth chamber experiment. a, b, Histograms indicate the distribution of seed sizes that remained dormant or were nonviable (a) or germinated or were dormant (b). Dashed lines signify the average seed mass in each category. Trend lines are derived from multinomial regressions and are portrayed $\pm 95\%$ confidence intervals. Stars indicate the elevation of the common garden.



high-elevation ambient conditions depended on the environment experienced by the parents (fig. 5c).

Plant Size. In the growth chamber experiment, plant size at the onset of vernalization was influenced by seed mass, the parental environment, and offspring environment (table S1). Plants derived from larger seeds grew to have a greater number of leaves at the onset of vernalization than those from small seeds, with every 1 SD unit increase in seed mass corresponding to a five-leaf increase in plant size (odds ratio = 1.05; 95% CI = 1.001–1.11; $F_{1,599} = 4.08$, $P = .044$; tables S1, S5). Furthermore, plants grew to a smaller size when their parents experienced the environments with the earliest snowmelt (low elevation, ambient and snow removal) or when they were exposed to the high-temperature and water-restricted treatments (fig. S4; table S5). Seed mass was the only factor to significantly influence survival to vernalization. For every 1 SD unit increase in seed mass, the odds of survival increased by 82% (odds ratio = 1.82; 95% CI = 1.39–2.39; $F_{1,969} = 18.8$, $P < .0001$; table S1). For the snow manipulation experiment, leaf number for seedlings surviving the first growing season did not differ among populations and was not influenced by parental or offspring environmental effects (table S8), nor did they affect growth rates or rosette size (data not shown).

Survival. For the snow manipulation field experiment, we detected elevation and offspring environmental effects on establishment and survival of offspring through the second growing season, but parental effects were not significant (tables S9, S10). Survival was highest in offspring from low-elevation populations across all parental and offspring environments ($\chi^2_1 = 694.4$, $P < .001$; tables S2, S11). Additionally, we detected an additive effect of offspring environment, with enhanced survival for offspring grown in the midelevation garden and the low-elevation removal environment and decreased survival in the high-elevation garden ($\chi^2_5 = 21.6$, $P = .001$; fig. S5; tables S9–S11).

Do Transgenerational and Within-Generation Plasticities Reinforce, Oppose, or Interact with Each Other?

We detected all possible combined effects of transgenerational and within-generation plasticities across traits and between

Figure 4: Probability of germinating varied as a function of source elevations for parental (a–c) and offspring (d, e) environments. We analyzed these data using mixed effects Cox proportional hazards models. Shown are only environments for which germination probabilities significantly differed across source elevations (see table S10, available online). To obtain trend lines, we calculated estimates of absolute risk $\pm 95\%$ confidence intervals from a fixed effects Cox proportional hazards model or 100 values equally distributed across the range of source elevations depicted on the X-axis. Stars denote the elevation of the relevant common garden.

experiments. In the growth chamber experiment, we found transgenerational plasticity in germination and dormancy, but the temperature treatment did not elicit within-generation plastic responses in these traits. In contrast, germination probabilities in the snow manipulation experiment were affected by an interaction between parental and offspring environments. Both forms of plasticity acted additively on plant size in the growth chamber experiment, but neither form affected size in the snow manipulation experiment. Survival probabilities in the snow removal experiment were affected by within-generation—but not transgenerational—plasticity. In summary, the cumulative effects of plasticity occurring across and within generations were dependent on the trait in question and the environmental conditions in which they are examined.

Is There Spatial Variation in Transgenerational or Within-Generation Plasticity?

On average, transgenerational plasticity for germination success (mean RDPI = 0.344 ± 0.032 SE) was greater than within-generation plasticity (mean RDPI = 0.244 ± 0.014 SE) across all genotypes (fig. 6a). Interestingly, the extent of both forms of plasticity differed significantly among genotypes according to their elevation of origin, with the probability of germination for low-elevation genotypes influenced more by transgenerational plasticity and less by within-generation plasticity ($F_{1,28} = 6.28$, $P = .018$; fig. 6b, 6c). Similarly, the variance in transgenerational plasticity across genotypes was nearly double that of within-generation plasticity ($F_{1,28} = 8.20$, $P = .008$) and was greatest for low-elevation genotypes ($F_{1,28} = 9.99$, $P = .004$). Estimates of both forms of plasticity were not significantly correlated ($r = 0.076$, $t_{14} = 0.29$, $P = .78$).

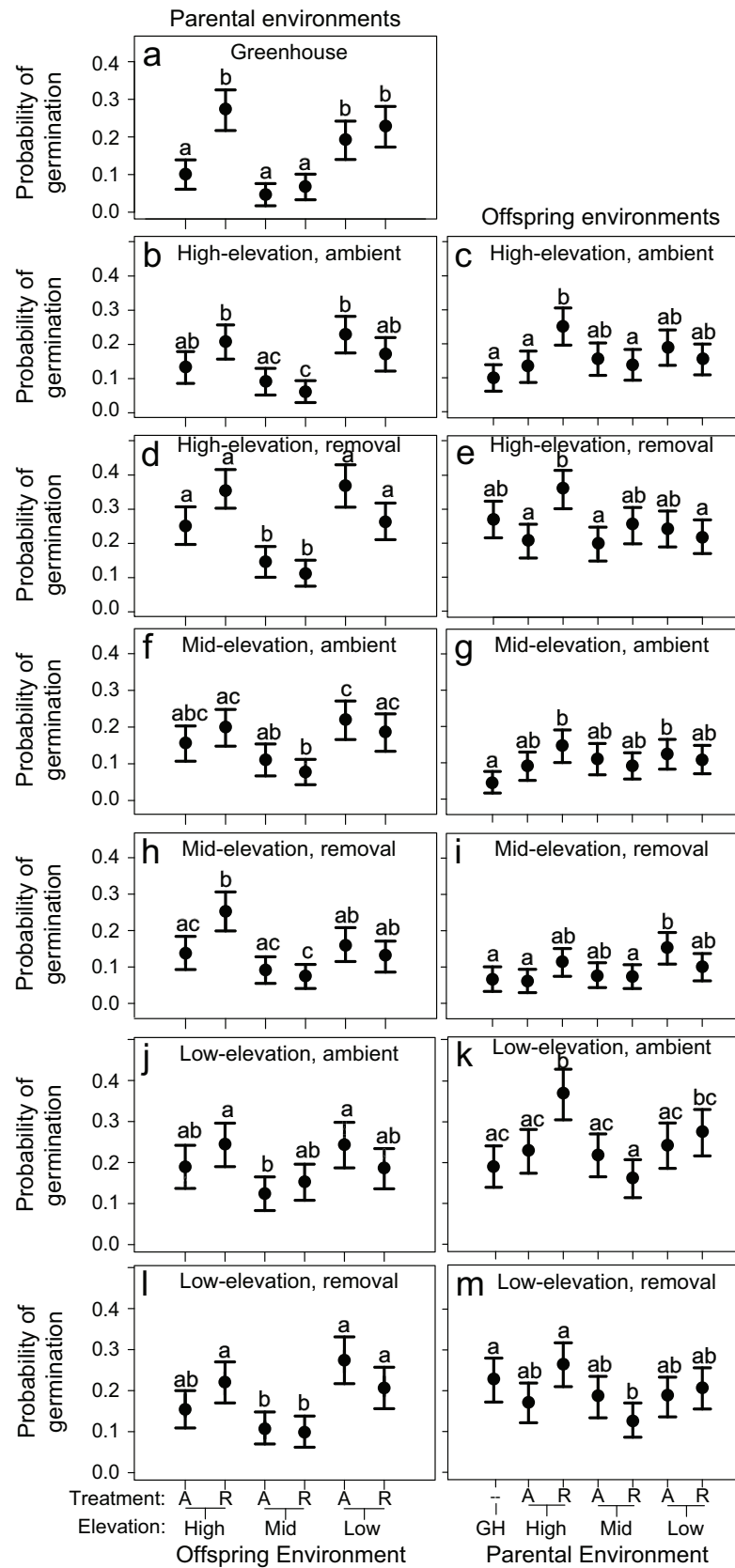
Discussion

Ongoing climate change is accelerating the risk of extinction for species around the globe (Urban 2015). As such, we have a pressing need to understand the contexts in which non-genetic processes can ameliorate or exacerbate the effects of climate change. In this study, *Boechera stricta* exhibited extensive transgenerational plasticity in traits expressed early in life history. Our growth chamber experiment revealed that the effects of transgenerational plasticity can persist in traits expressed after establishment, even when accounting for parental effects on seed mass. Our snow manipulation experiment in the field demonstrated that transgenerational and within-generation plasticities can interact and that environmental gradients may have generated clines in the extent and form of plasticity among populations. These findings suggest that transgenerational plasticity can influence traits known to affect fitness and demographic processes and should be incorporated in studies of adaptation. We discuss phenomena

that may have given rise to the patterns observed here, the contexts in which transgenerational plasticity may be adaptive or maladaptive in our study system, and promising future avenues of investigation.

Differences among populations in the extent or form of plasticity reflect spatial variation in the degree of environmental variability across the landscape (Lind and Johansson 2007). Whether transgenerational or within-generation plasticity evolves will depend on the temporal scale of fluctuating environmental conditions, with transgenerational plasticity more likely to evolve when conditions change regularly and abruptly across generations (Kuijper and Hoyle 2015). Our field experiment demonstrated greater transgenerational than within-generation plasticity in germination success in the low-elevation populations. In contrast, both forms of plasticity affected germination similarly in high-elevation populations. In our study system, these clines in plasticity may have evolved if decadal fluctuations in winter and summer precipitation disproportionately affected low-elevation sites, as generally found in the mountains regions in the western United States (Mote et al. 2005; Regonda et al. 2005). Our growth chamber experiment confirms that differences in germination can be partially attributed to transgenerational plasticity in dormancy. Multiple plant species exhibit geographic clines in seed dormancy (Allen and Meyer 2002; Kronholm et al. 2012; Waggmann et al. 2012; Debieu et al. 2013; Fernández-Pascual et al. 2013; Baskin and Baskin 2014; Zhou and Bao 2014; Burghardt et al. 2015). Future work should assess the relative roles of transgenerational and within-generational plasticities in mediating germination and dormancy dynamics among genetically differentiated populations in other systems.

Our growth chamber and field experiments reveal that there are limitations to accurately extrapolating results from controlled experiments to patterns that would be found under natural settings (Poorter et al. 2016). For instance, germination probabilities were much higher in both temperature treatments of the growth chamber experiment than in any of the field environments. In addition, we detected additive effects of transgenerational and within-generation plasticities on plant size in the growth chamber experiment, but neither parental nor offspring environmental effects significantly influenced size when measured in the field. In fact, we did not detect an influence of transgenerational plasticity on any offspring traits expressed after germination in the field experiment. This finding is not surprising, given that theory and empirical work predict the greatest influence of transgenerational plasticity on traits expressed early in life history (Roach and Wulff 1987; Mousseau and Fox 1998; Donohue 2009; Burgess and Marshall 2011). Furthermore, individuals are at their most vulnerable at the germinant and seedling stages (Donohue et al. 2010; Anderson 2016), and naturally occurring high mortality rates could preclude the detection of parental effects at juvenile and adult stages. Last, analyses from



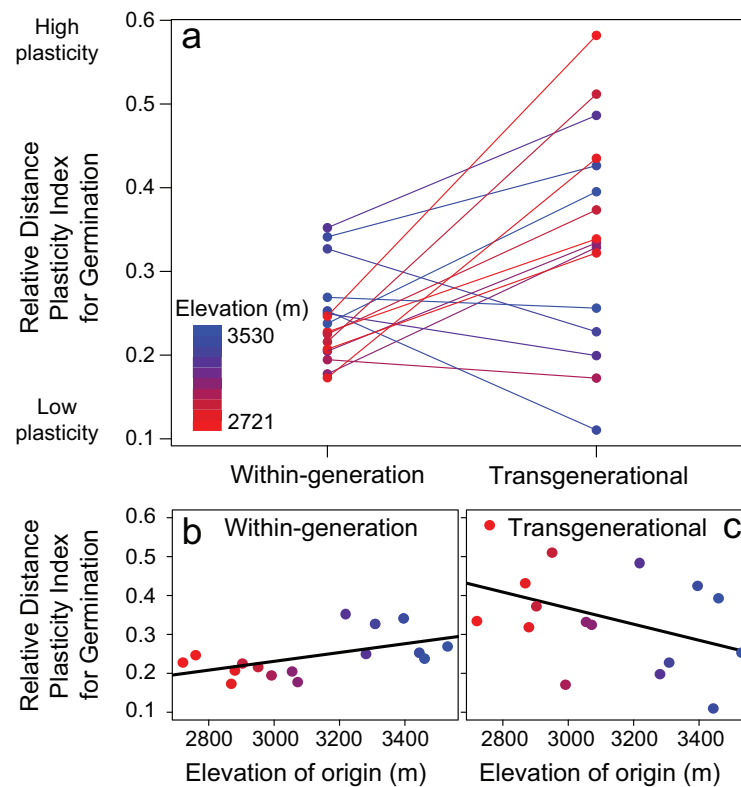


Figure 6: *a*, Measures of the relative distance plasticity index (RDPI) for the probability of germination for each genotype across offspring environments (reflecting the extent of within-generation plasticity) and parental environments (reflecting the extent of transgenerational plasticity). The index is bounded by 0 and 1, with high values indicating a greater extent of plasticity. Lines connect each genotype and are shaded to signify their source elevation. *b*, *c*, Within-generation (*b*) and transgenerational (*c*) RDPI values plotted against source elevation.

our growth chamber experiment included seed mass as a covariate, and it is possible that we were unable to detect the actions of transgenerational plasticity on later-expressed traits in the field via maternal resource provisioning. Our results suggest that researchers take caution when interpreting results from experiments conducted solely under laboratory or greenhouse conditions (Verdú and Traveset 2005; Kellermann et al. 2015; Poorter et al. 2016).

Plants originating from larger seeds have persistent fitness advantages over individuals derived from smaller seeds (Leishman et al. 2000; Metz et al. 2010; Larios et al. 2014). Indeed, in our experiment, larger seeds tended to germinate, grow larger, and have enhanced survival, while smaller seeds tended to be nonviable or remain dormant. In our field experiment, early snow removal increased the size of seeds produced by

parents in the high-elevation site. Furthermore, we detected a genetic cline in seed size across elevations, where high-elevation accessions produce larger seeds than those from low elevations. The prevalence of small seeds in lower-elevation populations may arise if there are trade-offs between the number and size of seeds produced by a mother within or across seasons. We found no evidence for such a trade-off in our study (data not shown). A more robust investigation of trade-offs in survival, growth, and fecundity in our experimental framework is forthcoming. Together, our results suggest that transgenerational plasticity in response to climate change may benefit the larger-seeded populations found at high elevation, while low-elevation populations will persistently face the fitness consequences of smaller-seeded individuals.

Figure 5: Probability of germination across parental (*left*) and offspring (*right*) environments, showing the significant interaction between transgenerational and within-generation plasticities. Within each panel, germination probabilities are shown across environments experienced by the other generation, where data are shown for the ambient (A) and early snow removal (R) treatments in the high- and low-elevation gardens (*left, right*) and for the greenhouse parental environment (*left*). Data were analyzed using a mixed effects Cox proportional hazards model, and estimates of absolute risk $\pm 95\%$ confidence intervals were calculated from a fixed effects Cox proportional hazards model. Letters denote significant differences among environments within a panel.

Dormancy allows seeds to avoid periods of time in which conditions are unfavorable for germination, establishment, and growth (Venable and Brown 1988; Rees 1996). Climate change can compromise seed and seedling vigor by inciting seeds to enter, exit, or forgo a dormant state at inappropriate times (Walck et al. 2011). Transgenerational plasticity in dormancy may thus function as a successful bet-hedging strategy in stressful environments (Venable and Brown 1988; Ooi et al. 2009). We found that transgenerational plasticity in *B. stricta* resulted in a greater probability of dormancy in seeds from parents that experienced warm and dry conditions. This result is surprising, given that increased temperatures during seed maturation are often associated with decreased levels of dormancy in many species (Baskin and Baskin 1998). The consequences of increased dormancy on fitness and population growth will depend on seed longevity and whether seeds encounter favorable conditions in subsequent years. In alpine environments, species with flat seeds like *B. stricta* persist for a shorter duration of time in the seed bank (Ceralbolini et al. 2003; Schwenbacher et al. 2010). In our study system, the date of snowmelt is negatively autocorrelated between consecutive years and positively autocorrelated every 3 years (fig. S3; see Wadgymar et al. 2018b). Transgenerational plasticity on dormancy may be maladaptive in our study system if seed longevity is short, if the timing of snowmelt is a primary determinant of the propensity of plants to produce dormant seeds, and if years with favorable conditions are likely to directly proceed stressful years. We note that all seeds experienced conditions favorable for germination in our growth chamber experiment, yet many remained dormant. Whether dormancy is an adaptive strategy when offspring bypass a suitable opportunity for establishment remains to be tested. Illuminating the fitness consequences of transgenerational plasticity on seed dormancy requires reciprocal seed burial and germination trials conducted across multiple years, sites, and generations.

The extent and adaptive value of transgenerational plasticity can be influenced by a myriad of factors that were not addressed or applicable to our study. In our experiments, all parental plants were the same age. However, maternal age can influence the presence and extent of transgenerational plasticity, suggesting that parental effects may be more dynamic in age-structured populations (Mousseau and Dingle 1991; Berkeley et al. 2004). Grandparental environmental effects can endure across generations, the impacts of which can reinforce (Herman et al. 2012) or oppose (Shama and Wegner 2014; Walsh et al. 2015) parental environmental effects. In outcrossing systems, transgenerational plasticity can also arise through paternal effects on offspring performance (Bonduriansky and Head 2007), and conflicts can arise when optimal strategies differ between both parents and their offspring (Shaanker et al. 1988; Uller 2008). As evidenced by our field experiment, transgenerational plasticity can arise as a result

of conditions experienced before reproduction (Lacey 1996; Burton and Metcalfe 2014; Chen et al. 2014). Thus, variation among parents in the environmental conditions experienced across their ontogeny can alter the extent or effects of transgenerational plasticity. Last, climate change can alter certain environmental variables at different rates, while other factors will remain unaffected (IPCC 2014; Wadgymar et al. 2018b). The effects of climate change on transgenerational plasticity are likely to be complex and context dependent. Future studies should examine the cumulative effects of climate and other agents of selection on parental effects, adaptation, and species persistence.

Our results illustrate how transgenerational plasticity can mediate the effects of climate change on seed and seedling performance unequally in populations across an elevational gradient. These insights suggest that conservation practices involving seed collection or species relocations should consider how transgenerational plasticity could promote or hinder successful establishment. Flexibility in the timing of restoration efforts (immediate vs. future) and the location of restoration sites (proximal vs. distant) may be limited by the presence of strong transgenerational plasticity. Additionally, most seed inventories are maintained and replenished by raising plants in a greenhouse or common garden. In our experiment, greenhouse-produced seeds were smaller than most field-produced seeds yet had a higher probability of germination. Thus, current management practices may inhibit the production of dormant seeds and neglect a valuable mechanism of stress avoidance (Ensslin et al. 2017). Subsequent studies should further explore the role of transgenerational plasticity in informing seed collection, maintenance, and transfer guidelines.

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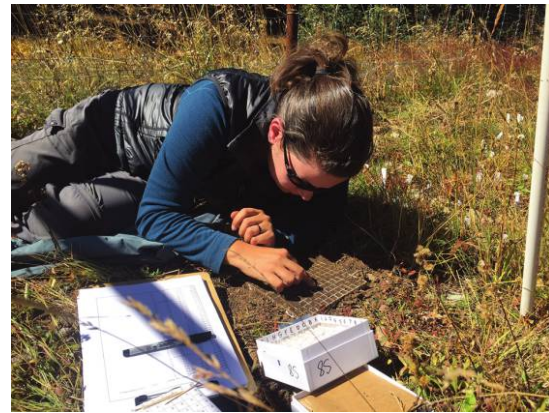
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Top left, view of Mt. Crested Butte from our low-elevation garden (elevation: 2,710 m) after snow removal but before snowmelt (April 2016). Polyvinyl chloride poles mark the locations of the plots. Photo credit: Susana M. Wadgymar. *Top right*, Susana M. Wadgymar planting seeds in a grid in a high-elevation experimental garden. Photo credit: Jill T. Anderson. *Bottom*, seed grid with several older *Boechera stricta* seedlings that have developed four true leaves while retaining their cotyledons. Photo credit: Jill T. Anderson.